STUDIES ON ANTI-OBESITY METABOLITES FROM *DIOSCOREA* SPECIES

A

THESIS SUBMITTED TO

SHIVAJI UNIVERSITY, KOLHAPUR

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

UNDER THE FACULTY OF SCIENCE AND TECHNOLOGY

BY

MR. PATIL RUTURAJ SUDHAKAR
M. Sc.

UNDER THE GUIDANCE OF

DR. MANSINGRAJ S. NIMBALKAR
M. Sc., Ph. D.

DEPARTMENT OF BOTANY
SHIVAJI UNIVERSITY,
KOLHAPUR - 416 004
MAHARASHTRA, INDIA

2022

DECLARATION

I hereby declare that the thesis entitled **"Studies on Anti-obesity Metabolites from *Dioscorea* species"** completed and written by me has not previously formed the basis for the award of any degree or diploma or other similar title of this or any other university or examining body. Further, I declare that I have not violated any of the provisions under Copyright and Piracy/ Cyber/ IPR Act amended from time to time.

In view of University Grants Commissions (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulations, 2018 dated 31st July 2018, I hereby submit an undertaking that this thesis is my original work and it is free of any plagiarism. Further, it is also to state that this thesis has been duly checked through a plagiarism detection tool approved by Shivaji University.

Place: Kolhapur

Date: **Mr. Ruturaj Sudhakar Patil**

Department of Biotechnology
Shivaji University, Kolhapur

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Anti-obesity Metabolites from *Dioscorea* species"** is being submitted herewith for the award of the degree of doctor of philosophy in Biotechnology to Shivaji University, Kolhapur. The work reported in this thesis is based upon the results of original experimental work carried out by **Mr. Ruturaj Sudhakar Patil** under my supervision and guidance. To the best of my knowledge and belief the work embodied in this thesis has not formed earlier the basis for the award of any Degree or Diploma or similar title of this or any other university or examining body.

In view of University Grants Commissions (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulations, 2018 dated 31st July 2018, this is also to certify that the work done by **Mr. Ruturaj Sudhakar Patil** is plagiarism free.

Place: Kolhapur

Date:

Dr. Mansingraj S. Nimbalkar
(Research Guide)

Prof. J. P. Jadhav
Head (I/C)
Department of Biotechnology,
Shivaji University, Kolhapur

ACKNOWLEDGMENTS

First and foremost, I would like to convey my deep sense of gratefulness to my research guide Dr. Mansingraj S. Nimbalkar This work would not have been possible without his guidance, unceasing support and inspiration.

I gratefully acknowledge to Prof. Dr. (Mrs.) J. P. Jadhav head of the department and Prof. Dr. V. A. Bapat for their valuable help, cognizance, and inspiration towards my research work which has provided root for the completion of Ph. D. work.

I gratefully acknowledge to Chhatrapati Shahu Maharaj Research Training and Human Development Institute (SARTHI), Pune, for financial assistance.

I wish to express my wholehearted gratitude to Prof. Dr. R. V. Gurav (Head, Department of Botany), Prof. Dr. (Mrs.) V. D. Jadhav and Prof. Dr. D. K. Gaikwad (Ex- Head Department of Botany), for granting me the opportunity to utilize excellent facilities available at the department.

It is my pleasant duty to offer my sincere thanks to Prof. Dr. G. B. Dixit, Prof. Dr. S. S. Kamble, Prof. Dr. N. S. Chavan, Prof. Dr. N. B. Gaikwad, Dr. (Smt.) K. B. Pawar, Dr. Nilesh Powar, Dr. V. B. Shimple, Dr. (Mrs.) S. A. Patil, Dr. M. M. Lekhak, and Dr. S. G. Ghane for encouragement and valuable suggestions.

It is my pleasant duty to offer my sincere thanks to Dr Ravikiran S. Pagare Sandip University, Nashik, and Maharashtra for helping in the plant identification and valuable suggestions.

I would like to thank teaching faculty of Vivekananda College, Kolhapur Mr. S. G. Kulkarni, Mrs. Salma Nadaf, Mr. Satish Parte, Mrs. Sarita Mankar who taught me how interesting and lively field of Science and Biotechnology is.

I am also grateful to Mr. Amol Jadhav, Nirav BioSolutions Pvt. Ltd. - A Biotechnology Resource Center Pune for providing the cell culture facility and his constant suggestions and timely help.

I would like to thanks teaching and non-teaching of staff Dr. Onkar Apinee, Dr. (Mrs.) Sushma Apinee Mrs. Supriya Sathe Dr. Sachin Otari for their valuable suggestions and encouragement throughout tenure.

During this work I have worked with many seniors and colleagues for whom I have great regard. I wish to extend my warmest thanks to Miss. Devashree Patil, Miss. Manali Rane, Mr. Chetan Aware, Mr. Suresh Suryawashi, Miss. Samidha Kakade, Miss. Partibha Mali, Mr. Subham Sutar, Mr. Rahul Jadhav from department of Biotechnology, Dr. Pramod Lawand, Dr. Chirag Narayankar, Mr. Aditya Magadum, Mr. Sudarshan Charpale, Dr. Indarjit Samant, Mr. Suraj Patil, Mr. Pradeep Deshmukh, Miss. Komal Walevekar colleagues from the department of Botany who have provided great time and company during the course of this work.

I am forever grateful to all non-teaching staff and gardeners from department of botany for their help. I sincerely thank Dr. (Smt.) S. S. Fernandes, Mrs. Seema Kumbhar, Mr. Gavade Mr. Harshal Vhatkar, Mr. Krishnat Patil, Mr. Mukund Kamble, Mr. Shambhuraje Warke, Mr. Vishal Tambekar, Mr. Vishal Nalawade, Mr. Sandeep Kesarkar, Mr. Santosh Kumbhar, Mr. Ramjan Pathan, Mr. Mahesh Powar and Mr. Abhijeet Powar.

I specially thank to Mr. Mahesh Mane for his support and encouragement from my B.Sc. till my Ph.D. work.

The technical difficulties in this time of technological development are painstaking. I am very much thankful to Mr. Ganesh Salokhe for their technical support.

The friends are the friends indeed useful in need too. I specially thanks to Mr. Pushrottam Jamdar, Miss. Varsharani Jamdar and Jamdar family, Mr. Omkar Patil, Mr. Auvdhut Medhe, Mr. Nikhil Patil, Mr. Abhijeet Shelake, Mr. Tejas Shaha, Mr. Nabarun Saha, Miss. Sasha Porenca, Miss. Sagarika Karmakar, Miss. Sayantani Chakraborty, Miss. Parjoti Palani, Mr. Chetan Urunkar, Mr. Yuvraj Kashid, Mr. Uttam More, Mr. Rohit Salunkhe, Mr. Rohit Nagtile, and Mr. Rahul Dongare.

I would also like to extend hug, warm thanks to my beloved brother Dr. Ajay Nalawade his generous support, constant encouragement, for making the photographic representation more attractive and accomplishing the work.

My family deserves a special mention for their inseparable support and prayers. My grandparents Mr. Dyandev Salokhe and Mrs. Sushila Salokhe and grandmother Late Sushila Dinkar Patil who are constant inspiration for me ever since I was child. I would like to express my thanks to in-laws Mr. and Mrs. Vijay Bhandari and all members of Bhandari family. I must express my appreciation for my father Mr. Sudhakar and mother Geeta. I also thanks to my brother Mr. Karan, sister-in-law Shivani.

In the end I express my deep hearted gratitude to my wife Amruta and my little champ Advait for their inspiration, love, and support constant encouragement during the work.

Thanks to all those who helped me knowingly and unknowingly for the fruitful realization of thesis, as well as expressing my apology to them whom I could not mention personally.

Place: Kolhapur

Date:

Mr. Ruturaj Sudhakar Patil

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

%	Percentage
µl	microliter
µg	microgram
^0C	Degree Celsius
ABTS	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
ASA	Acetylsalicylic acid
C/EBP α	CCAAT/Enhancer binding protein
CE	Colchicine equivalent
CO_2	Carbon dioxide
CPT 1	Carnitine palmitoyltransferase 1
DE	Diosgenin equivalent
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenylpicrylhydrazyl
EDTA	Ethylenediamine tetraacetic acid
ESI+	Electrospray ionization positive mode
FASN	Fatty acid synthase
FC	Folin–Ciocalteu *reagent*
$FeCl_3$	Ferrous chloride
g/l	Gram per litre
GAE	Gallic acid equivalent
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase.
gm	Gram
H_2SO_4	Sulphuric acid
HMDB	Human metabolic database
HPLC	High pressure liquid chromatography
hrs	Hours
IC_{50}	Half-maximal inhibitory concentration
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LC-MS	Liquid chromatography-mass spectroscopy
M	Molar
m/z	Mass charge ratio
mg / ml	Milligram per millilitre
mg/gm	Milligram per grams
mg/ml	Milligram per microlitre
mins	minutes
ml	Millilitre
mM	Millimolar
MS	Murashige and Skoog
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide

NaCl	Sodium chloride
NCBI	*National Centre for Biotechnology Information*
NLRP3	NLR family pyrin domain containing 3
OD	Optical density
PCR	Polymerase chain reaction
PPAR ϒ	Peroxisome proliferator-activated receptor-gamma
PSI	pound per square inch
QE	Quercetin equivalent
Q-TOF MS	Quadrupole time-of-flight mass spectrometry
rpm	Revolutions Per Minute
RT	Retention time
SREEBP F1	Sterol regulatory element binding protein 1
Temp.	Temperature
UAE	Ursolic acid equivalent
v/v	Volume by volume
w/v	Weight by volume
µg/ml	Microgram per microlitre
µm	Micrometre
µM	Micromolar


Introduction

1. INTRODUCTION

1. Obesity:

Obesity is rapidly expanding around the world. It is estimated that there are more than 300 million obese people in the world today (Ahmad *et al.,* 2010). It is one of the foremost health problems reflected by the accumulation of an excessive amount of body fat. Obesity, also known as adiposity, is the "New World Syndrome," and its prevalence is increasing rapidly in both developed and underdeveloped world. Swinburn *et al.,* (2011) found that obesity is characterized by an increase in adipose cell size, which is determined by the amount of fat accumulated in the cytoplasm of adipocytes. It is a serious and chronic disease with an associated increased risk of insulin resistance, type 2 diabetes, cardiovascular disease, cancer, gallstones, fatty liver disease, osteoarthritis, and oxidative stress and inflammation-based pathologies in the human population (Devlin *et al.,* 2000).

Obesity and being overweight are described as abnormal or excessive fat accumulations in adipose tissue that lead to health problems. Obesity or overweight is measured in the form of a body mass index (BMI) on the basis of the BMI. Obesity is categorized into six different classes. These are as follows:

Table No 1.1 Categories of obesity

Category	**BMI (kg/m^2)**
Underweight	< 18.5
Normal weight	18.5 – 24.9
Overweight	25.0 – 29.9
Obese (Class I)	30.0 – 34.9
Obese (Class II)	35.0 – 39.9
Obese (Class III)	≥ 40.0

A BMI of 25 or above is considered overweight, while a BMI of 30 or more is considered obese. According to the global burden of illness, the issue has reached epidemic proportions, with over 4 million people dying each year as a result of being overweight or obese in 2017.

According to the WHO (2016), there are slightly less than 2 billion overweight individuals worldwide. Over 600 million of them are classified as obese. In 2016, almost 40% of adults were overweight, and slightly under 15% were obese. Between 1975 - 2016, the global prevalence of obesity climbed threefold. Many of the obesity-related comorbidities are represented in metabolic syndrome, which was originally defined arbitrarily by WHO on the basis of insulin resistance along with other obesity-related problems. Obesity is also linked with malnutrition the change in the diet and lifestyle leads to obesity and related problem.

Obesity and being overweight are substantial risk factors for a variety of chronic illnesses, including cardiovascular disorders like heart disease and stroke, which are the leading causes of mortality worldwide. Obesity can also lead to diabetes and its complications, such as blindness, limb amputations, and the need for dialysis. Diabetes prevalence has doubled worldwide since 1980. Excess weight can cause musculoskeletal diseases such as osteoarthritis. Obesity has also been associated with endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon cancers.

2. Management of obesity:

Obesity's complicated pathophysiology necessitates the discovery and development of novel medications and alternatives for its prevention and treatment. Natural goods, particularly medicinal herbs, are thought to be promising anti-obesity agents as conventional medicines fail to provide long-term remedies. Different natural product combinations operate synergistically on multiple molecular targets in diverse ways, either boosting weight reduction or avoiding weight gain (Liu *et al.,* 2003).

Medicinal plants use mechanisms such as inhibition of lipid hydrolyzing and metabolizing enzymes, disruption and modification of adipogenic factors, and appetite suppressants to battle obesity (Rayalam *et al.,* 2008; Freitas *et al.,* 2017).

There are two approaches to treating obesity. One of these is the inhibition of pancreatic lipase. Enzyme lipase act on the dietary lipids in the intestine and helps to absorb fat in the body. Inhibition of the lipase reduces the excess absorption of the free fatty acid in the body. In the market, drugs like Orlistat reduce fat absorption through inhibition of pancreatic lipase, are available. Scientists have reported that some plant extracts inhibit pancreatic lipase, (Lei *et al.,*2007, Ado *et al.,* 2013, Roh and Jung 2012).

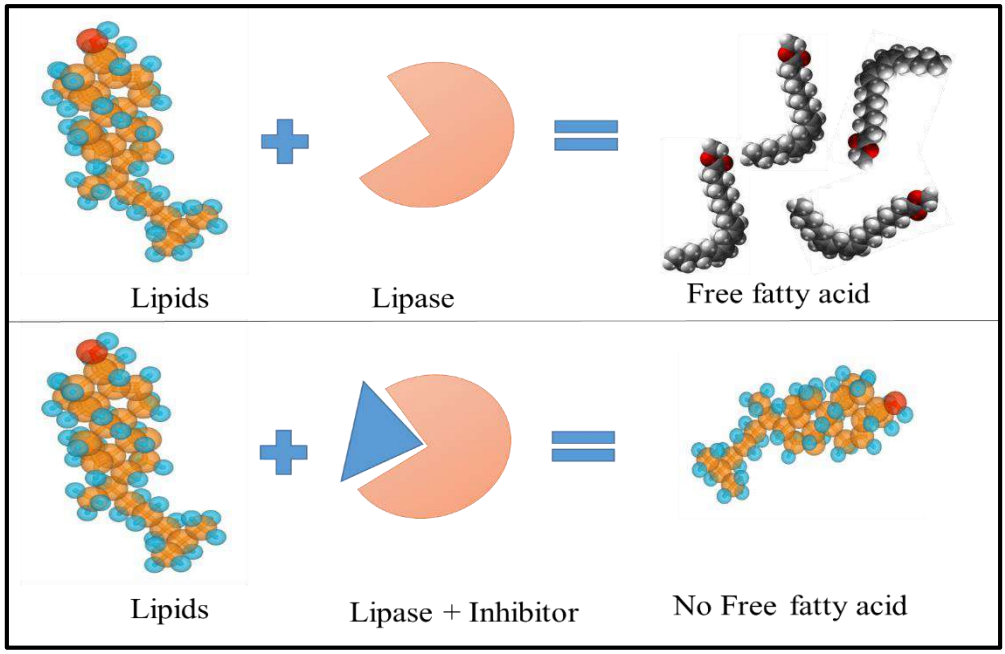


Fig 1.1 Activity of Lipase

Another approach to reducing obesity is the degradation of accumulated fat. Adipose tissues are the major part of the body that stores excess fat. Some medicines and herbal products are available in the market that down regulates adipogenic genes or by stimulating lipolysis of the stored fat. There are some plant extracts that stimulate gene transcription that activates the lipolytic pathway and restricts enzymes like 5'AMP activated protein kinase (AMPK), which inactivates Acetyl-CoA carboxylase (ACC) and enhances fatty acid oxidation by up-regulating the expression of CPT-1, PPARɣ, and uncoupling protein.

Adipose tissue, an endocrine organ, regulates metabolism and homeostasis through the release of many physiologically active adipokines. Three key transcription factors are peroxisome proliferator-activated receptor (PPAR) ɣ, CCAAT/enhancer-binding protein (C/EBP) α, and sterol regulatory element-binding protein (SREBP) 1c control the expression of these lipid-metabolizing enzymes during adipose tissue development. 5' AMP-activated protein kinase (AMPK) plays a major role in glucose and lipid metabolism by inactivating acetyl-CoA carboxylase (ACC) and stimulating fatty acid oxidation by up-regulating the expression of carnitine palmitoyltransferase-1 (CPT-1), PPARα, and uncoupling protein (Patra *et al.*, 2015).

Plant extracts are known to have multiple effects that can be used for obesity management. It is worthwhile to explore the potential of unexplored medicinal or endemic plants. Saponins, flavonoids, phenols, and alkaloids are plant components having anti-obesity potential. The existence of numerous phytochemical combinations in plant

medications may result in synergistic effects by acting on many molecular targets, providing advantages over therapies that typically employ a single ingredient.

India has tremendous sources of medicinal plants in the region of the Western Ghats. Some of these plants are used as a traditional medicine to treat obesity problems. These medicinal plants are easily available and are non-harmful compared to modern allopathic drugs. Thus, various combinations of the active components of these plants after isolation and identification can be made and have to be further assessed for their effects. The preparation of standardized doses and dosage regimes may play a critical role in the management of obesity. It seems to be an effective effort for the good health of humans. There is a broad scope to derive potent anti-obesity bioactive natural compounds from medicinal plants.

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Monocots
Order: Dioscoreales
Family: Dioscoreaceae
Genus: *Dioscorea* L.

3. Plant *Dioscorea* L.:

There are around 630 species in the genus *Dioscorea* (Mabberley, 2008). Yams are recognized as one of the most important basic foods. It is mostly found in the tropical and subtropical regions of Africa, Central and South America, and Asia (Coursey 1967). 50 *Dioscorea* species have been cultivated for food. Thirty of them are cultivated for medically relevant chemicals such as steroids, sapogenins, and dioscorin (Bhattacharjee *et al.,* 2011).

Dioscorea species are herbaceous vines with starchy tubers. Tubers include steroids, saponins, and sapogenins that are significant in the manufacture of anti-inflammatory, androgenic, estrogenic, and contraceptive medicines. Cytotoxic, anticancer, antifungal, immunoregulatory, hypoglycemic, and cardiovascular effects are also demonstrated by the active substances. They are used in folk medicine to relieve menstrual cramps as well as to alleviate labor discomfort.

Tubers also include monacolin-K (a cholesterol-lowering agent) and monascin (an anti-inflammatory substance). The majority of the species have commercial importance and are also significant ethnically as secondary foods. (Edison *et al.*, 2006).

Dioscorea is important in food and medicine, these species should be explored more for their medicinal properties, which include anti-obesity properties. In this study, four *Dioscorea* species from Kolhapur, Maharashtra, India were screened for their anti-obesity potential.


Review of Literature

2. REVIEW OF LITERATURE

2.1 The *Dioscorea*:

Yam is the popular name for the monocotyledonous family Dioscoreaceae. Brown (1810) established the family name '*Dioscoreae*,' which was later altered to Dioscoreaceae by Lindley in 1836 (Coursey, 1967). The biggest genus within the family is *Dioscorea* L. Linnaeus (1737) created it with eight species in his Species Plantarum. The genus name was chosen to honor Pedenios Dioscorides, a Greek physician who served in the army and gathered medicinal plants throughout the Roman Empire during his lengthy travels (Borzelleca and Lane, 2008). His five-volume work, "De Materia Medica (On Medical Material)," in native Greek, served as the foundation for all current pharmacopeias (Forbes *et al.*, 2013). Charles Plumer named the genus of edible yams after Pedenios Dioscorides in 1703. (Osbaldeston, 2000). *Dioscorea* evolved in the tropical regions of Africa, Southeast Asia, and South America (Edison *et al.,* 2006).

The Dioscoreaceae family is a pantropical group that is found in natural environments. However, members of this family are uncommon in arid places (Thapyai, 2004). Though rainfall patterns dominate the distribution, certain members have adapted to biologically severe environments such as rainforests and savannahs. Some members live on coastal plains, while others live in mountains at high elevations of several thousand meters. According to Coursey (1967), *Dioscorea* species were discovered in the desert near the tropical area, where rainfall averages 40/50 cm per year, as well as high altitude mountains with significant frost. Although *Dioscorea* is widespread, the other members of the family are not.

As a staple meal, *Dioscorea* tubers have had an essential influence on the current distribution of several edible species. The tubers were transferred from area to region by migrating people. Climate change and environmental whims appear to had a significant effect on *Dioscorea* migration and distribution (Thapyai, 2004). According to Coursey (1967), yams were chosen on ships owing to their abundant energy supply in the form of stored food and their long shelf life, which greatly aided global distribution. Furthermore, because of the commercial importance of yams, they were transported at an international level during the sixteenth century, and the rise of sea transportation greatly assisted this (Coursey, 1967). Hornel (1934) published a thorough examination of the evidence for a theory based on societal cultural aspects, linguistic and physical characteristics in its dispersion. By the end of the Cretaceous epoch, around 75 million years ago, *Dioscorea*

had spread all across the earth (Alexander and Coursey, 1969). It was widely distributed in the southern world, and its first expansion appears to have occurred via the Antarctic Continent in Asia (Coursey, 1967).

2.2 Obesity:

Obesity is connected with higher triglyceride accumulation and a slower rate of lipid breakdown. Obesity-related lipid metabolism is mostly due to decreased catecholamine-stimulated lipolysis. Blunted catecholamine-stimulated fatty acid metabolism in obesity appears to be irrespective of fat mass since it is evident in obese participants' first-degree nonobese relatives and after weight reduction in obese insulin-resistant people. Hormone sensitive lipase (HSL) and Adipose triglyceride lipase (ATGL) expression levels were shown to be lower in separated adipocytes from obese subjects, as well as in insulin-resistant states unrelated to obesity (Sam & Mazzone 2014). Obese and insulin-resistant individuals have lower expression of lipolytic b2-adrenoceptors70 and higher antilipolytic properties of a2-adrenoceptors77, both of which contribute to a decrease in catecholamine-induced lipolysis. Other variables, such as leptin, may also influence ATGL and HSL expression in obese people. Despite the fact that catecholamine-induced lipolysis is inhibited in obese patients, FAA levels are enhanced due to greater basal lipolysis associated with larger adipose tissue mass (Sam & Mazzone 2014).

2.3 Adipose Tissue:

Adipose tissue (AT) is a wide and highly specialized connective tissue made of various cell types. It serves as a significant energy reservoir in the body, storing surplus energy as lipids and releasing it as needed, as well as providing temperature control. AT is regarded as a significant endocrine organ that mediates biological effects on metabolism, hence contributing to the maintenance of energy balance and the prevention of obesity-related metabolic disorders (Wozniak *et al.,* 2009).

AT is divided into two categories based on its position and colour. AT is classified as brown adipose tissue (BAT) and white adipose tissue (WAT) based on colour, with major variations in shape and function. AT-tissues are characterised as visceral or subcutaneous based on their location (Rezaee and Dashty, 2013; Farkas *et al.*, 2018).

2.3.1 BAT-Adipocytes feature many tiny lipid droplets in their cytoplasm and are multilocular. As a result, energy stored in the form of triglycerides in lipid droplets is

available for fast hydrolysis and oxidation of fatty acids. BAT generates energy through non-oxidative phosphorylation by uncoupling protein 1. BAT possesses an abundance of mitochondrial chromogens, which are responsible for the brown colour. They have a high vascular supply and respond to the sympathetic nervous system to induce lipolysis, which results in the release of free fatty acids (FFA) and an increase in energy expenditure (Redinger, 2009; Frühbeck *et al.*, 2009).

2.3.2 WAT- Adipocytes are unilocular and have a distinct lipid droplet capable of storing triglycerides with high energy density (Trayhurn 2007). They are key factors of adipose tissue and play a role in metabolic control via energy balance, adipocyte development, and insulin sensitivity. Mature WAT adipocytes differentiate into a range of cells (pre-adipocytes, macrophages, fibroblasts, and endothelial cells) that drain FFA and inflammatory mediators in portal circulation and influence metabolism (Juge-Aubry *et al.*, 2005; Gesta *et al.*, 2007).

2.4 Role of the transcription factor in the adipocyte differentiation:

2.4.1 Peroxisome proliferator-activated receptor gamma (PPARɣ): It is both required and sufficient for WAT adipogenesis in animals, and is regarded as an adipogenesis "master regulator." PPARɣ is necessary for adipocyte differentiation both in vitro and in vivo in mice and plays a key role in placental vascularization, monocyte differentiation, and heart development. PPARɣ loss-of-function (LOF), severe lipodystrophy, insulin resistance, and hyperglycaemia can all result from naturally occurring mutations within the PPARɣ coding sequence in humans. Furthermore, in mice, adipocyte-specific deletion of PPARɣ leads to the complete lack of WAT. Surprisingly, PPARɣ expression, in conjunction with the presence of an activating ligand, is sufficient to launch an adipogenic programme and maintain an adipocyte phenotype in previously non-adipogenic cells. As a result, PPARɣ plays an important role in mammalian adipogenesis, as seen by PPARɣ LOF in humans, which is linked to severe lipodystrophy, metabolic disorders, and illness. (Wafer *et al.*, 2017).

2.4.2 CCAAT/enhancer-binding protein alpha (C/EBPα): The C/EBPα has been linked to adipoblast differentiation regulation. In this work, they have studied C/EBPα ability to induce the adipogenic pathway in a range of fibroblastic cells. The C/EBPα gene is transduced into eight mouse fibroblastic cell lines using retroviruses and DNA transfection, resulting in adipocyte colonies at varying frequencies. When the C/EBPα gene is

transduced by retroviruses, the percentage of G418-resistant colonies with an adipocyte shape is reproducibly greater than 50% in NIH-3T3 cells. The capacity to boost the adipogenic programme needs C/EBP alpha's powerful transcriptional activation domain, which C/EBPβ lacks. Despite its antimitogenic properties, clonal cell lines expressing high levels of C/EBPα can be easily generated. Stable expression of C/EBP α in BALB/c-3T3 cells improves their potential to develop into adipocytes. They have reported that C/EBPα may successfully enhance the adipogenic programme in a range of mouse fibroblastic cells, including those with little or no spontaneous adipogenic potential. (Freytag *et al.,* 1994).

2.4.3 Sterol regulatory element binding protein 1 (SREBP 1): When the mammalian liver cells are treated with a high carbohydrate diet, it increases the transcription of triglyceride production (lipogenesis) enzymes. The treatment activates hepatic insulin signalling, which helps in sterol regulatory element-binding protein-1c transcription (SREBP-1C). They have investigated the role of SREBP-1c in the expression of lipogenic genes in glucose- and insulin-treated primary rat hepatocytes by using an inducible adenovirus system. They found that overexpression of fatty acid synthase, S (14), and acetyl-CoA carboxylase mRNAs results in a mild elevation of fatty acid synthase, S (14), and acetyl-CoA carboxylase mRNAs in response to insulin therapy, but not lipogenic enzyme genes. Restoring insulin overexpression in cells did not result in an increase in mRNA levels. This study indicates that SREBP 1 regulates the expression of the fatty acid synthase and related genes. This is the essential gene for adipocyte development (Stoeckman and Towle 2002). Downregulation of these genes helps in the treatment of obesity.

2.4.4 Fatty acid synthase (FASN): It helps to catalyse the synthesis of palmitate (C16:0, a long-chain saturated fatty acid) from acetyl-CoA and malonyl-CoA in the presence of NADPH, and produces the fatty acid chain in the adipose tissue. Extra energy is converted to the stored energy in adipose tissue. Berndt *et al.,* (2007) stated that enhanced FASN mRNA expression in fatty tissue has been connected to visceral fat accumulation, impaired insulin sensitivity, increased circulating fasting insulin, IL-6, leptin, and RBP4, indicating that lipogenic pathways play an important role in the causal association between the consequences of excessive caloric intake and the development of obesity and type 2 diabetes. While considering the above statement, we need to search for a compound that controls or downregulates the fatty acid synthase gene expression to develop an anti-obesity compound.

2.4.5 Carnitine palmitoyl transferase (CPT-1): The presence of brown adipose tissue (BAT) in mature humans, as well as its reduction in obese and diabetic individuals, has highlighted this tissue as a major actor in obesity-induced metabolic problems. Because BAT controls energy expenditure via thermogenesis, boosting its thermogenic fat-burning capacity is an appealing therapeutic strategy. For this, they try to increase BAT fat burning by enhancing the rate of fatty acid oxidation (FAO). For the study, they used adenoviral infection to produce carnitine palmitoyl transferase 1 AM (CPT1AM), a permanently active mutant version in a rat brown adipocyte (rBA) cell line. CPT1AM-expressing rBA had higher FAO, lipolysis, UCP1 protein levels, and mitochondrial activity. Furthermore, increased FAO decreased the palmitate-induced rise in triglyceride level as well as the production of obesity and inflammatory markers. As a result, CPT1AM-expressing rBA exhibited increased fat-burning ability and reduced lipid-induced dysregulation. This suggests that CPT1AM-mediated increases in brown adipocytes FAO might be a novel method for treating obesity-related diseases. (Calderon-Dominguez *et al.*, 2016).

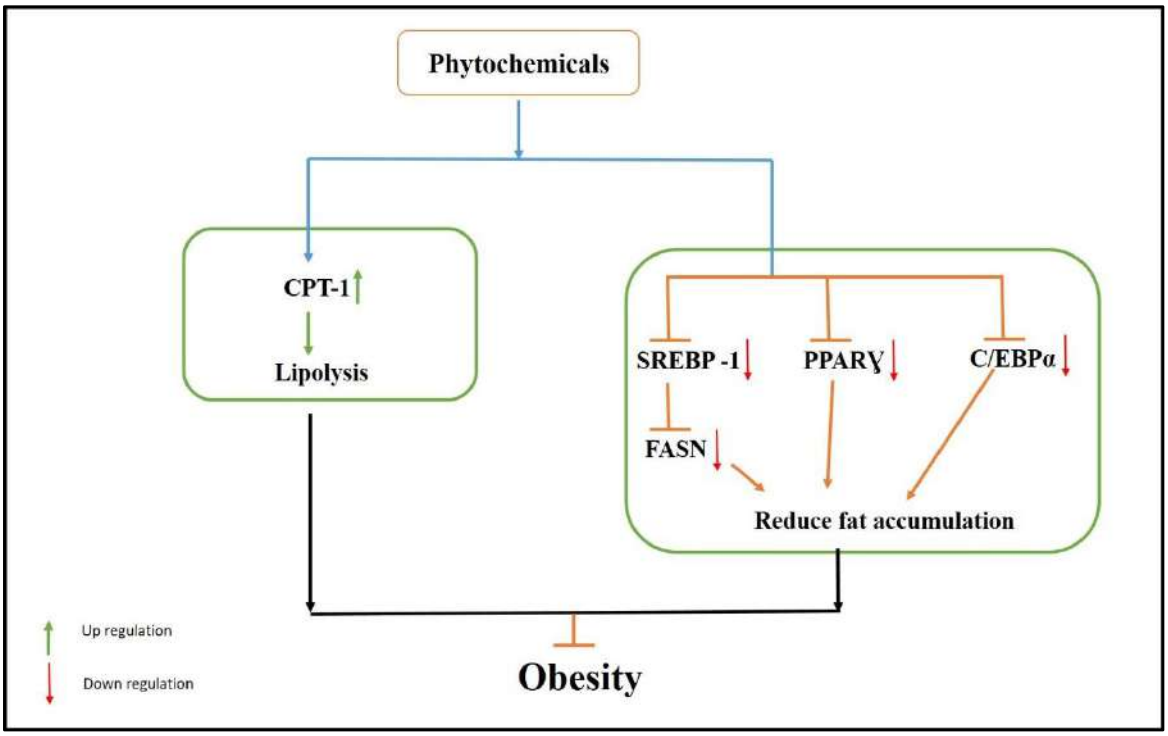


Figure No. 2.1: Over view of the gene involved in the obesity

2.5 Oxidative Stress and Obesity:

Oxidative stress is linked to the development of obesity-related co-morbidities. Hyperglycaemia, higher tissue lipid levels, vitamin and mineral deficiencies, and food type are all potential factors for oxidative stress in obesity. Antioxidant enzymes Cu-Zn superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity were shown to be decreased in obese participants' erythrocytes compared to non-obese controls. Insulin resistance and diabetes, as well as cardiovascular issues, sleep disorders, asthma, oncological problems, reproductive, rheumatological problems, and liver failure, are all caused by obesity-induced oxidative stress. (Manna and Jain, 2015).

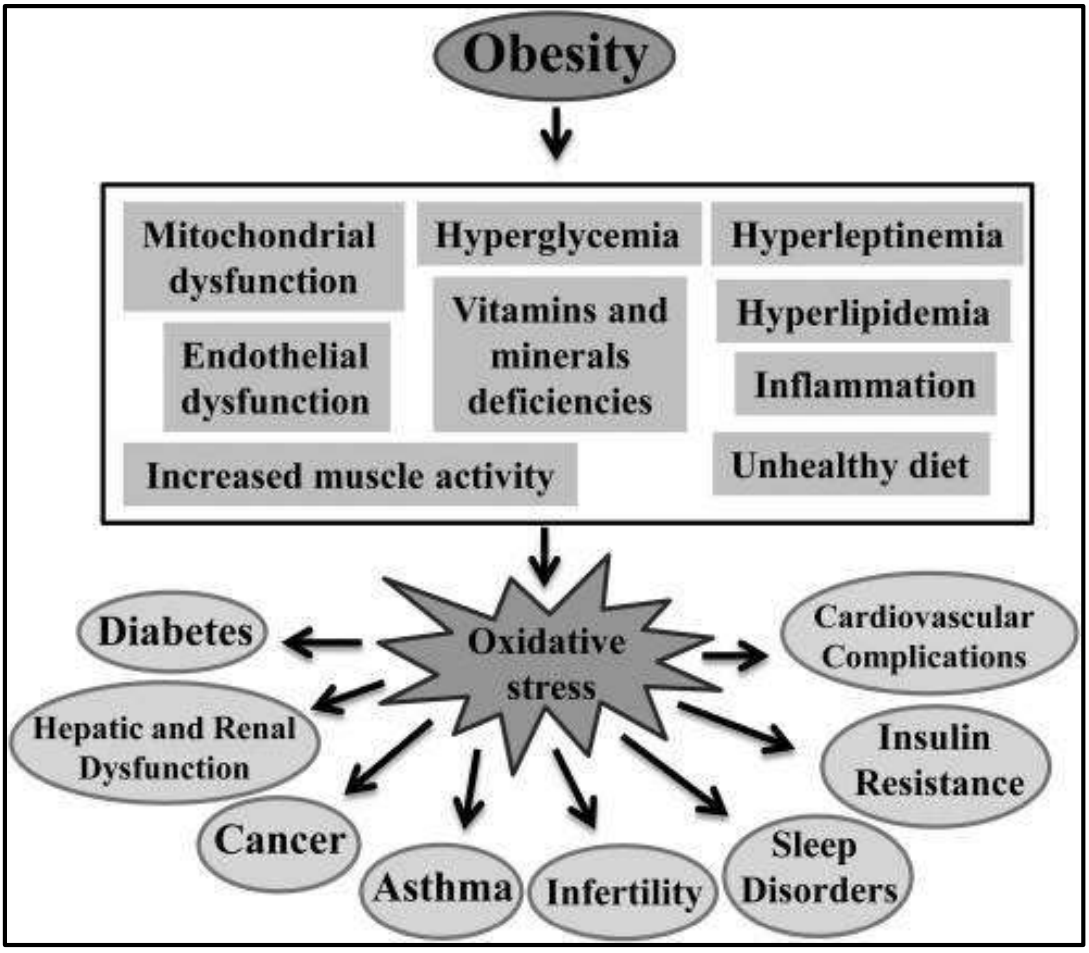


Figure No. 2.2: The role of oxidative stress in the development of obesity-related health risks and the conditions that cause oxidative stress in the pathogenesis of obesity (Manna and Jain, 2015)

To lower oxidative stress, possible antioxidant molecules from natural resources must be identified; plants are an excellent source of antioxidants. Due to their ability to donate hydrogen atoms to free radicals, phenolic and flavonoid molecules are key antioxidant

components that are responsible for deactivating free radicals. They also have excellent structural properties for free radical scavenging. Several studies have found a linear relationship between total phenolic and flavonoid concentration and antioxidant capability. (Aryal *et al.,* 2019).

Table No.2.1: Role of antioxidants in oxidative stress and obesity (Abdali *et al.,* 2015)

Antioxidant	Plant sources	Supplementation's effects
Polyphenols and carotenoids		
Procyanidins	*Cinnamomum cassia*, *Malus pumila*, Cranberries, Avocado, Red beans, Almonds, Peanuts	*Cinnamomum cassia* is the most studied plant. The extract has very little coumarin, making it safer; it may reduce FBG and HbA1c, oxidative stress, and perhaps fat mass.
Catechins	Green tea (*C. sinensis*), Apples, Blackberries	Increased thermogenesis and fat oxidation may result in weight loss; green tea also includes caffeine, which may be required for diabetic benefits; excessive caffeine (>300 mg/day) produces tolerance
Chlorogenic Acid	Green Coffee, Plums, Peaches, Dates	Fruits have the ability to help you lose weight; they also include ferulic acid, which is a potentially useful antioxidant.
Resveratrol	Red wine, Japanese knot wood, Acai, Blueberry, Bilberry, Cranberry, Pomegranate, *Ziziphus*	Reduces oxidative stress
Lycopene	Guava, Papaya, Watermelon, *Asparagus*, Tomato, Eggplant, Potato	There were minor impacts on oxidative stress state, IL-6, TNF-, or CRP; no effects on weight, FBG, HbA1c, or glucose tolerance impairment.

2.6 Current available anti-obesity drugs:

2.6.1 Pharmacotherapy:

Anti-obesity medications or weight reduction pharmaceuticals of pharmacological substances lower or regulate weight by affecting essential human body processes such as regulation, appetite regulation, neuroendocrine regulation, or calorie absorption. (Mohamed *et al.,* 2014)

Table No. 2.2: Available synthetic drugs available in market for long term and short-term treatment

Drugs	Action
Orlistat	Inhibits pancreatic lipase
Cetilistat	Inhibits pancreatic lipase
Rimonabant	Inverse agonist f or the cannabinoid receptor CB1. Its primary mode of action is appetite suppression.
Diethyl propionfluxetine	Reduce food intake through selective inhibition serotonin re uptake
Phentermine	Works on the central nervous system to suppress appetite.
Lorcaserin	Reduces appetite by activating a type of serotonin receptor
Sibutramine	A selective serotonin and noradrenaline re-uptake inhibitor

The synthetic medications that are now accessible have several adverse effects. Drugs that imitate the sympathetic nervous system induce the body to become stressed or anxious, resulting in headaches, sleeplessness, irritability, breathlessness, and agitation. Drugs that inhibit enzyme function induce diarrhoea, bloating, and stomach discomfort (Gadde *et al.,* 2018). As a result, there is an urgent need for the development of novel medications with low or no long-term negative effects.

2.6.2 Herbal approaches to treating the obesity:

Herbal extracts or compounds help to reduce obesity by inducing different pathways such as inhibiting the breakdown and absorption of dietary lipids, appetite suppressants, adipocyte differentiation as well as increasing intestinal transportation, β-oxidation and metabolic rate. Plant extracts have anti-obesity properties through several mechanisms; possibly the suggested method for obesity therapy might be achieved by the synergism of

different plant extracts with varied activities. As a result, natural plant products provide a great prospect and promise for new preventative and therapeutic approaches to obesity (Rayalam *et al.,* 2008). Some of the drugs are listed below in **Table No. 2.4**

Table No. 2.3: Herbal products available in the market

Drug	Content	Mode of action	Reference
Lipotrim	*Garcinia cambogia* extract 50 mg. Chromium 100 mcg	Diet	Brown 1999
Slimax	Aqueous extract of *H. vulgare, P. multiflorum, D. longan, L. sinense, L. brownii* and *Z. officinale*	Modification of lipid metabolism, with accumulation and the release of lipid from adipose tissue, increase lipid metabolism	Ignjatovic *et al.,* 2000
Phase 2	Fractionated *Phaseolus vulgaris* extract	Decrease carbohydrate absorption by inhibiting Alpha amylase.	Udani *et al.,* 2004
Forslean	Extract of *Coleus forskohlii* forskolin (25 mg)	Increase camp activates the hormone sensitive lipase results in lipolysis	Lieberman 2004
Slim339	Combination of *G. cambogia* extract with calcium pantothenate and extracts of *M. chamomilla, R. damascena, L. officinalis and Canangaodorata*	Appetite suppression, inhibition of pancreatic alpha amylase and intestinal alpha glycosidase	Toromanyan *et al.,* 2007
Ayurslim	*G. cambogia, B. mukul, G. sylvestre, T. chebula and T. graecum*	Reducing the consumption of fats and carbohydrates	Singh *et al.,* 2008 Asdaq *et al.,* 2015;

2.7 Mechanism of herbal products for anti-obesity:

2.7.1 Gene Regulation during adipocyte differentiation:

Adipocytes are required for lipid regulation and energy balance. Adipose tissue stores triglycerides and produces free fatty acids in response to energy needs. As a result, herbal extract expected at suppressing adipogenesis is being developed. Many transcription factors, including C/EBPα and PPARϒ, are inhibited, resulting in the suppression of adipocyte development. (Kang *et al.,* 2013).

2.7.2 Lipase inhibition effect:

Pancreatic lipase, a major enzyme in dietary triglyceride absorption, breaks down fat enzymatically; pancreatic lipase hydrolyses triglyceride to monoglyceride and FA (Marrelli *et al.,* 2013). Lipase inhibition, which interferes with fat absorption in the Gastrointestinal (GI) tract, is a potential approach for obesity. Lipase inhibitors are therefore commonly considered to be a successful treatment for obesity.

2.7.3 Rise in energy expenditure:

Some anti-obesity herbal drugs manage body weight by increasing energy consumption through the activity of BAT, which facilitates in the conversion of energy from food into heat by uncoupling protein1 (UCP1/thermogenin) As a result, plant extracts that can up-regulate UCP1 gene expression may be a promising technique for delivering an anti-obesity impact by boosting energy expenditure. (Kajimura *et al.,* 2014).

2.7.4 Appetite suppressant:

Many plant extracts stimulate the sympathetic nervous system, increasing satiety and energy expenditure by suppressing hunger, as well as increasing fat oxidation via neural signal peptides such as serotonin, histamine, and dopamine, and their associated receptor activities, which are associated with satiety regulation. These brain signal peptides and their receptors might be prospective targets for the development of anti-obesity dietary items by reducing calorie intake while improving satiety (Morton *et al.,* 2014).

2.7.5 Stimulating lipid metabolism:

Accelerated fat oxidation promotes triglyceride hydrolysis, which reduces fat storage and combats obesity by upregulating adrenergic receptors, which trigger lipolysis

via activation of adenosine monophosphate-activated protein kinase (AMPK), resulting in increased fatty acid oxidation (O'Neill *et al.*, 2013). In a conclusion, plant extracts that overexpress lipolysis can be explored in the development of anti-obesity therapies**.**

Obesity has become a serious global concern, necessitating the development of effective anti-obesity natural products. Several plant extracts and bioactive molecules have been studied in order to reduce obesity and related problems. Some of the plant extract and active components having anti-obesity potential are listed below in **Table No. 2.4.**

2.8 Indian scenario about Medicinal plants:

Herbal plant extracts are a type of traditional medicine that has been utilised by humans since the beginning of civilization and is still widely used today. According to the WHO, almost 80% of the population in undeveloped countries depend on traditional medicines for their primary health concern. India was a pioneer in the creation and recorded usage of traditional medicine, namely Ayurveda, Siddha, and Unani. More than 2000 medications of natural origin originating from various folkloric traditions are used in Indian medicine (Narayana *et al.*, 1998). In past decades, there has been a surge in the use of alternative medications all over the world to modify obesity, prevent obesity, and obesity-related illnesses. Several plant products, vegetables, herbs, and fruits are habitually consumed as part of a diet that provides significant health advantages by providing valuable nutritional and therapeutic qualities. Allopathic drugs' use has been limited due to their negative side effects.

Table No. 2.4: List of the plants reported for the anti-obesity properties

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
1	*Acorus calamus* Linn (Araceae)	Rhizome, roots and leaves	Ethyl acetate extract of *A. calamus* inhibits α-glucosidase activity.	HTT-T15 cell line	Avadhani (2013)
2	*Aegle marmelos* Linn (Rutaceae)	Leaves	The active ingredients Umbelliferone and Esculetin reduce Hyperlipidemia in obese rats fed a high-fat diet by depleting lipid content in adipocytes.	3T3-L1 preadipocytes	Karmase *et al.*, (2013)
3	*Agrimonia pilosa* Ledeb (Rosaceae)	Aerial parts	Active substance 1beta-hydroxy-2-oxopomolic acid suppresses adipocyte development and the expression of adipogenic marker genes such as PPAR-Ɣ, C/EBP alpha, GLUT4, adiponectin, aP2, ADD1/SREBP1c, resistin, and fatty acid synthase. By inhibiting PPAR-Ɣ and C/ EBP alpha expression, it also suppresses adipocyte development by downregulating certain adipocytokines.	3T3-L1 preadipocytes	Ahn *et al.*, (2012)
4	*Alnus hirsuta* (Spach) Rupr. (Betulaceae)	Leaves	Platyphyllonol-5-O-β-d-xylopyranoside suppresses the induction of PPARγ and C/EBPα protein expression, and inhibits adipocyte differentiation.	3T3-L1 preadipocyte	Lee *et al.*, (2013)
5	*Amomum cardamomum* L. (Zingiberaceae)	Seeds	C/EBPα, C/EBPβ, and PPARƔ gene and protein expression were regulated by seed extract.	3T3-L1 preadipocyte	Park (2014)
6	*Bauhinia variegate* L. (Fabaceae)	Flowers, flower buds, stem, roots, stem bark, seeds, leaves	It lowers elevated levels of total cholesterol, triglycerides, and LDLP while increasing levels of HDLP and brain serotonin. β-sitosterol in the stem causes serotonin release in the brain, which has anti-obesity properties.	Human neutrophils.	Bansal (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
7	*Brassica rapa* L. (Brassicaceae)	Root	Lipolysis-related genes, such as 3-adrenergic receptor, hormone-sensitive lipase, adipotriglyceride lipase, and uncoupling protein, are increased in white adipocytes of rats treated with B. campestris extract. In EBR-treated 3T3-L1 cells, cyclic AMPK, HSL, and extracellular signal-regulated kinase are activated.	3T3-L1 preadipocytes	An *et al.,* (2010)
8	*Caesalpinia sappan* L. (Leguminosae)	Heartwood	Brazilein decreases the induction of peroxisome PPAR-Ɣ (PPAR) and inhibits intracellular lipid accumulation during adipocyte development in 3T3-L1 cells.	3T3-L1 preadipocytes	Liang *et al.,* (2013)
9	*Citrus aurantium* L. (Rutaceae)	Fruits, leaves	It prevents AKT activation and GSK3 phosphorylation, which causes lipid build up and lipid metabolising genes to be down-regulated, preventing adipocyte development.	3T3-L1 preadipocytes	Moro and Basile (2000) Kim *et al,* (2012) Stohs *et al.,* (2012)
10	*Coptis chinensis* Franch. (Ranunculaceae)	Rhizome	In 3T3-L1 cells, it prevents lipid build up. Several adipocyte marker genes, such as proliferator activated receptor and CCAAT/ enhancer-binding protein, are drastically reduced by the five alkaloids found in this plant. Adipogenesis was shown to be inhibited by isolated alkaloids.	3T3-L1 preadipocytes	Choi *et al.,* (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
11	*Cucurbita moschata* Duchesne (Cucurbitaceae)	Stems	Decrease lipid accumulation by inhibiting peroxisome PPAR-Ɣ, CCAAT/ enhancer-binding protein α, fatty acid-binding protein 4, sterol response element-binding protein-1c, and stearoyl-coenzyme A desaturase-1 expression.	3T3-L1 preadipocytes	Lee *et al.*, (2012)
12	*Curcuma longa* L. (Zingiberaceae)	Rhizomes	AMPK causes lipolysis by increasing hormone-sensitive lipase and adipose triglyceride lipase mRNA levels and decreasing perilipin mRNA levels. Curcumin inhibits macrophage infiltration and nuclear factor kB activation produced by inflammatory agents in adipose tissue.	3T3-L1 preadipocytes	Bradford (2013) Ho (2013)
13	*Cyclopia falcata* (Harv.) Kies (Leguminosae)	Stem	Phloretin-3′,5′-di-C-glucoside, a flavonoid, decreases intracellular triglyceride and suppresses adipogenesis in vitro.	33T3-L1 preadipocytes	Dudhia *et al.*, (2013)
14	*Cyclopia maculata* (Andrews) Kies (Leguminosae)	Stems	Hesperidin and Mangiferin suppress intracellular triglyceride and fat accumulation, as well as PPAR2 expression, and can prevent adipogenesis in vitro.	3T3-L1 preadipocytes	Dudhia *et al.*, (2013)
15	*Eremochloa ophiuroides* (Munro) Hack (Poaceae)	Whole Plant	The key transcriptional regulators of adipogenesis, C/EBPα and PPARƔ, were expressed. Furthermore, this plant reduces AKT and GSK3 phosphorylation levels.	3T3-L1 preadipocytes	Park *et al.*, (2012)
16	*Glycine max* (L.) Merr. (Leguminosae)	Bean	In 3T3-L1 preadipocyte cells, it suppresses adipocyte development. Triglyceride accumulation was prevented, and AMPK is stimulated.	3T3-L1 preadipocytes	Kim (2014) Singh *et al.*, (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
17	*Houttuynia cordata* Thunb. (Saururaceae)	Leaf	Reduces fatty acid synthase, sterol regulatory element-binding protein-1, and glycerol 3-phosphate acyltransferase expression. In mice, the extract prevents an increase in plasma TG levels. The extracts may inhibit NEFA and glycerol absorption by inhibiting FAT/CD 36, as well as aquaproin-7.	Human HepG2 hepatocytes	Kang and Koppula (2014) Miyata *et al.*, (2010)
18	*Ilex paraguariensis* A.St.-Hil. (Aquifoliaceae)	Leaves and unripe fruits	There is also a modulatory influence on the expression of adipogenesis-related genes such as PPAR2, leptin, TNF, and C/EBPα.	3T3-L1 preadipocytes	Arçari *et al.*, (2009) (2013) Gosmann *et al.*, (2012) Resende *et al.*, (2012)
19	*Ipomoea batatas* (L.) Lam (Convlvulaceae)	Tuber	SREBP-l, Acyl-CoA Synthase, Glycerol-3-Phosphate Acyltransferase, HMG-CoA Reductase, and Fatty Acid Synthase all have high levels of expression in liver tissue.	3T3-L1 preadipocytes	Ju *et al.*, (2011)
20	*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (Irvingiaceae)	Seed	Adipogenesis in adipocytes is inhibited. The impact appears to be mediated by decreased expression of adipogenic transcription factors (PPAR-) and adipocyte-specific proteins (leptin), as well as increased expression of adiponectin.	3T3-L1 preadipocytes	Ngondi (2009)
21	*Morus australis* Poir. (Moraceae)	Root	Increases lipolytic effects such as decreased intracellular triglyceride and the release of glycerol.	3T3-L1 preadipocytes	Kim *et al.*, (2010)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
22	*Nelumbo nucifera* Gaertn. (Nelumbnaceae)	Seed epicarp, leaves, seed, petals	Preadipocyte differentiation was inhibited by the extracts. In cultured human adipocytes, flavonoids suppress both adipocyte development and pancreatic lipase activity, accumulation, and expression of PPARγ, GLUT4, and leptin, showing that it reduces the differentiation of pre-adipocytes into adipocytes.	3T3-L1 adipocyte, NIH3T3 mouse fibroblast, L-02 normal hepatocyte cells CHO-K1, and U2OS cells,	Ahn *et al.,* (2013) Du (2010) Velusami *et al.,* (2013) You *et al.,* (2014)
23	*Nepeta tenuifolia* Benth. (Lamiaceae)	Whole plant	Triglyceride accumulation was inhibited in 3T3-L1 adipocytes, implying anti-obesity action.	3T3-L1 preadipocytes	Roh and Jung (2012)
24	*Pericarpium zanthoxyli* (Rutaceae)	Seed	Reduces the expression of the adipogenesis-related transcription factor, PPAR-Ɣ and PPAR-Ɣ target genes, such as adipocyte protein 2 (aP2), fatty acid synthase (FAS), and other adipocyte indicators, as well as CCAAT/enhancer-binding protein β (C/EBPβ) levels in a dose-dependent manner.	OP9 cells	Kim *et al.,* (2014)
25	*Petasites japonicus* (Siebold & Zucc.)	Flower buds	The extracts inhibit three adipogenic transcription factors: peroxisome PPAR-Ɣ2, CCAAT/enhancer-binding protein, and sterol regulatory element-binding protein 1c.	3T3-L1 preadipocytes	Watanabe *et al.,* (2010)
26	*Peucedanum japonicum* Thunb. (Apiaceae)	Leaves	SREBP-1c, fatty acid synthase, and acetyl-coenzyme A are all downregulated by pteryxin. A carboxylase-1 upregulates lipid catabolizing genes in treated 3T3-L1 adipocytes and HepG2 hepatocytes. Another study found that the extract inhibits SREBP1 c, a major lipogenic activator, and paternally expressed gene 1/mesoderm-specific transcript (PEG1/MEST), an adipocyte size marker gene, in vivo in adipose tissue.	Both 3T3-L1 and HepG2 cell lines, 3T3-L1 and HepG2 cells	Nugara *et al.,* (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
27	*Rubus chingii* var. suavissimus (S.K.Lee) L.T.Lu (Rosaceae).	Leaves	Adipogenesis and the expression of adiponectin and leptin both are increased by the extract. Extract stimulates the mRNA production of adipogenic transcription factors CCAAT/ enhancer binding protein and PPAR-γ in the early stages of adipocyte differentiation.	3T3-L1 preadipocytes	Ezure and Amano (2011)
28	*Salicornia herbacea* L. (Amaranthaceae)	Whole plant	Isorhamnetin 3-O—D-glucopyranoside inhibits adipogenic development via inhibiting PPARγ, CCAAT/enhancer-binding proteins, SREBP1, and adipocyte-specific proteins. Activation of AMPK confirms a specific mechanism mediating the effects of isorhamnetin 3-—D- glucopyranoside.	3T3-L1 preadipocytes	Kong and Seo (2012)
29	*Siegesbeckia pubescens*L. (Amaranthaceae)	Whole plant	CCAAA/enhancer binding proteins, as well as peroxisome proliferator-activated receptor, gene, and protein expressions, influence the anti-obesity impact.	3T3-L1 preadipocytes	Park (2013)
30	*Smilax china* L. (Smilacaceae)	Leaves	Polyphenol and flavonoid with anti—glucosidase and anti-lipid buildup activities.	3T3-L1 preadipocytes	Kang (2013)
31	*Tetrapanax papyriferus* (Hook.) K.Koch (Araliaceae)	Whole plant	CCAAT/enhancer binding proteins, as well as peroxisome proliferator-activated receptor, gene, and protein expression, all influence the anti-obesity impact.	3T3-L1 preadipocytes	Park (2013).
32	*Veratrum nigrum* L. (Melanthiaceae)	Whole plant	In 3T3-L1 cells, it reduces lipid accumulation and the expression of two main adipogenesis factors, PPARɣ and C/EBPα.	3T3-L1 preadipocytes	Park *et al.,* (2013)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
33	*Vitis labrusca* L. (Vitaceae)	Seed	Seed extract reduces lipid accumulation in C3H10T1/2 and 3T3-L1 cells in a dose-dependent manner. Inhibition is related with decreased PPAR- Ɣ expression.	C3H10T1/2 and 3T3-L1 preadipocytes	Oh (2013)
34	*Vitis vinifera* L. (Vitaceae)	Seed flours, peel, roots, fruit	By increasing the expression of hepatic genes involved in cholesterol (CYP51) and bile acid (CYP7A1) production, as well as LDL-cholesterol absorption. Mlxp1, Stat5a, Hsl, Plin1, and Vdr, which are involved in lipid metabolism, were all down-regulated. As evaluated by real-time polymerase reaction, the extract treatment reduces expression of aP2, Fas, and Tnfa, which are recognised indicators of adipogenesis. PPAR-γ expression in the liver and adipose tissue is reduced by controlling lipid metabolism and suppressing obesity.	3T3-L1 preadipocytes, high-fat diet- induced mice, murine 3T3-LI adipocytes,	Aguilar Santamaría *et al.,* (2012) Hsu *et al.,* (2014) Kim *et al.,* (2014) Jeong (2012, 2011) Kang *et al.,* (2011) Kim (2014) Zhang *et al.,* (2012)
35	*Ziziphus jujube* Mill. (Rhamnaceae)	Fruit	Decrease fat build up and glycerol-3-phosphate dehydrogenase activity. Attenuates the expression of major adipogenic transcription factors, including PPAR- γ and CCAAT enhancer binding proteins (C/EBPs), eliciting the greatest inhibitory impact.	3T3-L1 preadipocytes	Gao *et al.,* (2013) Mostafa *et al.,* (2013) Kubota *et al.,* (2009)
36	Germinated brown rice, germinated waxy brown rice, germinated black rice, and germinated waxy black rice	Seed	This seed extract reduces body weight growth and lipid build up in the liver and epididymal adipose tissue. The seed extract reduces the mRNA levels of adipogenic transcriptional factors such as CCAAT enhancer binding protein (C/EBP)-α, SREBP(SREBP)-1c, and peroxisome proliferator activated receptors (PPAR)-, as well as associated genes (aP2, FAS).	3T3-L1 preadipocytes	Ho *et al.,* (2012)

2.9 Anti-obesity effect of plant extract on the high-fat diet-induced rats:

Oh *et. al.,* (2014) studied the effect of *Achyranthes bidentata* Blume root extract on the high-fat diet-induced rat they observed that the drug shows an effect on the differentiation of adipocytes and decreases phospho- Akt expression. Halfordinol, ethyl ether aegeline and esculetin were the active chemical elements *of Aegle marmelos* leaves responsible for the reduction in adipocyte formation. Umbelliferone and esculetin are active substances that reduce hyperlipidemia by depleting lipid content in adipocytes in high-fat diet-induced obesity in male SD rats Karmase *et al.,* (2013).

In high fat-fed rats, *Allium cepa* peel extract reduces mRNA levels of activating protein (AP2) while increasing levels of carnitine palmitoyl transferase-1 (CPT-1) and fatty acid binding protein 4 (FABP4) (Kim *et. al.,* 2012). In diet-induced obese male SD rats, it is also hypothesised that *A. cepa* raises levels of PPAR-2 mRNA (mesenteric fats) and IL-6 mRNA (perirenal and mesenteric fats) (Moon *et al.,* 2013).

Galangin, the principal component of *A. galangal* Rhizome was extracted and studied for lipase inhibition activity. After that, Galangin was fed to the obese female rats. Obesity was induced in rats by feeding a cafeteria diet. The treatment with galangin decreases serum lipids, liver weight, lipid peroxidation and the accumulation of hepatic TGs. (Kumar and Alagawadi 2013). Ethanolic extracts of *Argyreia speciosa* root were studied on cafeteria diet-induced obesity rats. The extract reduces the serum contents of leptin, total cholestrol, LDL, and triglycerides. (Kumar *et al.,* 2011).

Shikov *et al.,* (2014) discovered that *Bergenia crassifolia* leaf extracts inhibited fat buildup in obese rats produced by a high-calorie diet. Galloylbergenin derivatives 3,11-Di-O-galloylbergenin and 4,11-Di-O-galloylbergenin are active chemicals found in the leaf extracts. The extract of *Boehmeria nivea* leaf decreases fat tissue weight as well as serum alkaline aminotransferase and lactate dehydrogenase activity. In animals given leaf powder, serum triglycerides, total cholesterol, LDL-cholesterol level, atherogenic index, and cardiac risk factors are reduced, while blood HDL-cholesterol levels are elevated. This investigation was conducted on male SD rats fed a high-fat/cholesterol diet (*Lee et al.,* 2011).

The hydroalcoholic extract of *Boerhaavia diffusa* root contains sitosterol, which is structurally identical to cholesterol and lowers LDL cholesterol and cholesterol levels in plasma in female SD rats on a high-fat diet (Khalid and Siddiqui 2012). Gupta *et al.,* (2013) investigated the ability of *Bombax ceiba* stem bark extract containing gemfibrozil to reverse the effects of a high-fat diet in wistar rats. This action might be attributed to the inactivation

of acetyl-coA carboxylase as a result of AMPK activation, which is responsible for thermogenesis and FAS inhibition.

C. japonica leaf extract regulates insulin, which modulates lipid synthesis via sterol regulatory element binding protein-1c (SREBP-1c). Insulin deficiency affects hepatic triglyceride production in SD rats fed a high-fat diet (Tamaru *et. al.*,2013).

The impact of *Undaria pinnatifida* and fucoxanthin on metabolic, physiological, and inflammatory markers linked to obesity, as well as the expression of genes involved in white adipose tissue lipid metabolism, was investigated in a diet-induced overweight rat model. By up-regulating PPARα, PGC1, PPARɣ, and UCP-1, the treatments increased energy expenditure, oxidative stress, and adipogenesis. Image processing of retroperitoneal adipose tissue, which measured cell area, perimeter, and cellular density, also stated adipogenesis. Furthermore, the treatments reduced lipid synthesis and swelling by decreasing acetyl-CoA carboxylase (ACC) gene expression, boosting serum concentration and expression of adiponectin, and decreasing IL-6 expression. Both fucoxanthin and *Undaria pinnatifida* might well be explored for treating obesity and other disorders associated. (Grasa-López *et al.,* 2016)

Yazici-Tutunis *et al.,* (2016) investigated the impact of an aqueous extract of *Phillies latifolia* L. (Oleaceae), which is frequently found in the Mediterranean area of Turkey and is utilized in folk medicine as medicinal teas for weight reduction and hyperglycemia. They fed the rats a high-energy meal with *P. latifolia* leaf extract. In the HED + PLE group, PLE treatment resulted in a significant reduction in body weight. PLE increased mechanical strength and reduced leukocyte migration in the liver and small intestine tissues. The HED group had significantly higher levels of blood glucose, leptin, total cholesterol, and LDL. In the HED group, PLE medication reduced these levels. HDL levels were greater in the HED + PLE group than in the control and HED groups. For the first time, the chemical makeup of a Turkish sample of EtOAc extract of leaves was studied, and luteolin 7-O-glucoside and chlorogenic acid were determined. *Phillyrea latifolia* leaves may help with obesity-related cellular issues and might be a suitable source of anti-diabetic therapy.

Ramírez *et al.*, (2017) investigate the effects of *Mangifera indica* L. leaves because they include mangiferin, total phenolics, and antioxidants, all of which have several functional qualities. Hot leaf extracts were fed to Wister rats on a high-fat diet. They looked at biometrics and serum biochemical factors relating to metabolic control, inflammation, and oxidative stress, as well as visceral adipose tissue histomorphometry and mRNA

expression of PPAR-Ɣ co-activator 1 α, lipoprotein lipase (LPL), and fatty acid synthase (FAS). The ingestion of the extract increased total antioxidant capacity and interleukin10 serum concentrations, decreased belly fat storage, elevated PPAR-Ɣ and LPL expression, and downregulated FAS expression. Extract offers therapeutic promise in the treatment of obesity and related illnesses by modulating the expression of transcriptional factors and adipogenesis-related enzymes.

Bounihi *et al.,* (2017) assessed the effects of fruit vinegars on high-fat diet-induced rats fed a high-fat diet. Vinegar treatments effectively reduced the HFD-induced rise in body weight and visceral fat mass, as well as plasma levels of CRP, fibrinogen, leptin, TNF-a, AST, CK-MB, and LDH. Moreover, the fruit vinegar containing pomegranate, apple, and prickly pear may prevent HFD-induced obesity and obesity-related cardiac problems, and this protection may be due to the vinegar' significant anti-inflammatory and anti-adiposity capabilities.

In 2017 El Ayed *et al.,* investigated the effect of a high-fat diet (HFD)on lung lipotoxicity, oxidative stress, fatty acid composition and proportions in lung and implications in asthma development to lower the effect of lipotoxicity on the body they used grape seed extract. The trial revealed that theta HFD caused a lipid profile imbalance, raising cholesterol and VLDL-C. HFD also caused oxidative stress, as seen by increased MDA levels and a decrease in antioxidant activity such as SOD, CAT, and POD. Overall, GSSE demonstrated a strong preventative impact against HFD-induced obesity, and so may be employed as an anti-obesity drug as well as a beneficial agent with prospective uses against lung tissue damage.

CitroliveTM is a trademark for the combination of citrus fruit and olive leaves. CitroliveTM may regulate metabolic disorders associated with obesity and their side effects because citrus fruit and olive leaves contain bioactive compounds such as biophenols, which were shown to improve adiposity conditions through anti-hyperlipidaemic and anti-inflammatory effects, as well as by regulating lipoproteins and saturated fat body levels. CitroliveTM was discovered to inhibit pancreatic lipase after an animal study revealed that the CitroliveTM treatment substantially lowered the liver-to-body-weight ratio, as assisted by lower plasma transaminases, but non - significantly depleted plasma low-density lipoprotein (LDL) and postprandial TAG plasma levels. Finally, therapy alleviates symptoms of hepatotoxicity that are increased by the consequences of a high-fat diet. (Merola *et al.,* 2017).

Ekeleme-Egedigwe *et al.,* (2017) investigated the impact of *Vernonia amygdalina* leaves on obese wistar rats on a high-fat diet. Serum lipid profile, blood glucose concentrations, body weight, adiposity index, feed intake, faecal loss, and relative organ mass were all measured after twelve weeks of feeding. The researchers discovered that obese rats fed *Vernonia amygdalina* extract prevented HFD-induced weight gain and adiposity in rats while simultaneously increasing serum TG and TC levels as compared to obese rats fed a regular diet. The results show that feeding obese rats *Vernonia amygdalina* extract reversed fatty infiltration, resulting in lower weight gain and visceral fat mass in the rats.

2.10 *Dioscorea* species and anti-obesity:

Some species from the genus *Dioscorea* were screened for anti-obesity activity. *Dioscorea* species shows the different modes of action like lipase inhibition, which inhibits adipocytes differentiation.

Table No. 2.5: *Dioscorea* species reported for anti-obesity activity

Name of the species	Mode action	Compound	References
Dioscore nipponica	Lipase inhibitory	Dioscin	Kwon *et. al.,* (2003)
Dioscorea steriscus	Lipase and α-amylase inhibitors	flavonoids	Dzomba and Musekiwa (2014)
Dioscorea oppositifolia	Liapse inhibition Inhibit adipogensis	batasin	Jeong *et. al.,* (2016) Yang (2014)
Dioscorea batatas	Liapse inhibition	Dioscin	Jeon *et. al.,* (2006)
Marketed	Inhibits adipogenesis through the AMPK/MAPK pathway	Dioscin	Poudel (2014)
Marketed	Reduce weight in high-fat diet rat	Diosgenin	Son *et. al.,* (2007)
Dioscorea tokoronis	Suppresses the expression of SREBP-1 as well as that of fatty acid synthase in adipose and liver tissues.	-----	Song *et. al.,* (2009)

Herbal extracts have been traditionally used as an anti-obesity treatment due to their low or no negative effects. The current study attempted to investigate the anti-obesity effects of selected *Dioscorea* species. The study's purpose was to prevent the development of obesity and obesity-related complications by suppressing adipogenesis and lipogenesis.


Material
and
Methods

3. MATERIAL AND METHODS:

3.1 Germplasm collection of different *Dioscorea* species:

3.1.1 Survey and collection of *Dioscorea* species:

Dioscorea species were collected through extensive field tours during June to November from 2018 to 2020 to different localities (Fig 3.1). The germplasm of collected *Dioscorea* species was maintained in Botanical Garden, Shivaji University, Kolhapur. The photographs were taken in field and after cultivation in garden by using Cannon Eos-1500D and mobile camera. The plant material was authenticated by consulting protologues and relevant literature. The herbarium and experts were also consulted for the purpose of identification of the species.

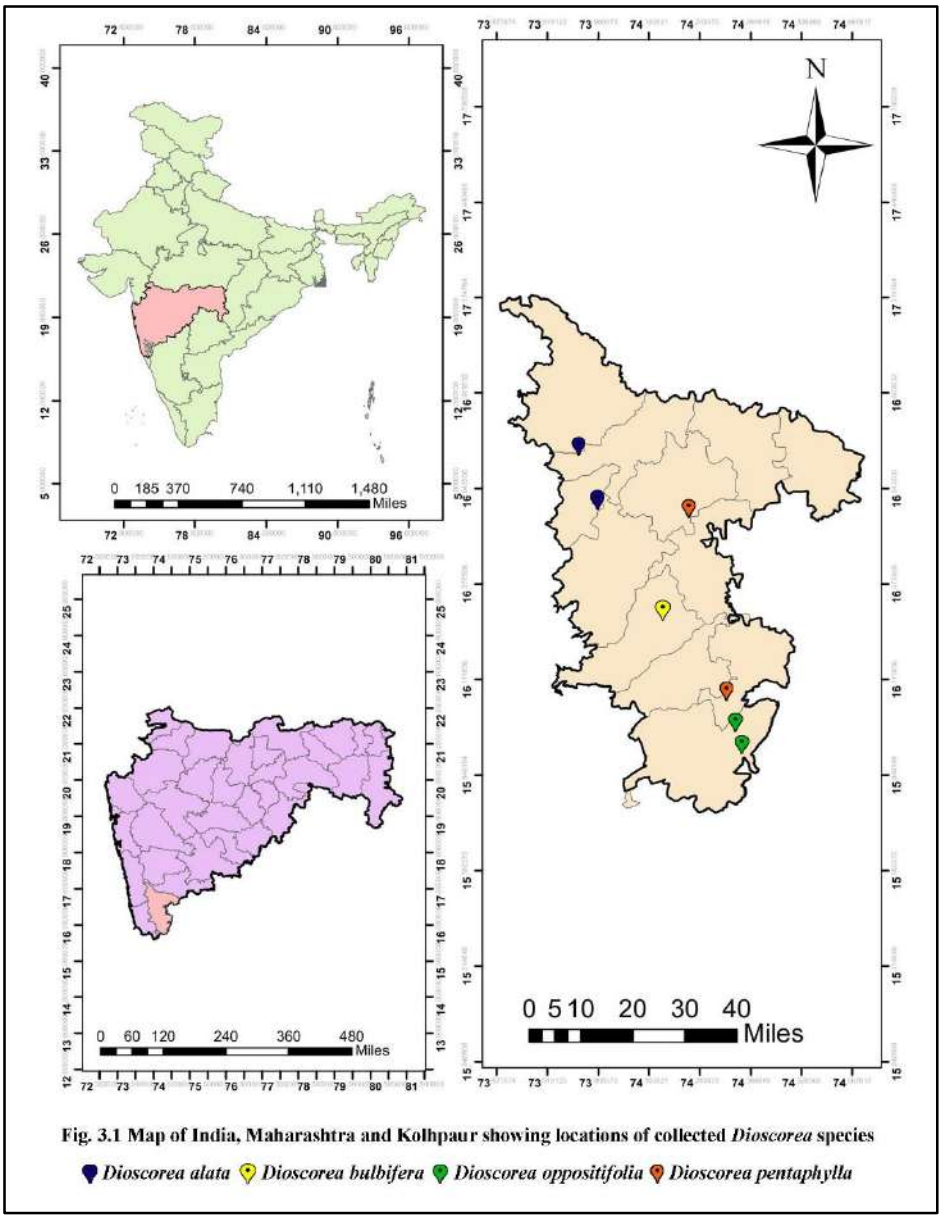


Fig. 3.1 Map of India, Maharashtra and Kolhpaur showing locations of collected *Dioscorea* species

Dioscorea alata *Dioscorea bulbifera* *Dioscorea oppositifolia* *Dioscorea pentaphylla*

3.1.2 Preparation of herbarium specimens:

Some of the specimens from collected plant material with proper phenological stage were used to prepare herbarium specimens. Specimens were dipped in 40% formalin for two days and pressed in blotting paper under wooden press. These specimens were dried using paper sheets with frequent changing. The dried specimens were poisoned, mounted and labelled on the herbarium sheet by standard method (Forman and Bridson, 1989). The processed herbarium specimens of *Dioscorea* species were placed in a well-labelled folder for each species containing photographs of habit and phenological stages**.** These specimens were deposited in the herbarium of Department of Botany, Shivaji University, Kolhapur.

3.1.3 Preparation of different extracts:

Extraction was carried out by a modified method by Sarker and Nahar, (2012). For extraction equal amount (10 gm) of dry plant powder and diatomaceous earth swas mixed and filled into a 66 ml stainless steel extraction cell. The cell was loaded in an accelerated solvent extractor (Dionex™ ASE™ 350 Thermo Scientific). The extraction was carried out using the following conditions, heating at 45˚C and pressurized for 5 min with static extraction under pressure 1300-1500 PSI. Then, flushed with fresh solvent for 0.5 min and purged with nitrogen for 1.0-2.0 min. Extracts [methanolic (350 mbar) and aqueous (72 mbar)] were collected and evaporated by rotary evaporator under vacuum at 64˚C and 100˚C, respectively. After evaporation, the dry residues were removed and dissolved in the respective solvents to obtain concentrations of 10 mg/ ml for each assay. And stored at -20˚C until used.

3.2. Chemo profiling of *Dioscorea species* for specific antiobesity molecules:

3.2.1 Estimation of Total Phenolic Content:

The total phenolic content was estimated as follows; 50µl of distilled water was distributed in all the wells; 12.5µl of plant extract and 12.5µl of Folin–Ciocalteu reagent were added, then incubated at room temperature for 10 min. after incubation 7% sodium carbonate was added and the reaction volume was made up with 100µl of distilled water and incubated in the dark at 37°C for 90 min. After incubation, optical density was measured at 760 nm on a 96-well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Phenolic content was determined by the calibration curve of standard gallic acid (20 to 100µg/ml). (Wolfe and Lui 2003)

3.2.2 Estimation of Total Flavonoid Content:

Total flavonoid content was estimated by the Luximon-Ramma *et al.,* (2002) method, with 100 µl of plant extract and 100µl of 2% aluminium chloride mixed together and incubated at room temperature for 10 min. Then optical density was measured at 368 nm on a 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Flavonoid content was determined by the calibration curve of standard quercetin (20 to 100µg/ml).

3.2.3 Estimation of Total Alkaloid Content:

The total alkaloid content was determined by using the method described by Singh *et al.,* (2004) with minor modification, 100 µl of plant extract was mixed with 100 µl of 0.05M Phenanthroline solution and 100 µl of 0.025M $FeCl_3$. The final volume was made to 1 ml using distilled water. The reaction mixture was incubated at 70^oC for 30 minutes in the water bath. Absorbance was measured at a 510 nm wavelength. Colchicine was used as a standard at varying concentrations (20 µg to 100 µg). The total alkaloid content was calculated from the calibration curve and the results were expressed as mg of Colchicine equivalent per gm fresh or dry weight.

3.2.4 Estimation of Total Terpenoid Content:

Total terpenoid content was determined by colorimetry using the following procedure (Chang *et al.,* 2012) 50µl of plant extract was mixed with the 75µl of 5% vanillin-glacial acetic acid solution and 250µl of perchloric acid, and the samples were heated at 45^oC for 60 min. After incubation cooled on an ice bath, 1.125 ml of glacial acetic acid was added and then optical density was recorded at 548 nm on a 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific).

3.2.5 Estimation of Total Saponin Content:

Saponin content in *Dioscorea* spices was determined according to the method described by Hiai *et al.,* (1976) with slight modification. In a 1.5 ml vial, 50µl of standard and plant extract were dispensed in triplicate. After that, 50µl of 8% vanillin was added to each tube. Sulphuric acid in the amount of 500µl was added to each tube. The reaction tubes were incubated in a water bath for 10 minutes at 60^oC. After cooling, optical density was recorded at 544 nm was measured using a 96well plate reader (Multiskan sky 96well spectrophotometer, Thermo scientific).

3.2.6 Anti-oxidant assays:

A. Determination of Free radical scavenging activity by DPPH:

The free radical scavenging activity of the plant extract was analysed with the method of Blois (1958), Rosidah *et al*,.(2008). For this, 10µl of plant extract was mixed with 290µl of DPPH solution (200µM in methanol). After addition, the plate was incubated at 37ºC for 30 min in the dark, and optical density was measured at 517nm on a 96-well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Ascorbic acid (20 to 100 µg/ml) was used as a standard. The formula was used to calculate the percentage of DPPH radical scavenging activity.

% Inhibition = [(Control OD – Sample OD)/Control OD] x100

B. Determination of ABTS scavenging activity:

The protocol of Patil *et al.,* (2020) was followed with some modifications to determine the ABTS scavenging poetical of plant extracts. To generate ABTS radicals, ABTS (7mM) and potassium persulfate (2.4mM) were mixed in equal parts and allowed to stand at room temperature for 16 hours in the dark. Then absorbance was measured at 734nm, to obtain O.D. of 0.700. After getting OD 0.700 the 10µl plant extracts were treated with 290µl of ABTS in 96 well plate. Before taking the readings at 734nm. Ascorbic acid (20 to 100µg/ml) was used as standard. % ABTS radical scavenging activity was estimated by using formula.

% Inhibition = [(Control OD – Sample OD)/Control OD] x100

C. Determination of FRAP (Ferric Reducing Antioxidant Power) assay:

The ferric reducing antioxidant power of plant extract was determined by the Benzie and Strain (1996) method. In a 10:1:1 ratio, 0.3M acetate buffer (pH 3.6), 10mM TPTZ (31.23 mg added to 10 ml of 40mM HCl), and 20 mM $FeCl_3$ (54.06 mg dissolved in 10 ml of D/W) were combined to make FRAP reagent. The test was performed in a 96-well plate.10 µl of plant extract was mixed with 290µl and incubated for 15 min at 37ºC. Then optical density was measured at 595nm on a 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Ascorbic acid (20 to 100µg/ml) was used as a standard.

3.3 To evaluate species from genus *Dioscorea* for antiobesity activity:

3.3.1 Testing of crude extracts for lipase inhibition activity:

The activity of Porcine Pancreatic Lipase (PPL, type II) was evaluated using the substrate p-nitropheny l butyrate (p-NPB). Earlier published technique for assessing pancreatic lipase activity was adapted for this study (Kim *et al.*, 2010 and Zheng *et al.*, 2010). PPL stock solutions (0.6 mg/ml) were prepared in a 0.5 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl and kept at 20˚C. The lipase inhibitory action was evaluated using extracts or Orlistat (concentrations ranging from 1 mg/mL to 5 mg/mL) For the lipase inhibition reaction, 100 µl of buffer, 50 µl of lipase enzyme, 25 µl of plant extract or Orlistat, and 25 µl of p-nitrophenyl butyrate substrate were added, and the optical density (OD) at 400 nm was measured using a spectrophotometer (Multiskan sky 96 well spectrophotometer, Thermo scientific). For control reaction, 125 µl buffer, 50 µl of lipase enzyme, and 25 µl of p-nitrophenyl butyrate substrate were added. The inhibitory activity was calculated according to the following formula:

$$\%\text{Inhibition} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

3.3.2 Testing of crude extracts on cell line 3T3-L1 pre adipocytes:

A. 3T3-L1 Cell Culture:

3T3-L1 preadipocytes were obtained from the National Centre for Cellular Sciences (NCCS) Pune, India. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with high glucose (Himedia laboratories India) containing 10% Fetal Bovine Serum (FBS) (Invitrogen, Waltham, Massachusetts, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin, in a humidified environment of 5% CO_2 at 37˚C. Cells were used for additional experiments once they reached 80% confluency.

B. Cell viability assay:

The effect of *Dioscorea* extract on the viability of 3T3-L1 preadipocytes cells was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Himedia laboratories India) assay. 3×10^4 cells were seeded on a 96 well plate and incubated for 24 hrs before treating with various *Dioscorea* species extracts at varying concentrations (6.25, 12.5, 25, 50, and 100 µg/ml). Extracts were diluted with DMEM media, then incubated for 24 hrs at 37˚C in 5% CO_2. Following incubation, cells were

treated with MTT for 4 hrs to form crystals. The mitochondrial reduction process of the live cell reduces MTT into formazan crystals that were dissolved in Dimethylsulfoxide (DMSO), and the O.D. was measured at 570 nm using a 96-well plate reader (Multiskan Sky Thermo fisher scientific).

C. 3T3-L1 Cells Differentiation:

For differentiation of 3T3-L1 preadipocytes into adipocytes, 3×10^4 cells were seeded in a 24 well plate and incubated for 48 hrs. After incubation, the cells were stimulated with a hormone cocktail comprising 0.5 mM 3-isobutyl-1-methylxanthine (sigma Aldrich, USA), 1 M Dexamethasone (Himedia laboratories India), and 1µg insulin (Sigma Aldrich, USA) coupled with various extracts of different *Dioscorea species* at varying concentrations (25 and 75 µg/ml) for 48 hrs. Then medium was changed with DMEM supplemented with 10% FBS and 1µg insulin, along with the plant extract, and kept at 37˚C with 5% CO_2 until day 8. Differentiated adipocytes were observed under microscope (Yang *et al.*, 2014).

D. Oil Red O Staining of Differentiated adipocytes:

For Oil Red O staining of differentiated adipocytes, on day 8 after induction of differentiation, the medium was withdrawn from the 24 well plates, and the cells were washed twice with 1X PBS. For 30 mins, the cells were fixed in 70% ethanol. The fixed cells were rinsed twice with 1X PBS to remove the ethanol. After that, Oil Red O stain [0.6% Oil Red O, Sigma Aldrich, USA in isopropanol: water (3:2)] was added 300µl to each well and incubated for 1 hr at room temperature and then washed twice with distilled water. The staining of differentiated adipocytes was examined under an inverted Microscope (INVI Inverted Microscope, Magnus Opto Systems India).

E. Quantification of lipid accumulation:

Accumulated lipids were quantified by the eluted Oil O Red stain that remained in the cells after rinsing with isopropanol and quantified by measuring the absorbance at 500 nm. The percentage of inhibition of lipid accumulation was calculated by the following formula:

% Inhibition of lipid accumulation = (OD of control – OD of the sample)/OD of control × 100

F. Gene expression studies:

Total mRNA was isolated by using the trizol method with some modification. The harvested cells were suspended in 500µl of trizol solution then 250µl of the chloroform was added and mixed vigorously and incubated at room temperature for 5 min. Then centrifuged at 10000 rpm for 10 min. After centrifugation upper aqueous layer was collected in to the fresh tube. The precipitation was carried out by adding 550µl of isopropanol in the aqueous layer and incubated at -20ºC for 15 min. Afterward centrifuged at 14000 rpm for 30 min. and supernatant was discarded. The pellet was washed twice with 75% ethanol. Later pellet was air dry at room temperature for 15 min. Dry pellet was then suspended in 50µl of the DEPC treated water and stored at -20ºC or used for c DNA synthesis. c DNA synthesized by using the High-Capacity cDNA Reverse Transcription Kits (4368814 thermofisher scientific) the 2X master mix was prepared according to instructions given in kit. **(Table no 3.1)** and 10µl of master mix was mixed with 10µl RNA. Then tube was kept in thermal cycler with condition given in table 3.2.

Table No. 3.1: Preparation of Master Mix for c DNA Reaction

Sr. No.	Content	Volume in µl for 1 reaction
1	10X RT Buffer	2.0
2	25X dNTP Mix (100 mM)	0.8
3	10X RT Random Primers	2.0
4	MultiScribe™ Reverse Transcriptase	1.0
5	Nuclease-free H_2O	4.2
	Total volume	**10µl**

Table No. 3.2: Thermal cycling conditions for cDNA Synthesis

parameter	Step 1	Step 2	Step 3	Step 4
Temp.	25ºC	37ºC	85ºC	4ºC
Time	10	120	5	∞

G. Real time PCR:

For gene expression study Applied Biosystems' Quant Studio™ 3D Digital qPCR instrument was used. Master mix was prepared as per mentioned in to the **Table 3.3** and mixed with 1.3µl c DNA and gene specific primers (kicqstart primer Sigma Aldrich USA) generated from sequences obtained from the NCBI nucleotide sequence database for

amplification**.** The polymerase chain reaction was carried out at the following temperatures (95ºC for 45 sec, gene specific **(Table 3.4)** annealing temp for 45 sec, 72ºC for 45 sec for 40 cycles) and fluorescence measurement during annealing and extension stage. All of the responses were carried out in triplicate. Glyceraldehyde 3 phosphate dehydrogenase was used as a reference gene to normalize the gene expressions.

Table No. 3.3: PCR reaction master mix (Invitrogen, Waltham, Massachusetts, USA).

Sr. No.	content	Volume in µl for 1 reaction
1	SYBR Green Master Mix	5.0
2	gene specific forward primers	1.1
3	gene specific reversed primers	1.1
4	Nuclease free water	1.5
5	cDNA	1.3
	Total volume	10

Table No. 3.4 Primer sequences used for qPCR reaction

Genes	Forward primer	Reverse primer	Temp. ^{O}C
PPARϒ	AAAGACAACGGACAAATCAC	GGGATATTTTTGGCACTCTG	60.0
SREBP 1	AATAAATCTGCTGTCTTGCG	CCTTCAGTGATTTGCTTTTG	59.7
FAS	GATTCAGGGAGTGGATATTG	CATTCAGAATCGTGGCATAG	59.55
CPT1	ACTAACTATGTGAGTGACTGG	TGGCATAATAGTTGCTGTTC	55.5
C/EBP α	AAGGGTGTATGTAGTAGTGG	AAAAAGAAGAGAAGGAAGCG	56.15
GAPDH	TGAACGGGAAGCTCACTGG	TCCACCACCCTGTTGCTGTA	60.2

(PPARγ): Peroxisome proliferator-activated receptor-gamma; **(SREBP 1):** Sterol regulatory element binding protein 1) ; (**FAS):** Fatty acid synthase; **(CPT1):** Carnitine palmitoyltransferase1; (**C/EBPα):** CCAAT/Enhancer binding protein**; (GAPDH):** Glyceraldehyde-3-phosphate dehydrogenase.

H. LCMS-MS analysis:

To analyse the metabolites the 6200 series TOF/6500 series Q-TOF B.09.00 (B9044.0) UHD accurate mass QTOF LC/MS was used. The extracted aliquot was filtered and diluted with mobile phase before injection for LC separation and subsequently to the hybrid mass analyser. For LCMS/MS analysis, 5 µl of extract was injected. The gradient mode was used to separate the samples. Mobile phase gradient is given in the **Table No. 3.5.**

Table No.: 3.5 Flow profile of mobile phase

Time (min)	Flow (ml/min)	Solvent A [0.1% formic acid in H_2O (v/v)]	Solvent B Acetonitrile
0- 18 min	300	95%	5%
18.10-25 min	300	5%	95%
25-25.10 min	300	95%	5%
25.10-30min	300	5%	95%

The column runoff was then continued for ionization under positive polarity and mass spectrometer m/z analysis. The Mass spectrometry was run in high-resolution mode with the following commands: The timer for the analytical run was set to 30 minutes, and the flow profile of the mobile phase, Gas Temp 325°C, Gas Flow (l/min) 8, Nebulizer (psig), Sheath Gas Temp 300°C, Sheath Gas Flow 10, etc., with a scan rate of 2 spectra/min and an Acquisition Mode of 60 -1700 (m/z). Mass Hunter Workstation Qualitative software was used to process the data (version B.06.00, Agilent Technologies, Santa Clara, CA, USA). For screened potential metabolites, the analysis of accurate mass and prediction of molecular formula in databases such as CAS, HMP, LMSD, KEGG, METLIN, and Mass Bank. METLIN's Personal Compound Database includes a mass MS/MS library with correct mass data (PCDL). All metabolites with extra accurate masses (Q-TOF MS/MS library reference spectra) are included in the METLIN PCDL.(Patil *et al.,* 2020)

3.4 Evaluation of anti-obesity of extracted compounds using cell line (3T3-L1 pro adipocytes):

3.4.1 Fractionation of the *D. oppositifolia* methanolic extract:

Methanolic extract was obtained through an accelerated solvent extractor. The methanol was evaporated by using a rotary evaporator to get a dry residue. Then, dry

residue powder was resuspended in to 30 ml distilled water to make a suspension. Then an equal volume of hexane was added, mixed well, and allowed to stand until two clear layers were formed. The hexane layer (upper one) was separated. Again, washed with hexane twice, and hexane fraction was collected. The process repeated with chloroform, ethyl acetate and n-butanol solvent. The remaining aqueous fraction contains water soluble compounds. All solvents were evaporated from each fraction, and the collected residue was suspended in DMSO at 1 mg/ml concentration. (Abubakar and Haque 2020)

3.4.2 Extraction of diosgenin:

Diosgenin was extracted using a modified of Shaha and Lee (2012) by acid hydrolysis process. 5 gm root powder was mixed with 100ml of isopropanol with 20% H_2 SO_4 and refluxed at 65^{o}C for 8 hours. Whatman filter paper was used to filter the refluxed material. The obtained filtrate was washed three times with hexane. The collected hexane extract was combined and washed three times with 5% NaOH. Again, rinsed with distilled water after the alkali wash. Finally, the solvent was evaporated in a rotary evaporator, and the dry powder produced was redissolved in ethanol.

3.4.3 All the fractions and extracted diosgenin were tested on the 3T3-L1 preadipocytes by following methods given in section 3.3.2 B, C, D, E, F, and G.

3.4.4 Identification of anti-obesity metabolites in chloroform fraction by LCMS-QTOF by following methods given in section **3.3.2 H** with modification in mobile phase gradient given in **Table No. 3.6.**

Table no 3.6: Flow profile of mobile phase

Time (min)	**Flow** (ml/min)	**Solvent A** [0.1% formic acid in H2O (v/v)]	**Solvent B** Acetonitrile
0-2 min	300	95%	5%
2-5 min	300	75%	25%
5-10 min	300	60%	40%
10-15 min	300	40%	60%
15-20 min	300	20%	80%
20-25 min	300	5%	95%
25-28 min	300	95%	5%
28-30 min	300	95%	5%

3.4.5 Detection of extracted diosgenin by HPLC

The JASCO Auto-sampler with UV-Visible detector was used for high performance liquid chromatography. Separations were performed on a C-18 column (250mmX 5mmX 5 microns) at a flow rate of 1 ml/min, at a temperature of 35°C, and peaks were detected at

absorption spectra 200 nm. With isocratic elution of Acetonitrile: water 90:10 (V/V) yielded well resolved chromatograms. The standard diosgenin retention time is 3.19 minutes. The extracted diosgenin was detected by matching the retention time with standard diosgenin.

3.5 *In vivo* studies

The animal study was carried out by using the Munshi *et al.,* (2014) method with some modification

3.5.1 Experimental design:

Obese model was developed by using the high fat diet containing 3ml/kg fat emulsion for 6 weeks along with normal diet and water. Animals were weighed, Labelled and randomly divided into 5 groups, each group containing 6 animals. All the animals were taken care with ethical consideration and protocol was dually approved by ethical committee **(1825/PO/EReBi/15/CPCSEA/2/2021, dated on 19 May 2021)**

Group I - Normal control (NC) - animal feed with Standard pellet diet and water for 24hr over a period of 6 weeks.

Group II – Diseased Control (DC) - animals feed with Standard pellet diet and water with 3ml/kg/day high fat diet.

Group III- Standard control (SC) - animal feed with Standard pellet diet and water with 3ml/kg/day high fat diet and 10 mg/kg/day of Orlistat.

Group IV- Test 1 group - animal feed with Standard pellet diet, water with 3ml/kg/day high fat diet and *D. oppositifolia* extract 200mg/kg/day.

Group V- Test 2 group - animal feed with Standard pellet diet, water with 3ml/kg/day high fat diet and *D. oppositifolia* extract 400mg/kg/day.

During the experiment daily weight of each animal was measured, and at the end of the experiment, Animals were taken by group wise and blood was collected using the retroorbital method.

3.5.2 Preparation of orlistat:

Orlistat was used as standard. Orlistat (Resheep) capsule content was dissolved in to the water to make 12mg/ml stock.

3.5.3 Experimental animals:

Wistar rats of 130 -150 gm were used for the study. Animals were housed in group of 6 animal each for 1 week in 12:12 h light and dark cycle in controlled temperature and humidity. After adaptation period the animals were used for the study.

3.5.4 High fat diet:

The high-fat diet was prepared by mixing Indian vanaspati ghee and coconut oil in a 3:1(V/V) ratio. It was fed to the rat every day at the dose of 1ml/kg of body weight.

3.5.5 Weight:

During the experiment the weight of each animal was recorded and noted for the determination of the dose of the drug every day.

3.5.6 Blood biochemical analysis:

At the end of the experiment the blood was collected group wise by retro orbital puncture and subjected to the centrifugation to separate serum. Total cholesterol, triglycerides (TG), LDL cholesterol, VLDL cholesterol and HDL cholesterol were analysed to check the lipids intake in the rat body.

3.6. Data analysis:

The data were shown as mean ± standard deviation and analysed using one-way ANOVA test with Dunnett's Multiple Comparison Test, and two-way ANOVA, $P < 0.05$ was considered as level of significance. The data were analysed using GraphPad Prism 5 Software, San Diego, California, USA.


Results
and
Discussion

4. RESULTS AND DISCUSSION

4.1 Germplasm collection

4.1.1 Germplasm collection of different *Dioscorea* species:

Dioscorea species were collected from various locations in the Kolhapur district during June to October 2018 to 2020. Frequent collection visits were conducted at various times of the year to obtain material in both vegetative and reproductive phases **(Plate No. 1-4). Table No. 4.1** shows the geographical coordinates with latitude, longitude, and voucher specimen numbers (**Plate No. 5**).

Table No. 4.1: Survey and collection sites of *Dioscorea* species

Sr. No.	Plant name	GPC location	Village name	Voucher specimen numbers.
1	*D. oppositifolia*	N $15^0$57'52.9, E$74^0$21'42.7 N $16^0$02'05.2, E$74^0$21'31.0	Kini Hadalge	RSP 001
2	*D. bulbifera*	N $16^0$20'21.3, E$74^0$08'47.1 N $16^0$20'11.9, E$74^0$08'46.4	Kalnakwadi Kalnakwadi	RSP 002
3	*D. alata*	N $16^0$38'36.5, E$73^0$57'02.4 N $16^0$38'37.33, E$73^0$57'01.51	Mandukali Karanjfen	RSP 003
4	*D. pentaphylla*	N $16^0$37'15.2, E$74^0$12'29.3 N $16^0$7'6.27", E$74^0$20'19.67	Katyayani hill Lakudwadi ghat	RSP 004

4.1.2 Preparation of plant extract and crude yield:

For the preparation of the crude extracts 10 gm the dry plant powder was used. From the 10 gm of dry plant powder in case of the methanolic extraction the yield of the crude residue is less than the aqueous extraction. The amount of the crude extract powder is mentioned in the **Table No. 4.2.** The highest yield was obtained in *D. alata* aqueous extract *i.e.,* 1700mg/10 gm of the dry powder and lowest yield was obtained in the *D. pentaphylla* methanolic extract *i.e.,* 250 mg/10 gm of the dry powder. While comparison between solvent the aqueous all the aqueous extract had the highest yield than the methanolic extract. For the further study extracts were diluted to the 1mg/ml.

Table No. 4.2: Amount of the crude extract powder after extraction

Sr. No.	Plant Name	Solvent	mg/10gm
1	*D. alata*	Methanol	720
		Aqueous	1700
2	*D. bulbifera*	Methanol	600
		Aqueous	1200
3	*D. pentaphylla*	Methanol	250
		Aqueous	740
4	*D. oppositifolia*	Methanol	700
		Aqueous	1500

4.2 Chemo profiling of *Dioscorea* species for specific anti-obesity molecules:

4.2.1 Estimation of Total phenolic content:

The total phenolic content of various parts of four yam species is shown in the **Table No. 4.3** and **Figure 4.1.** The phenolic content of four yam species differed significantly. The *D. oppositifolia* dry whole plant methanolic extract has the highest phenolic content, i.e. 128.14±1.70 mg/gm GAE of dry weight, while the lowest content was found in *D. pentaphylla* methanolic extract i.e. 34.23±0.70 mg/gm GAE of dry weight. When comparing the extraction solvents, the methanolic extract had a higher phenolic content than the aqueous extract of the plants. Phenolics are widely distributed throughout plants. Antioxidant and other biological activity features have been linked to Phenolics. Bhandari *et al.,* (2004) investigated the four *Dioscorea* species found in Nepal: *D. bulbifera, D. versicolor, D. deltoidea, and D. triphylla,* and found that *D. bulbifera* has the greatest phenolic content, while *D. triphylla* has the lowest. Sakthidevi and Mohan (2013) worked on the *D. alata* and reported that *D. alata* have 0.68mg/100gm of phenolic content in the methanolic extracts. The phenolic content of the different vaterites of *D. alata* and *D. esculenta* from the Philippine were studied by the Cornago *et al.,* (2010) reported the 69.9 to 421.8 mg gallic acid equivalent (GAE)/100 gm dry weight. Ghosh *et al.,* (2013) prepared *D. bulbifera* bulbs extract in different solvents and examined the phenolic content and reported that methanolic extract contains the highest phenolics *i.e.,* 145.446± 3.29 µg/ml than the other solvents used.

Table No. 4.3: Comparison of the phenolic, flavonoids, alkaloid, terpenoids and saponins content in different *Dioscorea* extract,

Extract	DAM	DBM	DPM	DOM	DAA	DBA	DPA	DOA
Total Phenolic Content	110.09 ± 1.80	37.68 ±2.04	34.23 ±0.70	128.14 ±1.70	68.94 ±1.33	39.63 ±2.07	55.03 ±2.08	65.38 ±7.83
Total Flavonoid Content	95.56 ± 0.645	39.50 ±0.308	34.11 ±0.867	94.45 ±0.308	82.15 ±0.760	24.11 ±0.760	47.10 ±0.534	42.83 ±0.226
Total Alkaloid Content	385.28 ±1.359	147.10 ±1.125	137.81 ±0.614	407.20 ±5.290	284.78 ±0.505	139.63 ±0.863	102.25 ±0.789	219.22 ±0.440
Total Terpenoid Content	288.90 ±5.94	307.77 ±18.26	282.70 ±5.12	349.70 ±2.54	14.00 ±4.04	6.93 ±12.60	-42.27 ±4.51	6.43 ±2.66
Total Saponins Content	543.87 ±4.06	350.53 ±3.53	458.87 ±2.33	547.53 ±2.19	424.87 ±5.84	482.87 ±2.40	420.53 ±3.76	579.53 ±5.33

Values presented in ± SE (n=3). DAM- *D. alata* methanolic extract, DBM- *D. bulbifera* methanolic extract, DPM- *D. pentaphylla* methanolic extract, DOM- *D. oppositifolia* methanolic extract, DAA- *D. alata* aqueous extract, DBA- *D. bulbifera* aqueous extract, DPA- *D. pentaphylla* aqueous extract, DOA- *D. oppositifolia* aqueous extract.

4.2.2 Estimation of Total flavonoid content:

Flavonoids are a class of naturally occurring polyphenol that are distinguished by their flavone nucleus. These are currently regarded an essential component in a wide range of nutraceutical, pharmaceutical, medical, cosmetic, and other products. Flavonoids promote good health and prevent illness (Karak 2019). While comparing the flavonoid content in different *Dioscorea* extract The maximum flavonoid content was found in *D. alata* methanolic extract *i.e.,* 94.45±0.308 mg/gm quercetin equivalent of dry weight and the lowest content found in *D. bulbifera* aqueous extract *i.e.,* 24.11±0.760 mg/gm quercetin equivalent of dry weight **(Table No. 4.3 and Figure No. 4.2)**. Both methanolic and aqueous the extracts of *D. bulbifera* and *D. pentaphylla* showed the lower flavonoid content than all other extracts. Sakthidevi and Mohan (2013) reported *D. alata* have 1.12gm/100gm of flavonoid content in the methanolic extracts. Ghosh *et al.,* (2013) have reported 27.866±0.18 µg/ml of the flavonoid content in ethyl acetate extracts of *D. bulbifera* bulbs, other three solvents show the less flavonoid content. Sonibare and Abegunde (2012) studied the Nigerian *D. dumetorum* (Kunth) Pax and *D. hirtiflora* for their biological activity. They have reported the flavonoid content 13.33±0.33 and 25.60±0.20 mg/gm of the dry weight respectively. Dzomba and Musekiwa

(2014) isolated the flavonoids from the *D. steriscus* and by using TLC and tested for the lipase inhibition activity.

4.2.3 Estimation of Total alkaloid Content:

Alkaloids are among the most significant and well-known secondary metabolites, alkaloids are the end products of nitrogen metabolism in plants, Because of toxicity in yam tuber, alkaloid content is crucial. These toxic metabolites can be found in variable amounts in yam tubers (Poornima and Rai 2009). While comparing the alkaloid content in the different *Dioscorea* extracts **(Table No. 4.3 and Figure No. 4.3)** the maximum alkaloid were found in the methanolic extract of the *D. oppositifolia i.e.,* 407.20±5.290 mg/gm of Colchicine equivalent and the minimum was found in the aqueous extract of the *D. pentaphylla i.e.,* 102.25±0.789 mg/ml Colchicine equivalent. Also, both the extracts of the *D. bulbifera* and *D. pentaphylla* showed less alkaloid content. Poornima and Ravishankar (2009) reported the alkaloids content 0.68 mg 100-1g in *D. belophylla.* Ogbuagu (2008) studied effect of cooking on the alkaloid content in *D. bulbifera and D. dumentorum*. After cooking in both the tubers the alkaloid content was reduced. Ezeabara and Anona (2018) investigated the alkaloid content in four different yams from Nigeria namely *D. alata, D. bulbifera, D. cayenensis and D. rotundata* where they found that highest alkaloid is present in the *D. bulbifera i.e.,* 0.64 ± 0.01 mg/100g.

4.2.4 Estimation of Total Terpenoid content:

Terpenoids, also defined as isoprenoids, are a wide and diversified group of naturally occurring organic compounds formed from isoprene, a 5-carbon molecule, and isoprene polymers called as terpenes. Terpenoids are the most common type of plant secondary metabolite and they have significant pharmacological bioactivity. The terpenoid content in the different extracts of the *Dioscorea* species shows in the **(Table No. 4.3 and Figure 4.4).** The highest terpenoid content was observed in the methanolic extracts of the *D. oppositifolia i.e.,* 349.70±2.54 mg/gm of ursolic acid equivalent. Adeosun *et al.,* (2016) studied the phytochemicals properties of the *D. bulbifera* and reported the 20.40 mg/gm of the terpenoids. Odimegwu *et al.,* (2013) extracted Essential oil from *D. floribunda* and *D. composite* and carried out the GCMS analysis they found 76 compounds in *D. floribunda* and 37 from *D. composita* essential oil, major compounds were terpenoids. The comparison in the methanolic

and aqueous extracts of *Dioscorea,* most of the terpenoid extracted in the methanol than the water.

4.2.5 Estimation of Total Saponins Content:

Saponins are major compounds found in the *Dioscorea* species, these are water soluble compound commonly found in the toxic plants. The *Dioscorea* tuber contains high amount of saponins. Saponins were estimated by using method described by Hiai *et al.,* (1976). The highest saponins content was found in the in the aqueous extract of the *D. oppositifolia i.e.,* 579.53±5.33mg/gm of Diosgenin equivalent and the lowest content of saponins observed in the methanolic extract of *D. bulbifera*. i.e. 350.53 ±3.5 mg/gm of Diosgenin equivalent **(Table No. 4.3 and Figure 4.5).** *D. belophylla* has the saponin content of 18.46 mg 100-1g (Poornima and Ravishankar 2009) in *D. bulbifera* and *D. diementorum* saponin content was reported as 79.48mg/100gm and 84.62mg/100gm respectively (Ogbuagu 2008).

Through multiple approaches, phytochemicals have a role in the treatment of obesity. Polyphenols have anti-obesity potential because they block fat metabolizing enzymes such as pancreatic lipase, lipoprotein lipase, and glycerol phosphate dehydrogenase. Polyphenols have the ability to lower blood glucose levels and lipid profiles, as well as boost energy expenditure and fat burning. Flavonoids are plant secondary metabolites that have previously been shown to have a key function in inhibiting pancreatic lipase activity. (Slanc *et al.*, 2009).

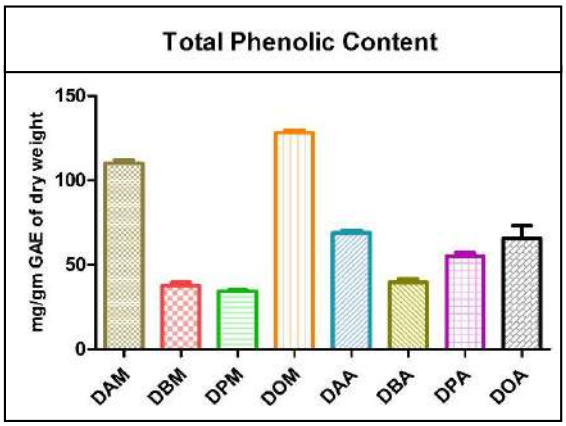


Figure No. 4.1: Total phenolic content in Different *Dioscorea* species.

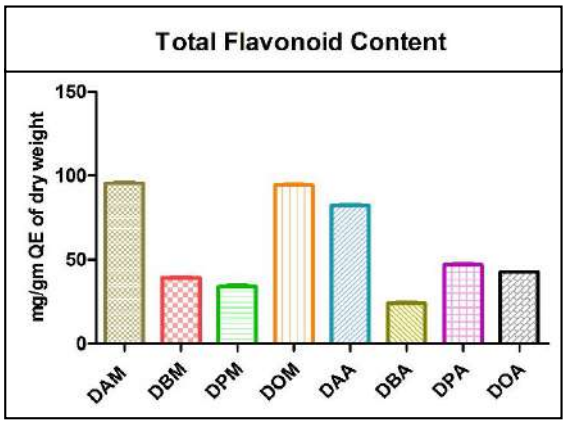


Figure No. 4.2: Total flavonoid content in Different *Dioscorea* species.

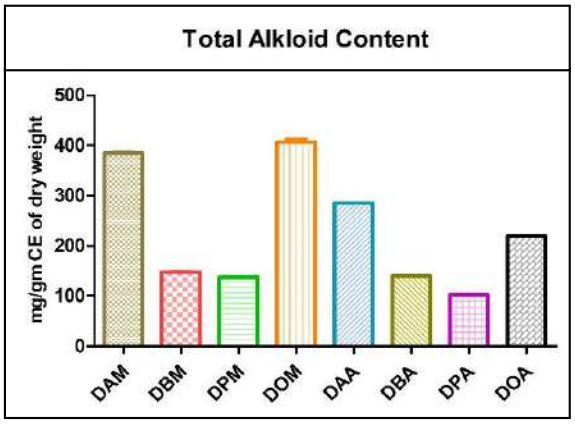


Figure No. 4.3: Total alkaloid content in Different *Dioscorea* species.

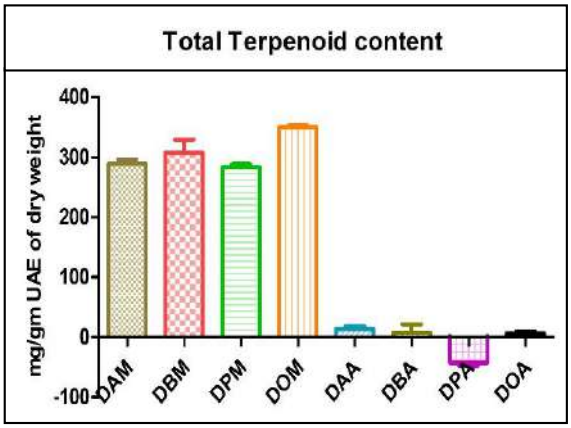


Figure No. 4.4: Total terpenoid content in Different *Dioscorea* species.

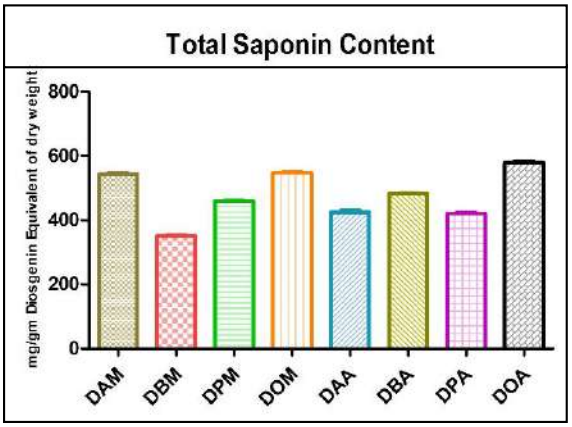


Figure No. 4.5: Total saponin content in Different *Dioscorea* species.

Results showed significantly difference in One-way analysis of variance (< *P=0.05*). (DAM - *D. alata* methanol, DBM - *D. bulbifera* methanol, DPM - *D. pentaphylla* methanol, DOM - *D. oppositifolia* methanol, DAA - *D. alata* aqueous, DBA - *D. bulbifera* aqueous, DPA - *D. pentaphylla* aqueous, DOA - *D. oppositifolia* aqueous)

4.2.6 Anti-oxidant activity:

During the obesity development inflammation and oxidative stress occur. Plant extracts have the ability to scavenge free radicals and reduce oxidative stress will be useful in the treatment of obesity. To evaluate the ability of the plant extracts to scavenge a free radicals developed during the obesity, antioxidant activity was analysed by an array of *in vitro* antioxidant assays **(Table No. 4.4)**.

The comparison of DPPH free radical scavenging activity of the Different *Dioscorea* extracts were presented in **Figure No.4.6**. The antioxidant activity is expressed as % inhibition. The highest DPPH radical scavenging activity was observed in methanolic extract of the *D. oppositifolia i.e.,* 80.30±4.919 % and the lowest was observed in *D. bulbifera* aqueous extracts *i.e.,* 5.65±1.326 %. In case of the ABTS radical scavenging activity the *D. oppositifolia* methanolic extract showed the highest % inhibition *i.e.,* 92.26± 0.484%. Also, both extracts of *D. alata* showed the above 90% inhibition of the ABST radical. **(Figure No 4.7)**. The lowest radical scavenging was shown by *D. bulbifera* methanolic extracts *i.e.,* 34.12± 1.345 %. While in the ferrous reducing antioxidant power assay the *D. alata* methanolic extract showed the highest ability i.e. 124.26±3.383 mM/gm of Ascorbic acid equivalent. Although minimum was observed in *D. bulbifera* aqueous extract 31.19± 1.136. **(Figure No 4.8)**

Table No.4.4: Comparison of the antioxidant activities Different *Dioscorea* extract.

Extract	DAM	DBM	DPM	DOM	DAA	DBA	DPA	DOA
DPPH % Inhibition	69.90± 7.388	16.09± 4.588	10.33± 0.393	80.30± 4.919	23.49± 2.690	5.65± 1.326	12.71± 1.117	25.16± 1.152
ABTS % Inhibition	92.11± 0.558	34.12± 1.345	50.48± 2.607	92.26± 0.484	91.82± 0.848	47.97± 2.014	91.24± 0.713	70.86± 1.302
FRAP mM/gm of AAE	124.26± 3.383	33.56± 4.608	31.43± 1.794	114.95± 6.461	80.26± 1.881	31.19± 1.136	51.58± 0.839	59.57± 1.639

Values presented in ± SE (n=3). DAM- *D. alata* methanolic extract, DBM- *D. bulbifera* methanolic extract, DPM- *D. pentaphylla* methanolic extract, DOM- *D. oppositifolia* methanolic extract, DAA- *D. alata* aqueous extract, DBA- *D. bulbifera* aqueous extract, DPA- *D. pentaphylla* aqueous extract, DOA- *D. oppositifolia* aqueous extract.

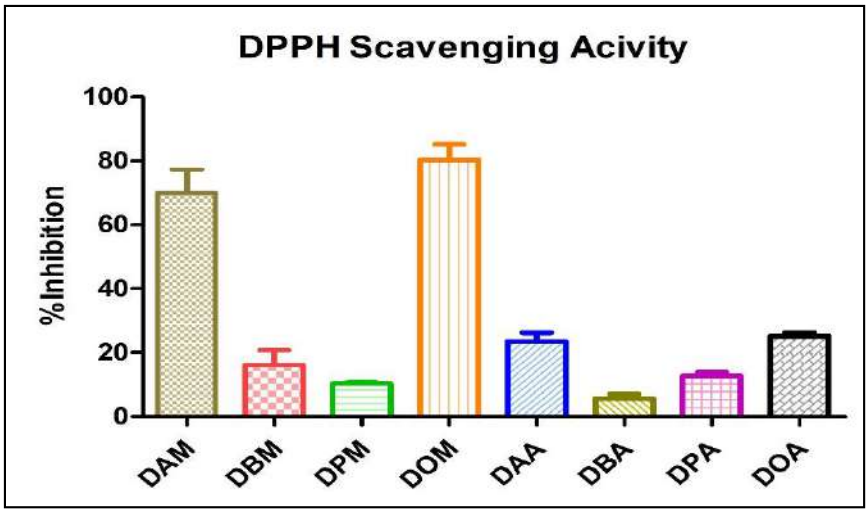


Figure No. 4.6: DPPH Free radical scavenging activity of different *Dioscorea* extracts

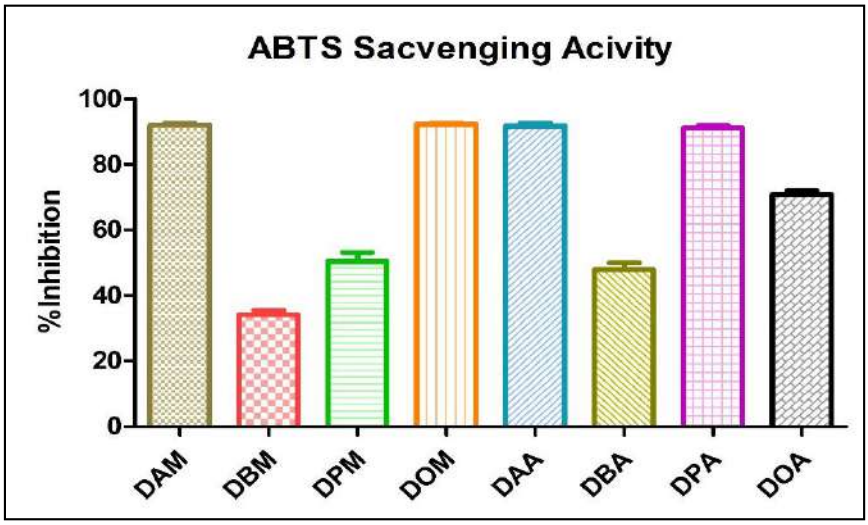


Figure No. 4.7: ABTS Free radical scavenging activity of different *Dioscorea* extracts

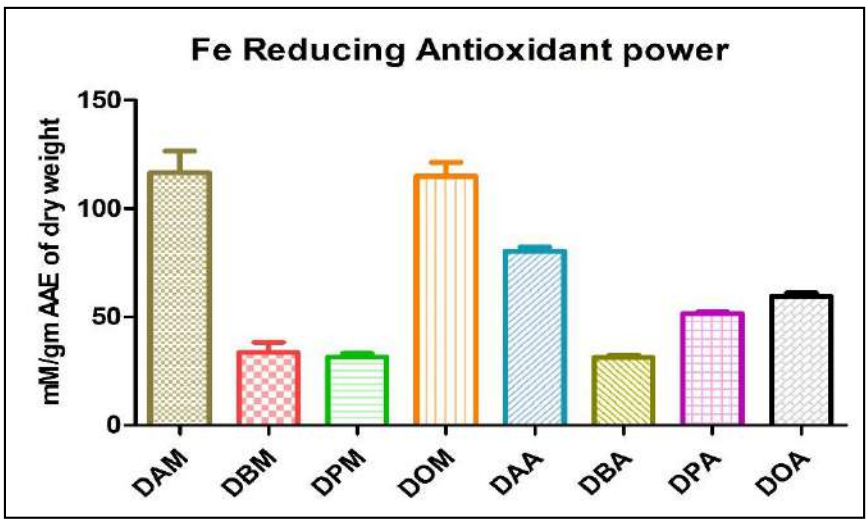


Figure No. 4.8: FRAP activity of different *Dioscorea* extracts

(DAM - *D. alata* methanol, DBM - *D. bulbifera* methanol, DPM - *D. pentaphylla* methanol, DOM - *D. oppositifolia* methanol, DAA - *D. alata* aqueous, DBA - *D. bulbifera* aqueous, DPA - *D. pentaphylla* aqueous, DOA - *D. oppositifolia* aqueous)

Obesity is characterised by low-grade inflammation caused by an increase in adipose tissue mass, which causes hypoxia and the release of pro-inflammatory cytokines. This results in cardiovascular disorders. Herbal extracts' ability to scavenge free radicals limits oxidation of macromolecules, hence avoiding a variety of obesity-related illnesses (Yang *et al.,* 2009; Mukherjee 2003).

It has been observed that phenolic chemicals have a variety of biological effects, including antioxidant activity. The existence of several antioxidant components in plant tissues, particularly fruits and vegetables, makes measuring each antioxidant component independently problematic. Bhandari *et al.,* (2004) evaluated the antioxidant activity of the four *Dioscorea* species from Nepal. They have reported *D. bulbifera* showing the strong DPPH scavenging activity. While the *D. deltoidea* showing the highest Fe reducing power than the other species. Also, the polyphenol content showing the correlation with the antioxidant activity. Sakthidevi and Mohan (2013) studied the antioxidant potential of the *D. alata.* They have reported leaf methanol extracts with strong antioxidant activity this activity may be because of the high phenolic and flavonoid content. Ghosh *et al.,* (2013) studied the antioxidant properties of the *D. bulbifera* by different *in vitro* assays and in all the studied assays the methanolic extracts exhibited the highest activity.

Yam (*Dioscorea* sp.) extracts have been shown to exhibit more than 70% antioxidant activity when tested using a model system including b-carotene and linoleic acid, and are classified as a vegetable with high antioxidant activity (Kaur & Kapoor, 2002). Farombi *et al.,* (2000) discovered a significant degree of antioxidant activity in several farmed yam species from Nigeria using the ABTS technique. Hsu *et al.,* (2003) discovered exceptional antioxidant properties in various cultivated yam species from Taiwan, as measured by DPPH radical scavenging, ferrous ion chelating, reducing power, and ABTS assays. Reports on the antioxidant activity of wild yam species, on the other hand, are quite rare in the literature. As a consequence, comparing our findings to those of earlier research is extremely challenging. Sonibare and Abegunde (2012) studied the antioxidant activity by DPPH activity of *D. hirtiflora, D. dumetorum* (edible) *D. dumetorum* (non-edible) and reported that *D. hirtiflora* exhibit the highest % of inhibition than *D. dumetorum*. Also, correlation between the total phenolic content and DPPH activity reported with R^2=0.892. Dzomba and Musekiwa (2014) isolate the different flavonoids from the *D. steriscus* and antioxidant potential was studied by

the DPPH assay. They have correlated the antioxidant and with the biological activity (lipase inhibition and amylase inhibition) also they suggested the flavonoids from the *D. steriscus* have the anti-obesity potential. Liu (2016) have reported the activity of the antioxidant activity of the fresh peel and flesh of *D. opposita* and got the highest free radical scavenging activity in the aqueous extract of peal, also studied the anticancerus properties of the extracts and reported the bioactive compound from the *D. opposita*.

4.2.7 Correlation of total phenolic, flavonoid, alkaloid, terpenoid, and saponin content of *Dioscorea* Species:

The Pearson correlation coefficients between antioxidant activities and TPC, TFC were represented in **Table No. 4.5** TPC strongly correlate with DPPH, and FRAP assays with r= 0.972, 0.968 respectively but with ABTS showing significant correlation with r=0.752, whereas TFC exhibited a strong correlation with FRAP activity with r = 0.962 and significant correlation with DPPH and ABTS with r=0.876, 0.790 respectively. Overall, the result revealed the existence of a positive and correlation between tested phytochemicals and antioxidant assays. TAC showing the stronger correlation with DPPH with r=0.940 and FRAP with r= 0.952. While with ABTS showing less correlation. Total terpenoid content and total saponin content were not showing significant correlation with any of the antioxidant assay.

Table No. 4.5: Correlation between antioxidant activity and phenolic, flavonoid, alkaloid, terpenoid, and saponins content of *Dioscorea* Species

Parameters	DPPH	ABTS	FRAP
TPC	0.972**	0.752*	0.968**
TFC	0.876**	0.790*	0.962**
TAC	0.940**	0.633	0.952**
TTC	0.583	0.426	0.558
TSC	0.572	-0.169	0.340

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed). TPC: Total phenolics content, TFC: Total flavonoids content, TAC: total alkaloid content, TTC: total terpenoid content, TSC: total saponin content. DPPH: 2, 2-diphenyl-1-picrylhydrazyl, ABTS: 2, 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant property assay.

4.3. Evaluation of species from genus *Dioscorea* for anti-obesity activity:

4.3.1 Lipase inhibition activity:

Pancreatic lipase is primarily responsible for the hydrolysis of triglycerides into free fatty acids and monoglycerides. It's a lipolytic enzyme that catalysis the hydrolysis of the triacylglycerol's' ester bonds. The enzyme operates by removing the fatty acids from sites 1 and 3 of the triglyceride, leaving a 2-monoglyceride and two free fatty acids in their place. (Lowe, 1997)

The inhibitory activities of extracts towards pancreatic lipase are represented in **Table No. 4.6.** Among the 8 extracts both methanolic and aqueous extracts of *D. pentaphylla* crude extracts showed lipase inhibition activity better than other extracts and *D. alata* and *D. bulbifera* extracts showing the moderate lipase inhibition at concentration 5 mg/ml **(Table No. 4.6).** The significant inhibition of Pancreatic lipase was observed in *D. pentaphylla* aqueous extract 75.83±1.661% with IC_{50} 0.986 mg/ml, although in *D. pentaphylla* methanolic extract with 71.73±1.729 % with IC_{50} 3.088 mg/ml. the lowest inhibition was observed in *D. oppositifolia* aqueous extract *i.e.,* 44.73±0.272% with high IC_{50} value 5.59mg/ml. The orlistat which was used as positive control showed the 86.33±0.832% inhibition at 5mg/ml concentration with IC_{50} value 0.957 mg/ml **(Figure No. 4.9).** Orlistat, a Hydrogenated derivative of lipstatin, is the pancreatic lipase inhibitor currently approved for a long-term treatment of obesity. Similarly inhibitory effect Dioscin, diosgenin, and saponins from *D. nipponica* were isolated and tested for lipase inhibitory efficacy in rats, finding a substantial inhibitory effect by Kwon *et al.,* (2003). Bioactive flavonoids were extracted from *D. steriscus* tubers that can inhibit lipase and α-amylase, making them useful for the development of anti-obesity therapeutics (Dzomba and Musekiwa, 2014). Jeong *et al.,* (2016) has reported n-butanolic extracts of *D. oppositifolia* has bioactive compound which inhibit lipase and reduces fat absorption in high fat induced mice.

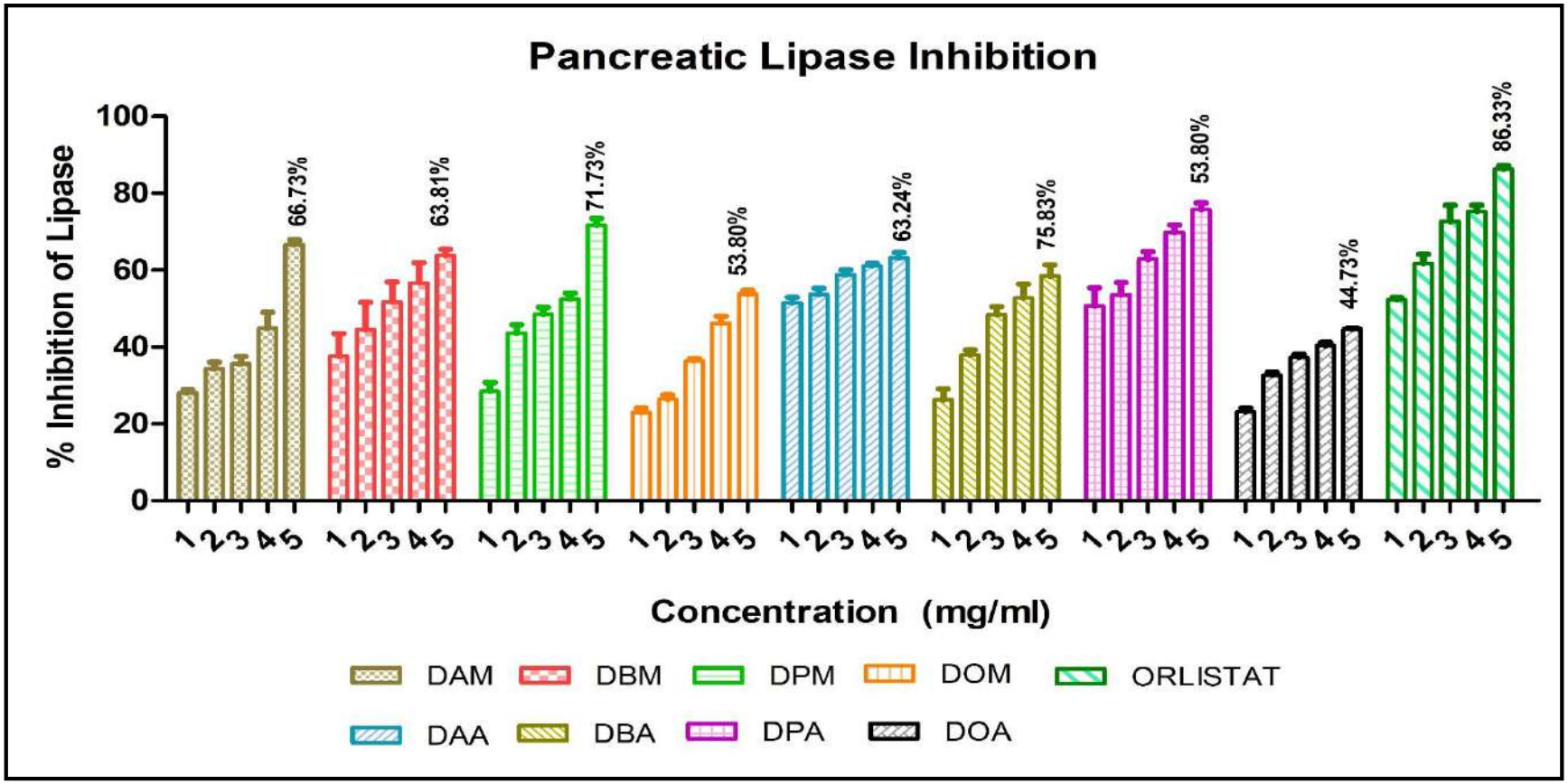


Figure No. 4.9: Inhibitory effect of orlistat and plant extract pancreatic lipase activity. Two-way ANOVA was calculated *p- value* is statistically significant (*P=< 0.0001*) (DAM= *D. alata* methanol, DBM= *D. bulbifera* methanol, DPM *D. pentaphylla* methanol, DOM= *D. oppositifolia* methanol, DAA =*D. alata* aqueous, DBA =*D. bulbifera* aqueous, DPA= *D. pentaphylla* aqueous, DOA =*D. oppositifolia* aqueous)

Table No. 4.6: Lipase inhibition activity of *Dioscorea* extracts values expressed in % inhibition with ± SE

Sr. No.	Plant Name	Solvent	%Inhibition at 5mg/ml	IC_{50} mg/ml
1	***D. alata***	Methanol	66.73 ± 1.109	4.461
		Aqueous	63.24 ± 0.889	0.971
2	***D. bulbifera***	Methanol	63.81 ± 1.658	2.903
		Aqueous	58.47 ± 2.925	3.097
3	***D. pentaphylla***	Methanol	71.73 ± 1.729	3.088
		Aqueous	75.83 ± 1.661	0.986
4	***D. oppositifolia***	Methanol	53.80 ± 0.889	4.332
		Aqueous	44.73 ± 0.272	5.59
5	**Orlistat**	DMSO	86.33 ± 0.832	0.957

4.3.2 Testing of crude extracts on cell line 3T3-L1 pre adipocytes

A. Cell viability assay:

The cell viability assay was carried out of the all the extracts was carried out for the determination of the IC_{50} value. From the IC_{50} value treatment concentration was decided for the differentiation assay. The toxicity of the extract was investigated using the MTT test at various doses (6.25-100 µg/ml). Methanolic extracts of all four plant extracts were shown to be toxic to cells at higher concentrations *i.e.,* 100µg/ml whilst aqueous extracts were found to be slightly toxic to cells at the tested doses. The IC_{50} values are mentioned in **Table No. 4.7,** and **Figure No. 4.10** and **4.11** are presenting the % viability of the 3T3-L1 cells at different tested concentrations.

Table No. 4.7: IC_{50} values of the methanolic and aqueous extracts.

Sr. No.	Plant Name	IC_{50} of methanol extract	IC_{50} of Aqueous extract
1	*D. alata*	81.31 µg/ml	97.50 µg/ml
2	*D. bulbifera*	83.90 µg/ml	93.17 µg/ml
3	*D. pentaphylla*	77.89 µg/ml	95.57 µg/ml
4	*D. oppositifolia*	78.59 µg/ml	69.04 µg/ml

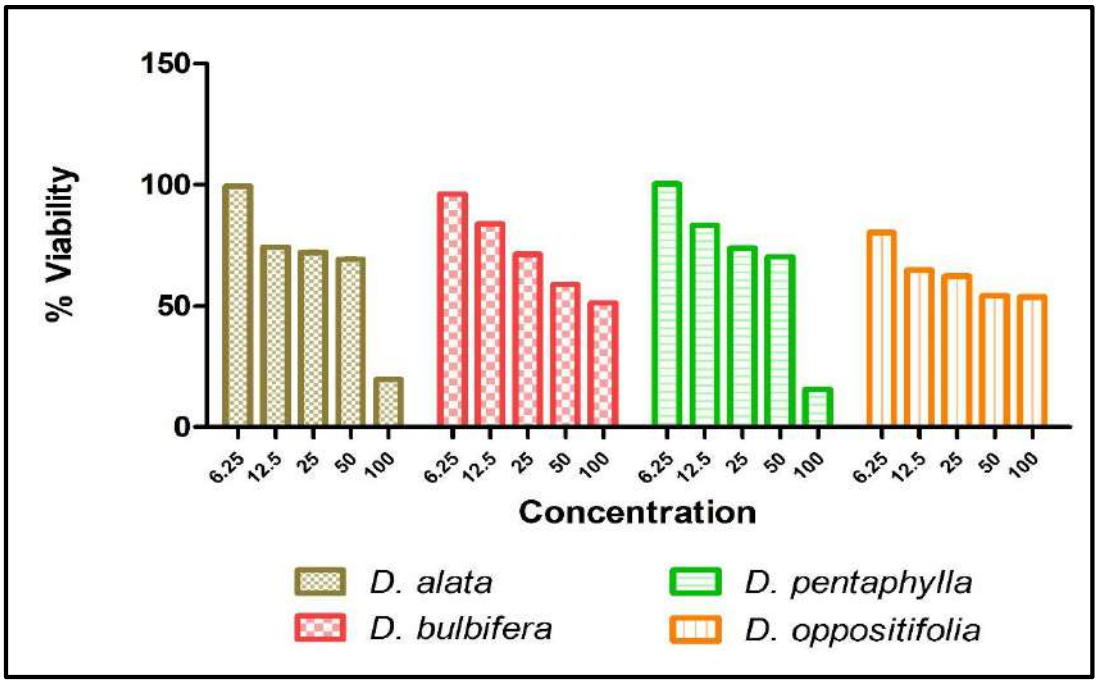


Figure No. 4.10: Cell viabilty test by MTT assay cells treated with methanolic extract (6.25 to 100µg/ml concentration)

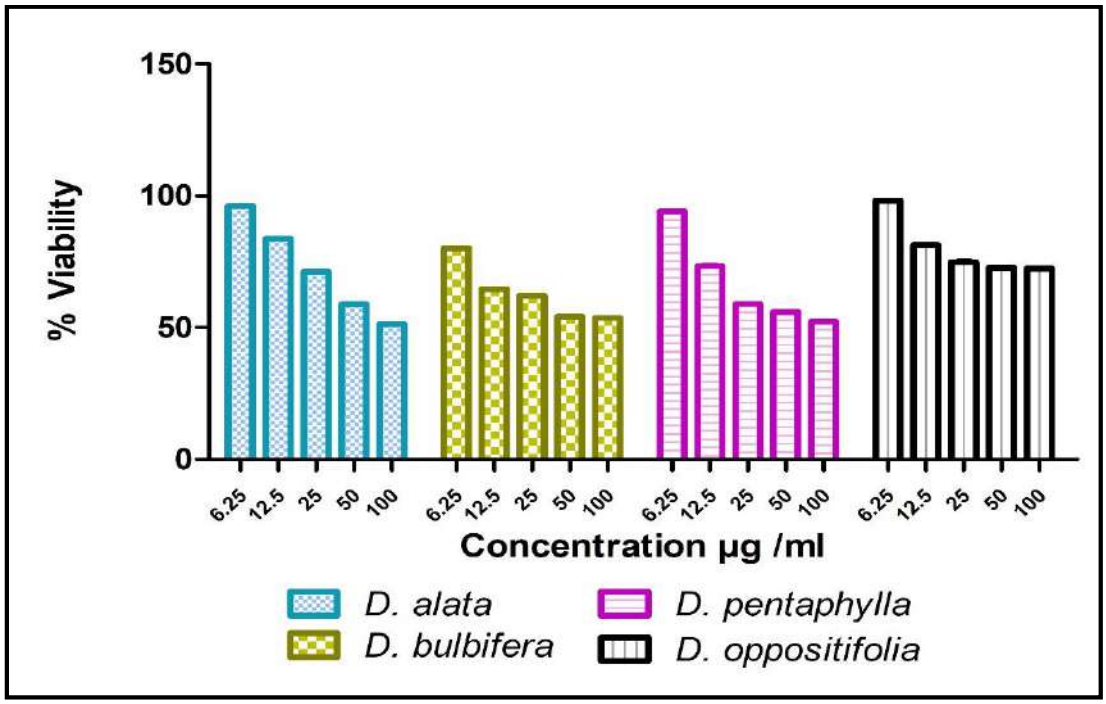


Figure No. 4.11: Cell viabilty test by MTT assay cells treated with aqueous extracts (6.25 to 100µg/ml concentration)

B. 3T3-L1 Cells Differentiation:-

The effects of the plant extracts were studied at different time intervals on 3T3-L1 cells. The treatments given on day 4 to day 6 and day 6 to day 8 did not exhibit inhibitory activity on the differentiation of the adipocytes. Whereas, the cells treated with plant extracts from day 2 up to day 8 with a hormone cocktail and insulin show a more effective inhibition of the adipocytes. The decrease in lipid deposition was measured by comparing the cells that had been stimulated with a hormone cocktail.

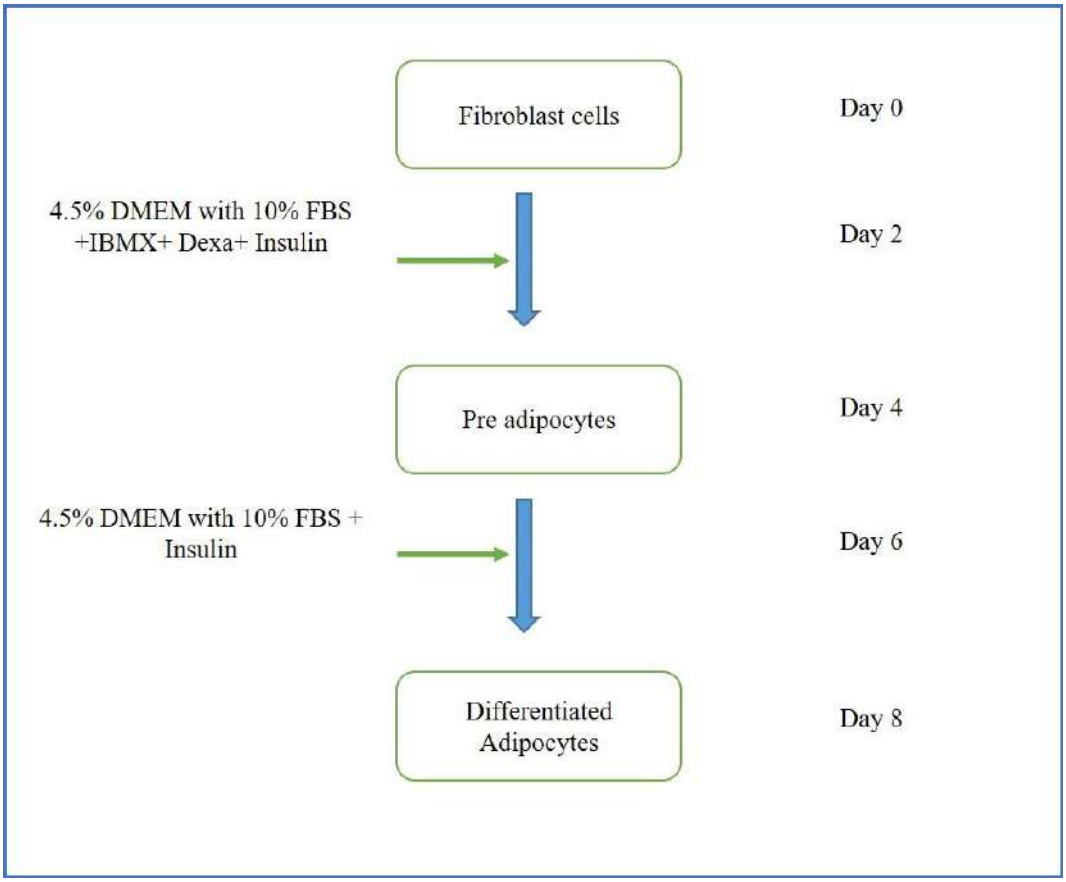


Figure No. 4.12: Flow chart of adipogenesis

C. Effect of plant extracts on inhibition of lipid droplet accumulation in differentiation of 3T3-L1 cell line:

The Lipid quantification was carried out on the eighth day of the experiment. Cells treated with a hormone cocktail had 2.19 times increase in lipid content as compared to non-induced cells. In comparison with control group the lipid accumulation was highly significant

(*P<0.001*) for induced group whereas in other tested groups the lipid accumulation was significantly decreased (*P<0.001*) in comparison with induced group, among the tested four *Dioscorea* species. The lipid deposition in cells treated with *D. oppositifolia* methanolic and aqueous extracts was found to reduce by 1.8 and 2.2 times, respectively. Furthermore, *D. pentaphylla* methanolic and aqueous extracts inhibit lipid formation in cells by 1.7 and 1.6 times, respectively **(Figure No. 3.13).** *D. pentaphylla* and *D. oppositifolia* were chosen based on the decrease in lipid accumulation among the four species for gene expression study **(Plate No. 6 and 7).**

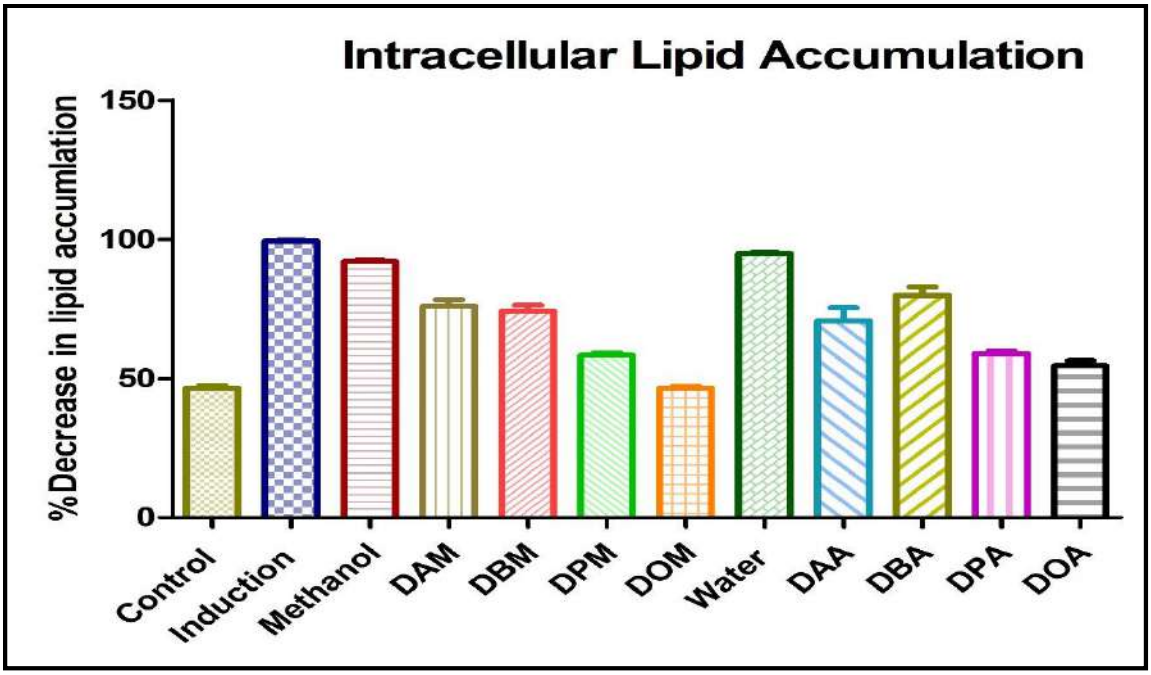


Figure No. 4.13: Intracellular lipid accumulation in 3T3-L1 cells by isopropanol extraction method ******P< 0.001* **as compare to induction and control using one way ANOVA followed by tukyes test. (DAM= *D. alata* methanol, DBM= *D. bulbifera* methanol, DPM *D. pentaphylla* methanol, DOM= *D. oppositifolia* methanol, DAA =*D. alata* aqueous, DBA =*D. bulbifera* aqueous, DPA= *D. pentaphylla* aqueous, DOA =*D. oppositifolia* aqueous)

D. Effect of *D. oppositifolia* and *D. pentaphylla* on gene expression in 3T3-L1 cells:

While considering the results obtained from the lipase inhibition activity and lipid droplet accumulation in differentiated of 3T3-L1 cell. The whole plant extracts of *D. pentaphylla* and *D. oppositifolia* were selected for further gene expression study.

Methanolic and aqueous whole plant extracts of *D. oppositifolia* and *D. pentaphylla* were tested on 3T3-L1 preadipocytes cells. Comparison of all four extracts of *D. oppositifolia* and *D. pentaphylla*, both methanolic and aqueous extracts of *D. oppositifolia* exhibited superior anti-adipogenesis action on 3T3-L1 cells than both the extracts *D. pentaphylla* extract.

D. oppositifolia methanolic and aqueous extracts can down regulates adipogenic gene expression. The expression of PPARγ **(Figure No. 4.14 A, 4.15 A)**, C/EBPα **(Figure No. 4.14 B, 4.15 D)**, and SREBP-1 **(Figure No. 4.14 C, 4.15 C)** genes were assessed in comparison to the control. At greater concentrations of the extracts, cells treated with methanolic and aqueous extracts down-regulated the adipogenic genes PPARγ, C/EBPα, and SREBP-1 by up to 99 %, 97 %, and 98 %, respectively. Methanolic extracts also regulated the fatty acid synthase (FASN) gene expression. The FASN was down-regulated up to 93 and 99 % of the extracts at 25 µg/mL and 75 µg /mL, respectively **(Figure No. 4.14 D)**. In the case of the treatment of the aqueous extract, the expression of FASN increased with the increasing concentration of the *D. oppositifolia* aqueous extract. The FASN up-regulation was observed, up-regulated up to 26% and 124% at 25 µg /mL and 75 µg /mL concentration respectively. **(Figure No. 4.15 D)** The methanolic and aqueous extracts of *D. pentaphylla* were found to up regulate the expression of PPARγ **(Figure No. 4.16 A and 4.17 A),** SREBP-F1 **(Figure No. 4.16 C and 4.17 C),** and C/EBPα **(Figure No. 4.16 B and 4.17 B).** Whereas the methanolic extract of *D. pentaphylla* inhibited FAS expression, the aqueous extracts stimulated it **(Figure No. 4.16 D and 4. 17 D).**

D. oppositifolia showed promising results in the down-regulation of the adipogenic genes. PPARγ was first identified as a factor that is activated during adipocyte differentiation (Ferré, 2004 and Matsusue *et al.,* 2004) and is well recognized for its function in regulating adipogenic and lipogenic pathways. Similarly, it is important for modulating gene networks involved in glucose homeostasis, especially boosting the expression of glucose transporter type 4 (Glut4). Expression of the C/EBPα is stimulated by the PPARγ (Ahmadian *et al.,* 2013). C/EBPα helps in the differentiation and development of the adipocytes. It is a key transcription factor that helps to regulate adipogenic genes. It is highly expressed in the liver and adipocytes in humans and rodents (Louise *et al.,* 2008). Previously, Yang *et al.,* (2014) had extracted batatasin I from the butanolic fraction of *D. oppositifolia* root extract and tested it against 3T3-L1 cells, obtaining a 90% down regulation of the PPARγ gene and a 20.9% decrease in C/EBPα

expression. *D. oppositifolia* methanolic and aqueous extract showed the 99% down-regulation the PPARγ gene. In the case of C/EBPα, a drop of up to 98% was observed. This significant down-regulation of PPARγ gene may be due to the cumulative action of phytochemicals from the extracts. This reduction of adipogenic transcription factors may aid in regulating adipocyte differentiation and lowering adipocyte lipid accumulation. There is a correlation between SREBP-F1 and FASN gene. If the level of SREBP-F1 increases, it ultimately elevates the level of the FASN gene and thereby increases the fat accumulation. During differentiation, FASN helps in the *de novo* synthesis of the new lipid molecules in the cells (Laliotis *et al.*, 2010). Thus, the target in the present study was to examine the effectiveness of the screened extract towards the gene expression. In the present investigation, methanolic extract of the *D. oppositifolia* reduced 99% expression of the FASN in 3T3-L1 cells, while in the case of the aqueous extract of *D. oppositifolia*, up-regulation of the FASN was observed at higher concentration of the extracts. But at a lower concentration, it down regulated the expression of the FASN up to 73%. **(Figure 4.15 D)**

There is another way to control obesity by triggering lipolysis. Up regulation of the CPT-1 helps increase the transport of the cytosolic long-chain fatty acids in the mitochondria for the β oxidation of the lipids (Ju *et al.*, 2011). If CPT-1 gene expression is inhibited, there is an increase in the risk of obesity (Yang *et al.*, 2014). The *D. oppositifolia* methanolic extract at 25 µg/mL and 75 µg/mL increased the CPT-1 expression by ≅ 2 and 6.9 times, respectively **(Figure No. 4.14 E).** In the case of the aqueous extract, the CPT-1 was up-regulated in dose dependent manner; 7.8 folds, and 5.3 folds increased as compared to the control at 25 µg /mL and 75 µg /mL, respectively **(Figure No. 4.15 E).** Both methanolic and aqueous extracts of *D. oppositifolia* increased CPT-1 gene expression by ≅ 7 times the control that will help to reduce fat accumulation in the cells by activating the assisting the lipolysis pathway.

Both *D. pentaphylla* extracts suppressed the expression of CPT-1 **(Figure No. 4.16 E and 4.17 E).** *D. pentaphylla* extracts stimulated adipogenesis by activating adipogenic transcription factors inhibiting the expression of CPT-1, which is a crucial enzyme that assists in lipolysis. When CPT-1 is inhibited, the rate of fatty acid oxidation is decreased, and fatty acid accumulation is increased. As a result, *D. pentaphylla* exhibited the anti-lipase activity but did not show the down-regulation of the adipogenic genes.

This study's goal was to assess potential *Dioscorea* species for anti-obesity effects. The lipase inhibition ability of all four *Dioscorea* species extracts were investigated, both methanolic and aqueous extracts of *D. pentaphylla* inhibited lipase more effectively than *D. alata, D. bulbifera*, and *D. oppositifolia* extracts. All extracts were also tested for antiadipogenic activity in 3T3-L1 preadipocytes. The uptake of the oil red O stain in cells provides information on lipid build-up in the cells. All extracts of *D. alata, D. bulbifera*, and *D. pentaphylla* revealed less antiadipogenic efficacy than *D. oppositifolia* methanolic and aqueous extracts. On the basis of the oil red o staining assay, both methanolic and aqueous whole plant extracts of *D. oppositifolia* and *D. pentaphylla* were carried forward for the gene expression study.

In a gene expression analysis, *D. oppositifolia* methanolic extracts suppressed adipogenic genes more than *D. oppositifolia* aqueous extracts and *D. pentaphylla* methanolic and aqueous extracts. From the obtained results, the *D. oppositifolia* whole plant extract was used for the solvent fraction extraction study and the animal study.

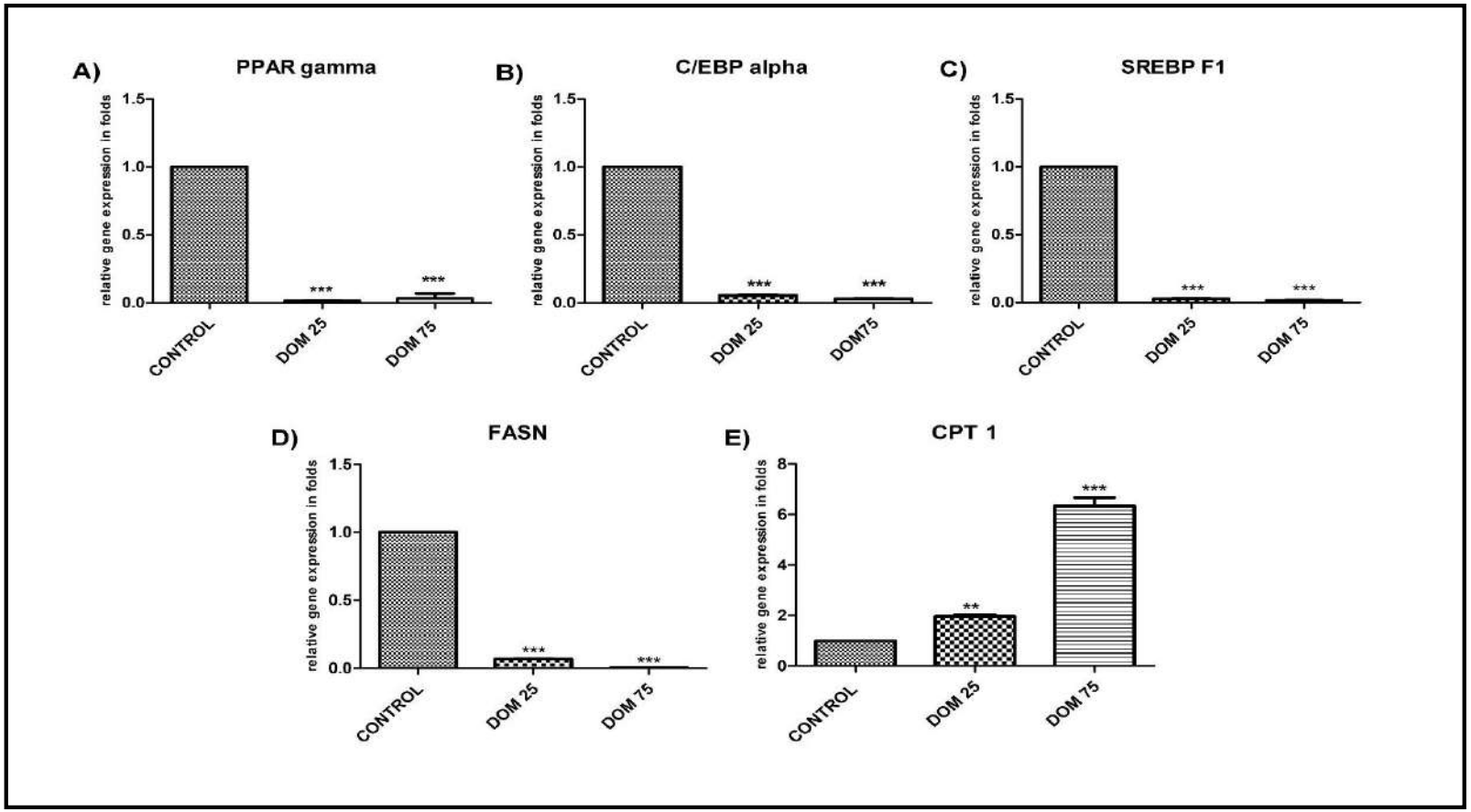


Figure No. 4.14: Effect of *D. oppositifolia* methanolic (DOM) extract on Relative mRNA expression of (a) peroxisome proliferator-activated receptor-gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one-way ANOVA followed by Dunnett's Multiple Comparison Test.**

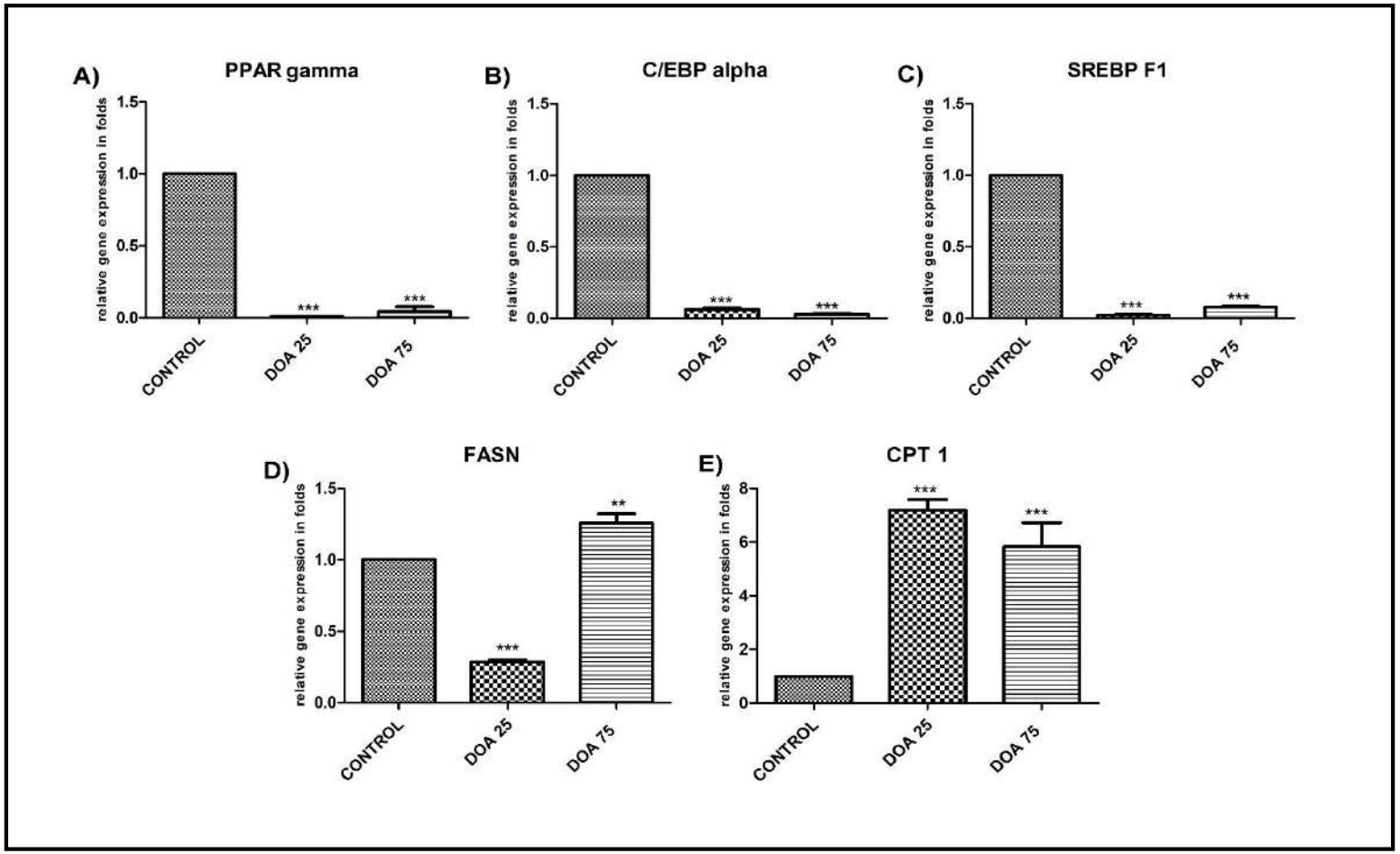


Figure No. 4.15: Effect of *D. oppositifolia* aqueous (DOA) extract on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

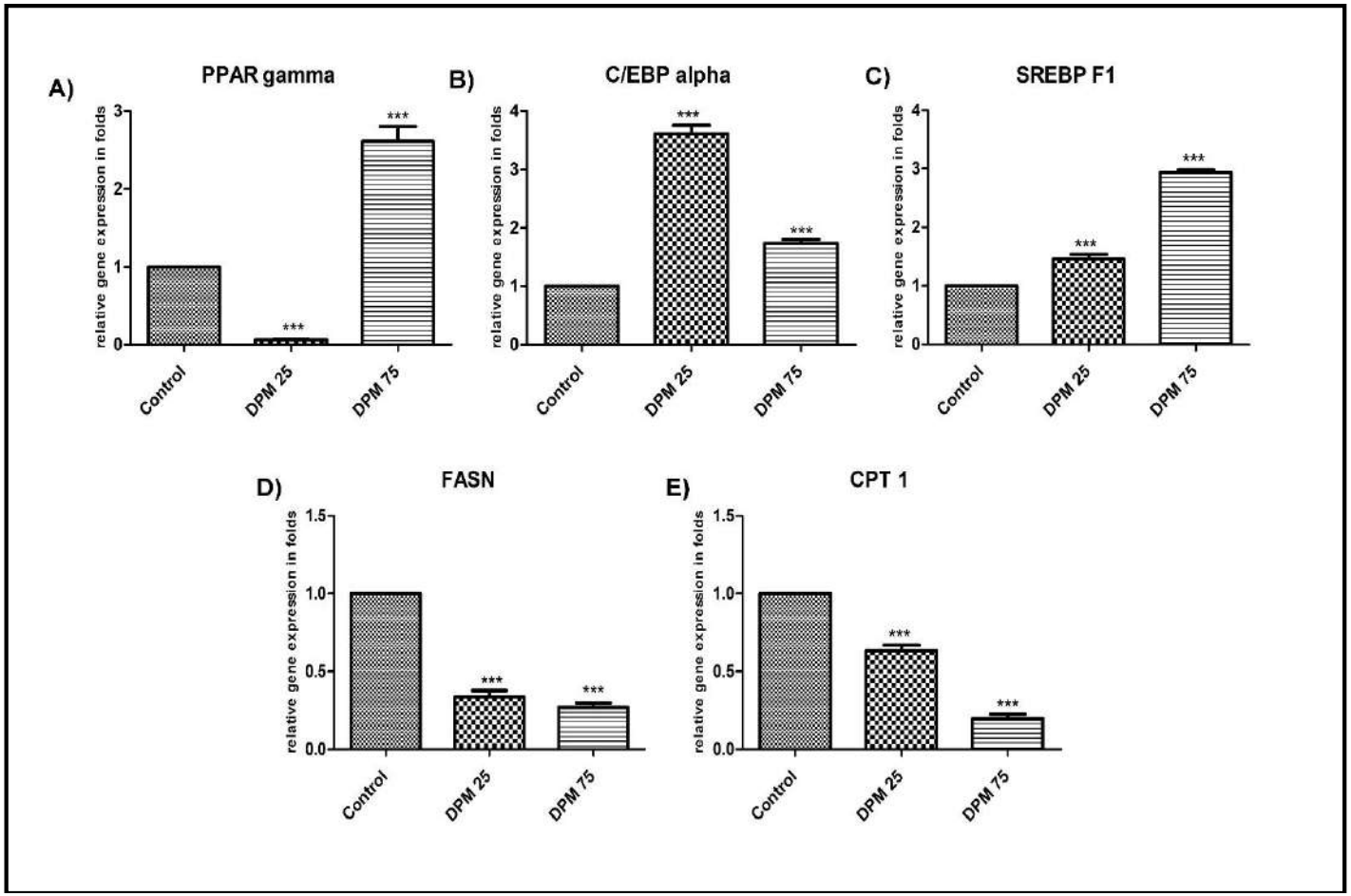


Figure No. 4.16: Effect of *D. pentaphylla* methanolic (DPM) extract on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

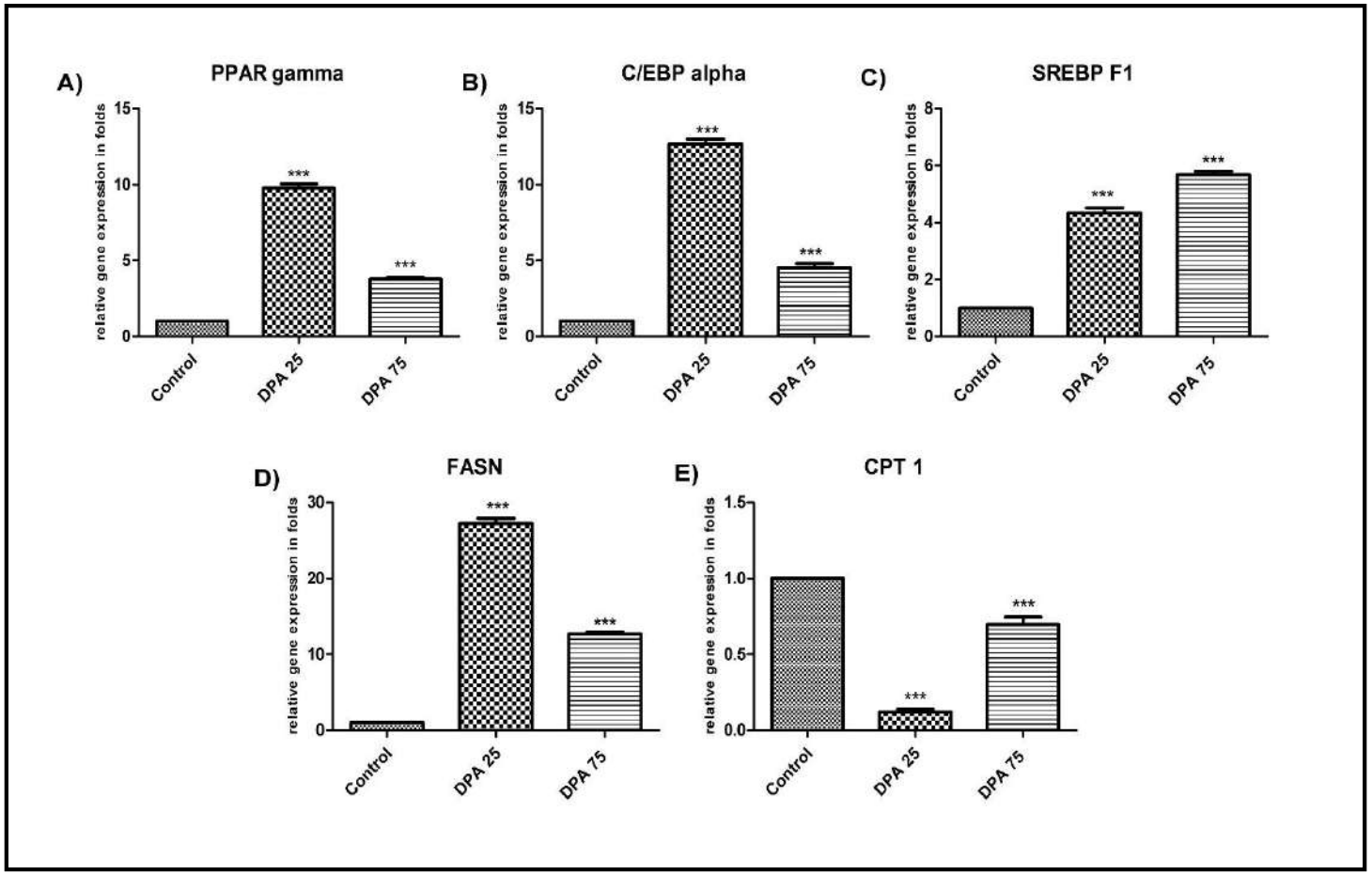


Figure No. 4.17: Effect of *D. pentaphylla* aqueous (DPA) extract on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

In context with results obtained from the lipase inhibition, lipid accumulation in 3T3-L1 cells, phytochemical analysis, and antioxidant assay the *D. oppositifolia* methanolic extracts has a good anti-oxidant potential and it also inhibiting the lipid accumulation in 3T3-L1 cells and *D. pentaphylla* has a good lipase inhibition potential so these two extracts were carried forward for the metabolite profiling by the LCMS method to identify the antiobesity compounds present in the plant extracts.

E. LCMS analysis of the *D. oppositifolia* and *D. pentaphylla* methanolic extract:

The LCMS-MS analysis of methanolic extracts of *D. oppositifolia* and *D. pentaphylla* was carried out. The collected data showed many classes of substances including fats/lipids, alkaloids, sugars, peptides, phenolic, steroids, glycosides, terpenoid, flavonoids, and cyanidins. Identified compounds are listed in **Table No. 4.8** and **4.9. Figure No. 4.18** and **4.19** shows the LCMS chromatogram of *D. oppositifolia* and *D. pentaphylla* respectively.

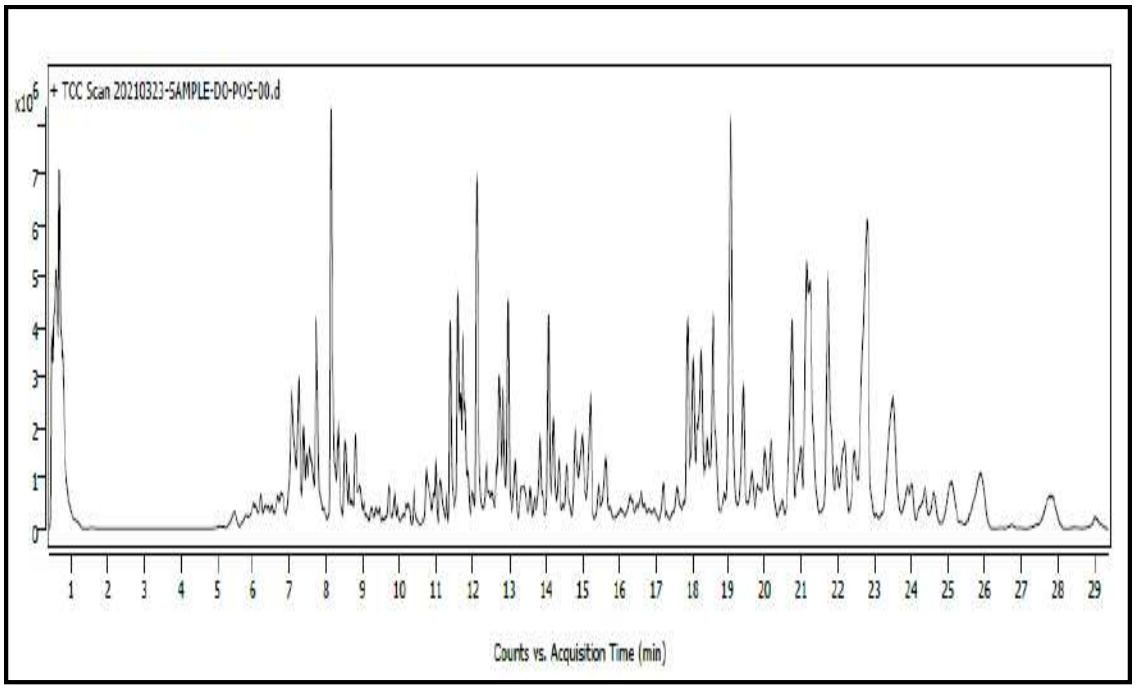


Figure No. 4.18: LCMS chromatogram of *D. oppositifolia* whole plant methanolic extract

Table No. 4.8: List of identified compound from *D. oppositifolia* using LSMS

	Name	Formula	RT	Mass
Phenolics	Crosatoside B	$C_{20}H_{30}O_{11}$	5.818	446.1785
	2,6-Dimethoxy-4- propylphenol	$C_{11}H_{16}O_3$	7.109	196.1099
	Octyl gallate/ gallic acid	$C_{15}H_{22}O_5$	8.557	282.1464
	3"-Hydroxygeranylhydroquinone	$C_{16}H_{22}O_3$	10.624	262.1567
	Nogalonic acid	$C_{20}H_{14}O_8$	12.524	382.0688
	R1128A	$C_{17}H_{14}O_5$	12.670	298.0833
	5-Heptadecyl-1,3- benzenediol	$C_{23}H_{40}O_2$	13.804	348.3021
	Ethyl vanillin isobutyrate	$C_{13}H_{16}O_4$	13.834	236.1043
	Oryzarol	$C_{26}H_{42}O_3$	14.797	402.3130
Flavonoid	Cinnamtannin A1	$C_{45}H_{38}O_{18}$	6.355	866.2065
	Apigenin 6-C-glucoside	$C_{26}H_{28}O_{14}$	6.595	564.1487
	"4'-O-Methyldelphinidin 3- O-beta-D-glucoside"	$C_{22}H_{23}O_{12}$	6.743	479.1194
	Moracin I	$C_{20}H_{20}O_4$	7.009	324.1363
	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	7.156	464.0952
	Cyanidin 3-rhamnoside 5-glucoside	$C_{27}H_{31}O_{15}$	7.454	595.1665
	7,8,3',4'-Tetrahydroxyisoflavone	$C_{15}H_{10}O_6$	7.457	286.0476
Terpenoid	"3-Hydroxy-6,8-dimethoxy-7(11)-eremophilen-12,8-olide	$C_{17}H_{26}O_5$	6.491	310.1770
	Gibberellin A38 glucosyl ester	$C_{26}H_{36}O_{11}$	7.083	524.2260
	(-)-trans-Carveol	$C_{10}H_{16}O$	7.375	152.1200
	Unshuoside A	$C_{16}H_{28}O_7$	7.518	332.1838
	Ganoderenic acid A	$C_{30}H_{42}O_7$	8.590	514.2936
	Acuminoside	$C_{21}H_{36}O_{10}$	8.785	448.2304
	Eremopetasidione	$C_{14}H_{20}O_3$	9.017	236.1414
	Valerosidatum	$C_{21}H_{34}O_{11}$	9.087	462.2100
	Neryl glucoside	$C1_6H_{28}O_6$	9.200	316.1883
	Zerumbone	$C_{15}H_{22}O$	9.354	218.1668
	"2,2,4,4,-Tetramethyl-6-(1-oxopropyl)-1,3,5-cyclohexanetrione	$C_{13}H_{18}O_4$	10.104	238.1201
	Ethyl menthane carboxamid	$C_{13}H_{25}NO$	12.038	211.1936
	2-oxophytanic acid	$C_{20}H_{38}O_3$	12.523	326.2821
	Celastrol	$C_{29}H_{38}O_4$	12.545	450.2770
	Armillaripin	$C_{24}H_{30}O_6$	12.756	414.2044
	Celastrol	$C_{29}H_{38}O_4$	12.759	450.2768
	Isomytiloxanthin	$C_{40}H_{54}O_4$	12.779	598.4029
	Pristanic acid	$C_{19}H_{38}O_2$	13.465	298.2867
	Isomytiloxanthin	$C_{40}H_{54}O_4$	13.731	598.4025
	Lactapiperanol D	$C_{18}H_{28}O_5$	13.833	324.1935
	all-trans-heptaprenyl	$C_{35}H_{60}O_7P_2$	13.907	654.3817

	Name	Formula	RT	Mass
Terpenoid	3L,7D,11D-phytanic acid	$C_{20}H_{40}O_2$	14.102	312.3025
	"all-trans-heptaprenyl diphosphate	$C_{35}H_{60}O_7P_2$	14.216	654.3821
	6-O-Acetylaustroinulin	$C_{22}H_{36}O_4$	17.674	364.2613
	22-Angeloyltheasapogenol A	$C_{35}H_{56}O7$	20.681	588.4030
	3-cis-Hydroxy-b,e-Caroten-3'-one	$C_{40}H_{54}O$	21.138	550.4177
Lipids	Cucurbic acid	$C_{12}H_{20}O_3$	8.125	212.1405
	9,10-Dihydroxy-12,13-epoxyoctadecanoate	$C_{18}H_{34}O_5$	10.169	330.2407
	2-Tetradecanone	$C_{14}H_{28}O$	10.790	212.2137
	Octadecanedioic acid	$C_{18}H_{34}O_4$	10.793	314.2455
	9,10,13-Trihydroxystearic acid	$C_{18}H_{36}O_5$	10.822	332.2560
	Phenethyl decanoate	$C_{18}H_{28}O_2$	11.769	276.2087
	(R)-2-Hydroxysterculic acid	$C_{19}H_{34}O_3$	11.779	310.2503
	(Z)-15-Oxo-11-eicosenoic acid	$C_{20}H_{36}O_3$	11.850	324.2666
	Phytosphingosine	$C_{18}H_{39}NO_3$	12.115	317.2930
	DGlucosyldihydrosphingosine	$C_{24}H_{49}NO_7$	12.385	463.3505
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	12.718	352.2608
	Octadecanedioic acid	$C_{18}H_{34}O_4$	12.761	314.2456
	Stearamide	$C_{18}H_{37}NO$	12.832	283.2873
	MG(0:0/18:1(11Z)/0:0)	$C_{21}H_{40}O_4$	12.922	356.2926
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	12.965	352.2614
	LysoPC(18:3(6Z,9Z,12Z))	$C_{26}H_{49}NO_7P$	13.130	518.3245
	MG(0:0/20:1(11Z)/0:0)	$C_{23}H_{44}O_4$	13.521	384.3236
	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	13.678	678.3814
	MG(0:0/18:3(6Z,9Z,12Z)/0:0)	$C_{21}H_{36}O_4$	13.837	352.2611
	22-Oxo-docosanoate	$C_{22}H_{42}O_3$	14.265	354.3126
	LysoPE(0:0/16:0)	$C_{21}H_{44}NO_7P$	14.460	453.2845
	alpha,alpha'-Trehalose 6-	$C_{28}H_{52}O_{12}$	14.988	580.3465
	Phenethyl decanoate	$C_{18}H_{28}O_2$	15.216	276.2089
	9-HOTE	$C_{18}H_{30}O_3$	15.217	294.2199
	"8, 11, 14, 17-icosatetraenoic acid;C20:4n-3,6,9,12	$C_{20}H_{32}O_2$	15.246	304.2400
Glycosides	Pelargonidin 3-(6-pcoumaroyl)glucoside	$C_{30}H_{27}O_{12}$	5.453	579.1508
	Cyanidin 3-rhamnoside	$C_{27}H_{31}O_{15}$	6.033	595.1669
	Lucuminic acid	$C_{19}H_{26}O_{12}$	6.188	446.1423
	Cyanidin 3-rhamnoside	$C_{27}H_{31}O_{15}$	6.203	595.1666
	Peonidin-3-galactoside	$C_{22}H_{22}O_{11}$	6.215	462.1162
	cis-3-Hexenyl b-primeveroside	$C_{17}H_{30}O_{10}$	6.652	394.1840
	Cyanidin 3-glucogalactoside	$C_{27}H_{31}O_{16}$	7.053	611.1625
	Linalool oxide D 3-[apiosyl-(1->6)-glucoside]	$C_{21}H_{36}O_{11}$	7.375	464.2253
	Petunidin 3-rhamnoside	$C_{28}H_{33}O_{16}$	7.543	625.1778
	Peonidin	$C_{16}H_{13}O_6$	7.549	301.0711

	Name	Formula	RT	Mass
Glycosides	Linalyl propionate	$C_{13}H_{22}O_2$	7.596	210.1623
	Isolariciresinol 9-O-beta-D-glucoside	$C_{26}H_{34}O_{11}$	7.667	522.2102
	Malvidin	$C_{17}H_{15}O_7$	7.723	331.0813
Alkaloids	N-Methylcalystegine C1	$C_8H_{15}NO_5$	0.605	205.0946
	Maculosine	$C_{17}H_{17}NO_6$	0.906	331.1055
Lignan	Isolariciresinol 9-O-beta-D-glucoside	$C_{26}H_{34}O_{11}$	6.992	522.2099
	Austrobailignan 7	$C_{20}H_{22}O_5$	15.043	342.1464
Coumarins	Coriandrone C	$C_{13}H_{10}O_5$	10.282	246.0529
	4-Hydroxy-8-methoxy-2H-furo[2,3-h]-1-benzopyran-2-one	$C_{12}H_8O_5$	13.425	232.0372
	Avocadenofuran	$C_{17}H_{28}O$	15.217	248.2142
Oligosaccharides.	((S)-Nerolidol 3-O-[a-LRhamnopyranosyl-(1->4)-a-Lrhamnopyranosyl-(1->2)-b-Dglucopyranoside]	$C_{33}H_{56}O_{14}$	12.719	676.3666
Amino Acid	Arginyl-Proline	$C_{11}H_{21}N_5O_3$	0.689	271.1647
	L-Leucine	$C_6H_{13}NO_2$	0.691	131.0942
	N(alpha)-t-Butoxycarbonyl-L-leucine	$C_{11}H_{21}NO_4$	0.729	231.1468
	N-(1-Deoxy-1-fructosyl)phenylalanine	$C_{15}H_{21}NO_7$	0.780	327.1320
	2-Carboxy-1-[5-(2-carboxy-1-yrrolidinyl)-2-hydroxy-2,4-pentadienylidene]pyrrolidinium	$C_{15}H_{21}N_2O_5$	7.826	309.1442
	NPhenylacetylphenylalanine	$C_{17}H_{17}NO_3$	7.933	283.1207
	Mucronine B	$C_{28}H_{36}N_4O_4$	13.389	492.2737
	L-Leucine	$C_6H_{13}NO_2$	0.691	131.0942
Steroids	Diosgenin 3-[glucosyl-(1- >4)-rhamnosyl-(1->4)-[rhamnosyl-(1->2)]-glucoside]	$C_{51}H_{82}O_{21}$	8.855	1030.5371
	4,4-Difluoropregn-5-ene-3,20-dione	$C_{21}H28F_2O_2$	10.383	350.2061

In case of LCMS profile of the methanolic whole plant extract of *D. oppositifolia* total 224 compounds were acquired from the LCMS data base. From that data 97compunds were identified by using pubchem, human metabolome database and KEGG database. The data revealed a wide range of metabolite, including 25 molecules from fats/lipids, 26 molecules from terpenoid, 13 molecules from glycosides, 9 molecules from phenolic, 7 molecules from flavonoids, 8 molecules from amino acids, 3 molecules from coumarins, and 2 molecules from each group of alkaloids, steroids, and lignin. (**Table No. 4.8)**

In LCMS data of the *D. pentaphylla* 316 compounds were found. From the data 132 compounds were identified including by using pubchem, human metabolome database and KEGG database. The results indicated a diverse set of metabolite including 55 molecules from fats/lipids, 19 molecules from terpenoid, 21 molecules from glycosides, 11 molecules from phenolic, 15 molecules from flavonoids, 8 molecules from steroids and 3 molecules from alkaloids. (**Table No. 4.9)** among these compounds 11 have been reported for the anti-obesity properties **Table No. 4.10**

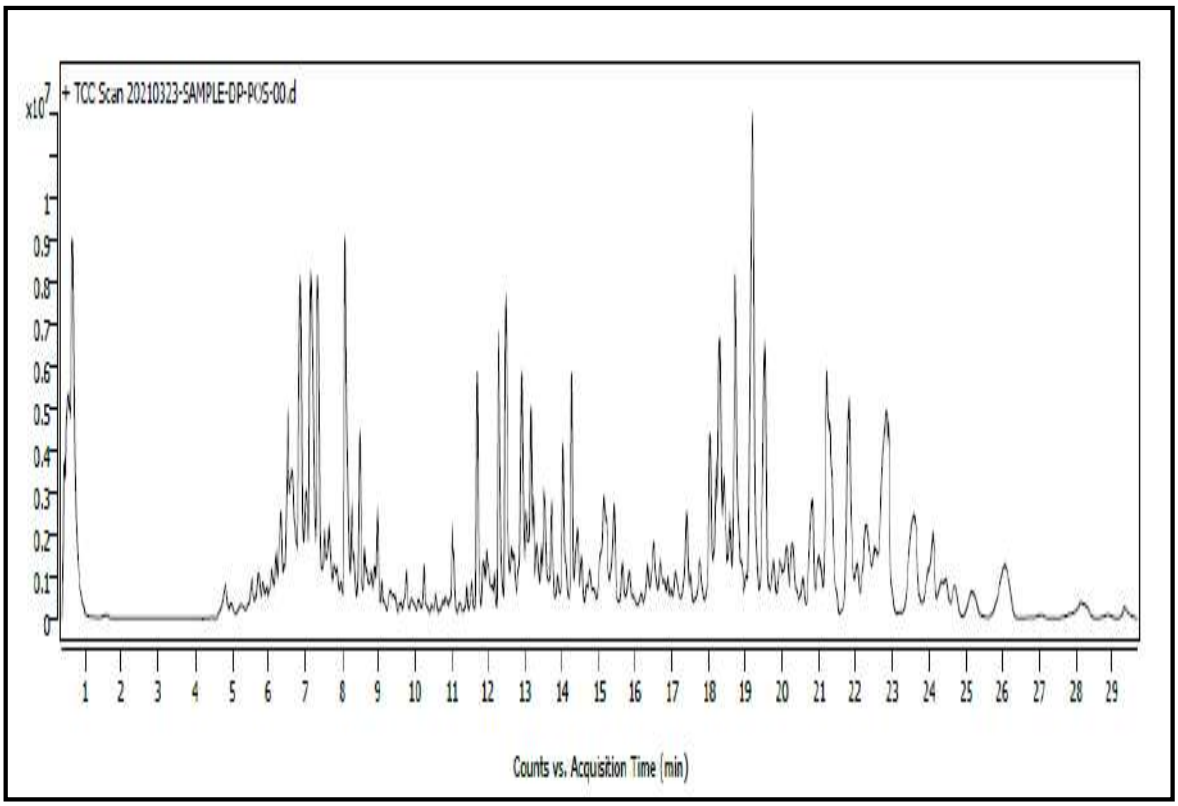


Figure no 4.19: LCMS-MS chromatogram of *D. pentaphylla* whole plant methanolic extract

Table No. 4.9: List of identified compound from *D. pentaphylla* using LSMS

	Name	Formula	RT	Mass
Phenolic	Reboxetine	$C_{19}H_{23}NO_3$	0.506	313.1679
	N-Methyltyramine	$C_9H_{13}NO$	0.685	151.0996
	Oxprenolol	$C_{15}H_{23}NO_3$	6.154	265.1675
	2,6-Dimethoxy-4-propylphenol	$C_{11}H_{16}O_3$	7.057	196.1097
	Alteichin	$C_{20}H_{14}O_6$	7.159	350.0783
	Linusitamarin	$C_{17}H_{22}O_9$	7.525	370.1266
	"3-Hydroxychavicol 1- glucoside	$C_{21}H_{30}O_{11}$	7.702	458.1788
	"1-Methoxy-3-(4- propenal 4'-glucoside"	$C_{16}H_{22}O_7$	7.95	326.1356
	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	8.689	307.2142
	Ethyl vanillin isobutyrate	$C_{13}H_{16}O_4$	14.004	236.1045
	Oryzarol	$C_{26}H_{42}O_3$	14.996	402.3139
Flavonoid	Coumestrin	$C_{21}H_{18}O_{10}$	6.528	430.0895
	Quercitrin	$C_{21}H_{20}O_{11}$	6.528	448.1001
	Rothindin	$C_{22}H_{20}O_{10}$	6.651	444.1062
	Prunitrin	$C_{22}H_{22}O_{10}$	7.119	446.1214
	6-beta-DGlucopyranosyl- 4',5- dihydroxy-3',7-dimethoxyflavone	$C_{23}H_{24}O_{11}$	7.319	476.1310
	6"-O-Acetyldaidzin	$C_{23}H_{22}O_{10}$	7.348	458.1212
	Barpisoflavone A	$C_{16}H_{12}O_6$	7.486	300.0635
	Pelargonidin 3-rhamnoside 5-glucoside	$C_{27}H_{31}O_{14}$	7.503	579.1717
	7,8,3',4'-Tetrahydroxyisoflavone	$C_{15}H_{10}O_6$	7.555	286.0478
	Molludistin 2"-rhamnoside	$C_{27}H_{30}O_{13}$	7.622	562.1698
	Coumestrin	$C_{21}H_{18}O_{10}$	7.816	430.0906
	Apimaysin	$C_{27}H_{28}O_{13}$	8.181	560.1533
	Maysin 3'-methyl ether	$C_{28}H_{30}O_{14}$	9.102	590.1646
	Grossamide	$C_{36}H_{36}N_{28}$	9.292	624.2472
	5-Hydroxyflavone	$C_{21}H_{20}O_8$	9.797	400.1159
Terpenoid	Leonuridine	$C_{15}H_{24}O_9$	7.654	348.1422
	6Z-8-Hydroxygeraniol 8- O-glucoside	$C_{16}H_{28}O_7$	7.456	332.1835
	(2S,4R,6S)-2-[2-(4- hydroxy-3-methoxyphenyl)ethyl]tetrahydro-6-(4-hydroxy-3,5-dimethoxyphenyl)- 2H-pyran-4-ol	$C_{22}H_{28}O_7$	7.641	404.1840
	Leonuridine	$C_{15}H_{24}O_9$	7.654	348.1422
	Eremopetasidione	$C_{14}H_{20}O_3$	8.990	236.1415
	2,2,4,4,-Tetramethyl-6-(1-oxopropyl)-1,3,5-cyclohexanetrione	$C_{13}H_{18}O_4$	10.117	238.1201
	(5alpha,10alpha)- 3,7(11)-Eudesmadien-2-one	$C_{15}H_{22}O$	9.362	218.1670
	Austroinulin	$C_{20}H_{34}O_3$	11.406	322.2499
	Celastrol	$C_{29}H_{38}O_4$	12.706	450.2768
	Armillaripin	$C_{24}H_{30}O_6$	12.918	414.2039
	Isomytiloxanthin	$C_{40}H_{54}O_4$	12.966	598.4028
	2-oxophytanic acid	$C_{20}H_{38}O_3$	13.349	326.2825
	Lactapiperanol D	$C_{18}H_{28}O_5$	14.002	324.1935

	Name	Formula	RT	Mass
Terpenoid	Tricycloekasantal	$C_{12}H_{18}O$	15.200	178.1356
	Erythrodiol	$C_{30}H_{50}O_2$	17.186	442.3811
	Triptohypol F	$C_{31}H_{52}O_2$	17.340	456.3967
	(ent-2b,4S,9a)-2,4,9-Trihydroxy-10(14)-oplopen-3-one 2-(2 methylbutanoate) 9-(3-methyl-2E-pentenoate)	$C_{26}H_{40}O_6$	17.519	448.2825
	6-O-Acetylaustroinulin	$C_{22}H_{36}O_4$	17.846	364.2611
	(2S,4R,6S)-2-[2-(4-hydroxy-3-methoxyphenyl) ethyl]tetrahydro-6-(4-hydroxy-3,5-dimethoxyphenyl)-2H-pyran-4-ol	$C_{22}H_{28}O_7$	7.641	404.1840
Glycosides	Mandelonitrile rutinoside	$C_{20}H_{27}N_{10}$	6.073	441.1635
	Cyanidin 3-rhamnoside	$C_{27}H_{31}O_{15}$	6.553	595.1664
	Cyanidin 3-sambubioside	$C_{26}H_{29}O_{15}$	6.240	581.1513
	Isopeonidin 3-galactoside	$C_{22}H_{23}O_{11}$	6.651	463.1241
	Netilmicin	$C_{21}H_{41}N_{57}$	6.664	475.2996
	Pelargonidin 3-rhamnoside 5-glucoside	$C_{27}H_{31}O_{14}$	6.958	579.1712
	Cyanidin 3-Cyanidin 3-glucogalactoside	$C_{27}H_{31}O_{16}$	6.978	611.1621
	Luteolinidin 3-Oglucoside	$C_{21}H_{21}O_{10}$	7.004	433.1137
	Prunitrin	$C_{22}H_{22}O_{10}$	7.119	446.1214
	Peonidin 3-rhamnoside	$C_{22}H_{23}O_{10}$	7.158	447.1280
	Linalool oxide D 3-glucoside]	$C_{21}H_{36}O_{11}$	7.307	464.2255
	6-beta-DGlucopyranosyl-4',5-dihydroxy-3',7-dimethoxyflavone	$C_{23}H_{24}O_{11}$	7.319	476.1310
	6''-O-Acetyldaidzin	$C_{23}H_{22}O_{10}$	7.348	458.1212
	6Z-8-Hydroxygeraniol 8-O-glucoside	$C_{16}H_{28}O_7$	7.456	332.1835
	Linusitamarin	$C_{17}H_{22}O_9$	7.525	370.1266
	Eriojaposide A	$C_{24}H_{38}O_{11}$	7.574	502.2419
	Malvidin	$C_{17}H_{15}O_7$	7.665	331.0817
	3-Hydroxychavicol 1-glucoside]	$C_{21}H_{30}O_{11}$	7.702	458.1788
	1-Methoxy-3-(4-propenal 4'-glucoside	$C_{16}H_{22}O_7$	7.950	326.1356
	Piceatannol 4'-galloylglucoside	$C_{27}H_{26}O_{13}$	8.906	558.1384
	(3b,16b,20R)-Pregn-5-ene-3,16,20-triol 3-glucoside	$C_{27}H_{44}O_8$	14.036	496.3031
Steroid	Polypodoside A	$C_{45}H_{72}O_{17}$	15.087	884.4781
	6-Azaequilenin+AN3:AS9	$C_{17}H_{17}NO_2$	9.968	267.1258
	3-Deoxyestradiol	$C_{18}H_{24}O$	12.111	256.1826
	3beta-(1-Pyrrolidinyl)-5alpha-pregnane-11,20-dione	$C_{25}H_{39}NO_2$	12.550	385.2988
	Terminaline	$C_{23}H_{41}NO_2$	13.296	363.3139
	Ophiopogonin C'	$C_{39}H_{62}O_{12}$	16.662	722.4252
	Lycoperoside D	$C_{39}H_{65}N_{12}$	16.662	739.4513
	26-Hydroxybrassinolide	$C_{28}H_{48}O_7$	16.720	496.3401
Alkaloid	Muricinine	$C_{18}H_{19}NO_4$	4.975	313.1318
	Hydrocodone	$C_{18}H_{21}NO_3$	5.556	299.1520
	Desacetylcolchicine	$C_{20}H_{23}NO_5$	8.916	357.1579

	Name	Formula	RT	Mass
	Malonylcarnitine	$C_{10}H_{18}NO_6$	0.567	248.1135
	Aminocaproic acid	$C_6H_{13}NO_2$	0.681	131.0947
	Ginkgolide C	$C_{20}H_{24}O_{11}$	7.221	440.1327
	Corchorifatty acid F	$C_{18}H_{32}O_5$	9.753	328.2254
	"9,10-Dihydroxy-12,13-epoxyoctadecanoate"	$C_{18}H_{34}O_5$	10.237	330.2409
	C16 Sphinganine	$C_{16}H_{35}NO_2$	10.832	273.2668
	"9,10,13-Trihydroxystearic acid"	$C_{18}H_{36}O_5$	10.936	332.2561
	MG(0:0/15:0/0:0)	$C_{18}H_{36}O_4$	11.530	316.2613
	"3-Methyl-5-pentyl-2-furanundecanoic acid"	$C_{21}H_{36}O_3$	11.557	336.2667
	"2-methoxy-hexadecanoic acid"	$C_{17}H_{34}O_3$	11.683	286.2509
	"10,20-Dihydroxyeicosanoic acid"	$C_{20}H_{40}O_4$	11.852	344.2926
	Phytosphingosine	$C_{18}H_{39}NO_3$	11.858	317.2928
	"(Z)-15-Oxo-11-eicosenoic acid"	$C_{20}H_{36}O_3$	12.000	324.2665
	10Z-nonadecenoic acid	$C_{19}H_{36}O_2$	12.466	296.2713
	"D Glucosyldihydrosphingosine"	$C_{24}H_{49}NO_7$	12.580	463.3509
	"Polyoxyethylene 40 monostearate"	$C_{20}H_{40}O_3$	12.634	328.2972
	Methyl linoleate	$C_{19}H_{34}O_2$	12.806	294.2556
	"Polyoxyethylene (600) mono- ricinoleate"	$C_{21}H_{40}O_3$	12.872	340.2973
	MG(18:0/0:0/0:0)	$C_{21}H_{42}O_4$	12.900	358.3079
Lipids	"MG(0:0/18:3(6Z,9Z,12Z)/0:0)"	$C_{21}H_{36}O_4$	12.901	352.2609
	Stearamide	$C_{18}H37NO$	13.015	283.2874
	Docosatetraenoyl	$C_{24}H_{41}NO_2$	13.047	375.3140
	"MG(0:0/18:3(6Z,9Z,12Z)/0:0)"	$C_{21}H_{36}O_4$	13.152	352.2610
	"(Z)-13-Oxo-9-octadecenoic acid"	$C_{18}H_{32}O_3$	13.155	296.2350
	LysoPC(18:3(6Z,9Z,12Z))	$C_{26}H_{49}NO_7$	13.315	518.3250
	Stearoylethanolamide	$C_{20}H_{41}NO_2$	13.367	327.3139
	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	13.609	678.3838
	LysoPC(16:0)	$C_{24}H_{51}NO_7$	15.056	496.3412
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	14.037	352.2610
	LysoPE(0:0/18:2(9Z,12Z)	$C_{23}H_{44}NO_7$	14.082	477.2861
	LysoPC(18:2(9Z,12Z))	$C_{26}H_{51}NO_7$	14.124	520.3404
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	14.269	352.2606
	LysoPC(18:2(9Z,12Z))	$C_{26}H_{51}NO_7$	14.383	520.3406
	22-Oxo-docosanoate	$C_{22}H_{42}O_3$	14.462	354.3137
	LysoPE(0:0/16:0)	$C_{21}H_{44}NO_7$	14.666	453.2860
	omega-hydroxy behenic	$C_{22}H_{44}O_3$	14.771	356.3291
	7-Oxostigmasterol	$C_{29}H_{46}O_2$	14.934	426.3501
	Phenethyl decanoate	$C_{18}H_{28}O_2$	15.418	276.2088
	9-HOTE	$C_{18}H_{30}O_3$	15.419	294.2197
	8, 11, 14, 17-icosatetraenoic acid;C20:4n3,6,9,12	$C_{20}H_{32}O_2$	15.447	304.2404

	Name	Formula	RT	Mass
	LysoPC(18:1(11Z))	$C_{26}H_{53}NO_7$	15.462	522.3564
	(22Alpha)-hydroxycampest-4-en-3-one	$C_{28}H_{46}O_2$	15.950	414.3496
	Tridecyl phloretate	$C_{22}H_{36}O_3$	16.434	348.2665
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	16.556	352.2608
	10-Hydroxy-2,8-decadiene-4,6-diynoicacid	$C_{10}H_8O_3$	17.088	176.0474
	Oleoyl Ethanolamide	$C_{20}H_{39}NO_2$	17.767	325.2975
	Docosatrienoic Acid	$C_{22}H_{38}O_2$	17.787	334.2867
Lipids	10-Hydroxy-2,8-decadiene-4,6-diynoicacid	$C_{10}H_8O_3$	18.000	176.0473
	2-Hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinol	$C_{38}H_{58}O_3$	18.098	562.4386
	MG(0:0/22:1(13Z)/0:0)	$C_{25}H_{48}O_4$	18.447	412.3549
	MG(0:0/16:0/0:0)	$C_{19}H_{38}O_4$	18.572	330.2770
	13Z,16Z-docosadienoic	$C_{22}H_{40}O_2$	18.656	336.3028
	Soyacerebroside I	$C_{40}H_{75}NO_9$	22.766	713.5432
	10-hydroperoxy-8E,12Zoctadecadienoicacid	$C_{18}H_{32}O_4$	9.332	312.2297
	10-Hydroxy-2,8-decadiene-4,6-diynoicacid	$C_{10}H_8O_3$	8.491	176.0474

The LCMS data provided insight into the phytochemical distribution in the extracts. When compared to previously reported data, the 11 compounds identified in the data exhibit anti-obesity action **Table No. 4.10.**

Rahim *et al.,* (2015) studied the lipase inhibition effect of the Apigenin 6-C-glucoside (IC_{50} was 5.28E+11 µM) and suggested this compound can be used to treat obesity. Song *et al.,* (2021) studied the anti-obesity effect of the *Vaccinium bracteatum* Thunb. fruit extract on high fat diet obese mice and found that extract reduces the body weight of mice. The phytochemicals analysis of the plant extracts found that Apigenin 6-C-glucoside one of the compounds that inhibit the pancreatic lipase.

The effect of delphinidin 3-O-beta-D-glucoside (D3G) was studied on the 3T3-L1 cells. D3G inhibit the lipid accumulation in the cells in doses dependent manner. D3G down regulate the expression of the PPARϒ, C/EBPα, SREBP-1, and FASN genes and up regulate the expression of the CTP-1 gene in the 3T3-L1 cells. D3G inhibits adipogenesis and enhances lipid metabolism through activating AMPK-mediated signaling, and hence has the potential to play a therapeutic role in the management and treatment of obesity. (Park *et al.,* 2019)

Yamamoto and Oue (2006) reported the effect of quercetin in rats fed with a high-fat high-sucrose diet they observed that quercetin reduce fat accumulation in 3T3-L1 preadipocytes by down regulating the PPARϒ.

Table No. 4.10: List of anti-obesity compounds from studied *Dioscorea* species.

Sr. No.	Compound name	Molecular Formula	RT	Mass	References
1	Apigenin 6-C-glucoside	$C_{26}H_{28}O_{14}$	6.595	564.1487	Rahim *et al.*, (2015) Song *et al.*, (2021)
2	Delphinidin 3-O-beta-D-glucoside	$C_{22}H_{23}O_{12}$	6.743	465.4121	Park *et al.*, (2019)
3	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	7.156	464.0952	Yamamoto and Oue (2006) Abdul *et al.*, (2017)
4	(-)-trans-Carveol	$C_{10}H_{16}O$	7.375	152.1200	Kazemipoor *et al.*, (2013) Ali-Shtayeh *et al.*, (2019)
5	Peonidin	$C_{16}H_{13}O_6$	7.549	301.0711	Sari *et al.*, (2020)
6	Octyl gallate/ gallic acid	$C_{15}H_{22}O_5$	8.557	282.1464	Totani *et al.*, (2011) Song *et al.*, (2020)
7	Ganoderic acid A	$C_{30}H_{42}O_7$	8.590	514.2936	Zhu *et al.*, (2018) Sharma *et al.*, (2019)
8	Diosgenin	$C_{51}H_{82}O_{21}$	8.855	1030.5371	Son *et al.*, (2007) Uemura *et al.*, (2010) Wang *et al.*, (2015) Hua *et al.*, (2016)
9	Zerumbone	$C_{15}H_{22}O$	9.354	218.1668	Tzeng *et al.*, (2014) Ahn (2017)
10	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	13.678	678.3814	Ilavenil *et al.*, (2016)
11	Oleoyl Ethanolamide	$C_{20}H_{39}NO_2$	17.596	325.2975	Romano *et al.*, (2014)

Chemical structures of all compounds represented in **(Plate 8-9).**

A study conducted by Kazemipoor *et al.*, (2013) reported that the effect of *Carum carvi* L. on overweight and obese women as clinical trial they treat the obese women with the aqueous 10% seed extract for the 90 days with their diet the observed that the significant weight loss. In the GCMS analysis of the extracts found the Trans Carveol as one compound. Trans-Carveol from the mentha has lipase inhibition activity Ali-Shtayeh *et al.,* (2019).

Sari *et al.,* (2020) did the virtual screening of anthocyanins from black rice for anti-obesity activity by targeting the TLR4 and JNK pathways. According to the findings of this study, cyanidin, peonidin, cyanidin-3-O-glucoside, and peonidin-3-O-glucoside directly inhibited TLR4 and JNK proteins at their critical regions. All black rice anthocyanins may have anti-obesity activity.

In 2011 Totani *et al.,* reported the effect of gallic acid ester and octyl gallate on the obese rats which are feed with 7% frying oil for the 12 weeks. In observation the octyl gallate and ester from the gallic acid were effectively reduces the weight in the rat.

The *Diospyros kaki* extract inhibits the development of 3T3-L1 pre-adipocyte cells into mature adipocytes. Gallic acid is a significant bioactive component of extract that Inhibit Fatty Acid Synthesis in 3T3-L1 Cells by activating AMPK. The observed that the fermented *Diospyros kaki* extract effect on metabolic parameters of mice feed with high-fat diet (HFD). Fermented extracts supplementation resulted in a 15% reduction in body weight also decreased abdominal and liver fat, and decreased blood levels of triglycerides, total cholesterol, and glucose Song *et al.,* (2020).

Zhu *et al.,* (2018) studied the effect of ganoderic acid A on the high fat diet induce mice and 3T3-L1 adipocytes. ganoderic acid A (GAA) inhibits SREBP expression and lowers cellular levels of cholesterol and fatty acids. Also reduces body weight gain and fat accumulation in the liver or adipose tissues, as well as improving serum lipid levels and insulin sensitivity in obese mice. GAA has the potential to be a leading compound in the development of drugs for the prevention of obesity and insulin resistance.

Fatty acid synthase is the key enzyme involved in the DE novo synthesis of the fatty acids in adipocytes and liver tissue. Kaushal *et al.,* (2019) did the bioinformatics study on the

interaction of the ganoderic acid and fatty acid synthase gene. The inhibition of the fatty acid synthase helps in the controlling the obesity.

Wang *et al.,* (2015) studied the effect of diosgenin on the 3T3-L1 cells and high fat diet induced mice. When treat the 3T3-L1 cells and mice with the diosgenin the PPARϒ gene was suppressed in both cases. They concluded that in response to diosgenin stimulation, ERβ interacted with RXR α and detached RXRα from PPARϒ resulting in a decrease in transcriptional activity. The study shows that ER-β mediated control of PPAR expression and activity is essential for diosgenin-inhibited adipocyte differentiation.

Son *et.al.* (2007) examined the effect of the diosgenin on the high fat diet induced rat which is feed with 0.1% to 0.5% diosgenin for 6 weeks. The treatment with diosgenin found reduction in the total cholesterol level in plasma and liver. From this study they conclude that diosgenin can be used for the treatment of the hypercholesterolemia.

In pregnant diabetic mice, Diosgenin reduced the expression of sterol regulatory element-binding transcription factor-1 (SREBP-1) and its target genes, including fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1) and acetyl coenzyme A carboxylase (ACC). Furthermore, overexpression of SREBP-1 by LV-SREBP-1 injection might significantly reduce diosgenin protective impact against disorders of glucose and lipid metabolism as well as oxidative stress in diabetic mice. The findings suggest that SREBP-1 is a significant target of diosgenin that mediates its anti-diabetic effects in diabetic mice. Hua *et.al,* (2016).

Uemura *et al.,* (2010) studied the effect of the *Trigonella foenum-graecum* (fenugreek) extract on the high fat induced the rat the examined the hepatic tissue for the lipid accumulation the fond reduction of the lipid accumulation in the liver tissue. Further they did the fractionation of the extracts and again did the same experiment. In this study they found fraction containing diosgenin inhibiting the lipid accumulation in the hepatic tissue.

Lipid- reducing effects of zerumbone was investigated by the Tzeng *et al.,* (2014) on high-fat diet-induced hyperlipidemic hamsters. Zerumbone is a natural cyclic sesquiterpene of *Zingiber zerumbet* Smith, in the experiment hamster feed with the high fat diet for two weeks. Then apply a dose of the zerumbone in different concentrations for 8 weeks. They

observed that the zerumbone decreases in plasma levels of TC, TG, and LDL-C, as well as hepatic lipid concentrations, were seen, with an increase in faucal lipids occurring simultaneously. Zerumbone down regulate the gene expression of fatty acid synthase, malic enzyme, sterol-regulatory element binding protein, and 3-hydroxy-3-methyl-glutaryl-CoA reductase in the liver.

The effect of the zerumbone on the high fat diet induced mice and gene regulatory effect on 3T3-L1 cells. Zerumbone dysregulated lipid metabolism in the white adipose tissues of mice. In 3T3-L1 induced AMPK activation and phosphorylation of acetyl-CoA carboxylase, and effectively decreased the adipogenesis. The administration of zerumbone was shown to efficiently reverse the significant increase of microRNA-146b. In zerumbone-treated differentiated adipocytes, the levels of SIRT1, a direct target of microRNA-146b, was increased Ahn *et al.*, (2017)

Abdul *et al.,* (2017) studied the antilipase activity of the eight herbs. From the studied plant *Cosmos caudatus* was used for the farther study. Ethanolic extracts of *Cosmos caudatus* was used for metabolite profiling by UHPLC-MS/MS. The presence of quercetin-3-rhamnoside, catechin, kaempherol, kaempherol glucoside, quercetin, quercetin-3-glucoside, quercetin-O-pentoside, quercetinrhamnosyl galactoside, quinic acid, 1-caffeyolquinic acid, monogalloyl glucose, and procyanidin B1 was found in the extracts. *Cosmos caudatus* was discovered to be a promising therapeutic plant for the creation of novel functional foods with enormous applications in obesity. lavenil *et al.*, (2016) examined the anti adipogenic effect of *Chlorella vulgaris.* Ethanolic extract inhibit the adipogenic expression. They did the LCMS analysis of the extracts in that Cecropiacic acid, briarellin-A, Platycodigenin, Martiriol, Ergost-7-ene-2,3,5,6,9,11,19- heptol, Gingerglycolipid-A, gingerglycolipid-B, gingerglycolipid-C are the major compound.

According to the behavioral satiety sequence paradigm, mice given identical dosages (5 or 10 mg/kg, i.p.) of Oleoyl Ethanolamide or rimonabant were studied for the gradual expression of spontaneous behaviors (feeding, grooming, rearing, locomotion, and resting) throughout the onset of satiety (BSS). Both medications lowered food intake (wet mash) to a similar level. The OEA therapy reduced eating activity within the first 30 minutes and induced a brief increase in resting time, which was not followed by a decrease in horizontal, vertical,

or total motor activity. Rimonabant, in addition to reduced eating activity, increased grooming time and decreased horizontal motor activity, changes that might be suggestive of adverse no motivational effects on feeding. These finding suggest that OEA reduces appetite by increasing fullness, and that its profile of action may indicate safer effects in humans as a potential anti-obesity medication. (Romano *et al.*, 2014)

These 11 chemicals discovered in the LCMS profiles of *D. pentaphylla* and *D. oppositifolia* provide evidence that this plant contains anti-obesity metabolites that work at the levels of lipase inhibition, adipogenic gene regulation, and appetite suppression.

4.4 Evaluation of anti-obesity activity of extracted compounds using cell line (3T3-L1 pro adipocytes):

4.4.1 Fractionation of the *D. oppositifolia* methanolic extract

In the gene expression study *D. oppositifolia* methanolic extract down regulated adipogenic genes and up regulated the expression of the CPT-1 gene. From these results the *D. oppositifolia* carry forward for the fractionation. Fractions were prepared by using the different solvent like Hexane, ethyl acetate, chloroform, n- Butanol and water. After fractionation the yield of each fraction was calculated. **(Table No. 4.11)** residue was diluted in DMSO for the required concentration.

4.4.2 Extraction of diosgenin from *D. oppositifolia* root

78 mg of crude diosgenin was recovered from 5 gm of *D. oppositifolia* dry root powder (Table no 4.11). After diosgenin extraction, the dry residue was weighed and diluted in methanol to make a 10 mg/ml stock.

Table No. 4.11: Amount of the crude extract powder after fractionation

Sr. No.	Plant Name	Residue in mg
1	Hexane fraction	300
2	Chloroform fraction	90
3	Ethyl acetate fraction	200
4	n- Butanol fraction	148
5	Remaining water fraction	247
6	Extracted Diosgenin	78

4.4.3 Cell viability assay:

The cell viability of all the fractions was carried out for the determination of the IC_{50} value. Form the IC_{50} value treatment concentration was decided for the differentiation assay. The toxicity of the fraction was investigated using the MTT test at various doses (6.25-100 µg/ml). All fractions were shown to be moderately toxic to cells at higher concentrations *i.e.*, 100µg/ml at the tested doses. The IC_{50} values mentioned in **Table No. 4.12, Figure No. 4.15** presenting the % viability of the 3T3-L1 cells at different tested concentrations.

Table No. 4.12: IC_{50} values of the fractions

Sr. No	Fraction Name	IC_{50}
1	Hexane fraction	58.97 µg/ml
2	Chloroform fraction	60.60 µg/ml
3	Ethyl acetate fraction	70.31 µg/ml
4	n- Butanol fraction	65.89 µg/ml
5	Remaining water fraction	66.88 µg/ml
6	Extracted Diosgenin	62.89 µg/ml
7	Std. Diosgenin	75.51 µg/ml

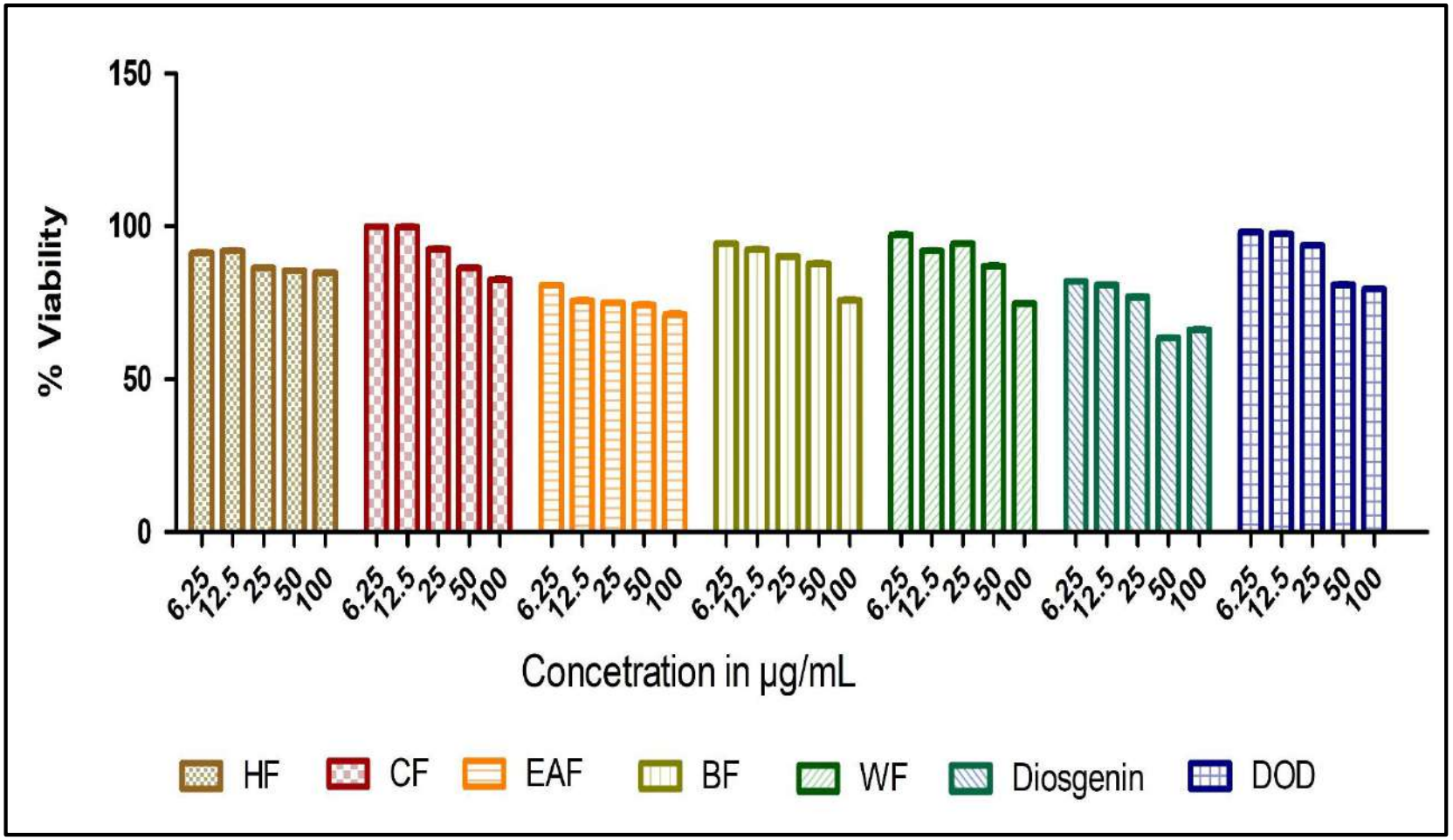


Figure No. 4.20 : Cell viabilty test by MTT assay cells treated with different fractions and extracted Diosgenin and Standerd Diosgenin (6.25 to 100 µg/ml concentrations) (HF: hexane fraction, CF: Chloroform fraction, EAF: ethyl acetate fraction, BF: n-butanolic fraction and WF: water fraction, SD: Standard Diosgenin, DOD: *D. oppositifolia* Diosgenin)

4.4.4 Effect of *D. oppositifolia* methanolic extract fractions on inhibition of lipid droplet accumulation in 3T3-L1 cell line:

The Lipid quantification was carried out, on the eighth day of the experiment. The decrease in lipid deposition was measured by comparing the cells that had been stimulated with a hormone cocktail. Cells treated with a hormone cocktail had 2.65 times increase in lipid content as compared to non-induced cells. In comparison with control group the lipid accumulation was highly significant (*P<0.001*) for induced group whereas in other tested groups the lipid accumulation was significantly decreased (*P<0.001*) in comparison with induced group. Among the tested 5 fractions and extracted diosgenin from the *D. oppositifolia,* the lipid deposition in cells treated with extracted Diosgenin from the *D. oppositifolia* and Standard diosgenin were found to reduce by 2.4 and 2.2 times, respectively in comparison with induced group. Furthermore, the cells treated with different fractions (hexane fraction, Chloroform fraction, ethyl acetate fraction, n-butanolic fraction and water fraction) of *D. oppositifolia* have not shown significant decrease in the lipid accumulation in the differentiated 3T3- L1 cells when comparing with induced group. In this chloroform fraction inhibit lipid accumulation in the cells by 1.1 times as compere to the induced cells. . The cells treated with all the fractions did not show significant difference in lipid accumulation, so all the fraction were selected for the gene expression study. (**Plate No. 10)**

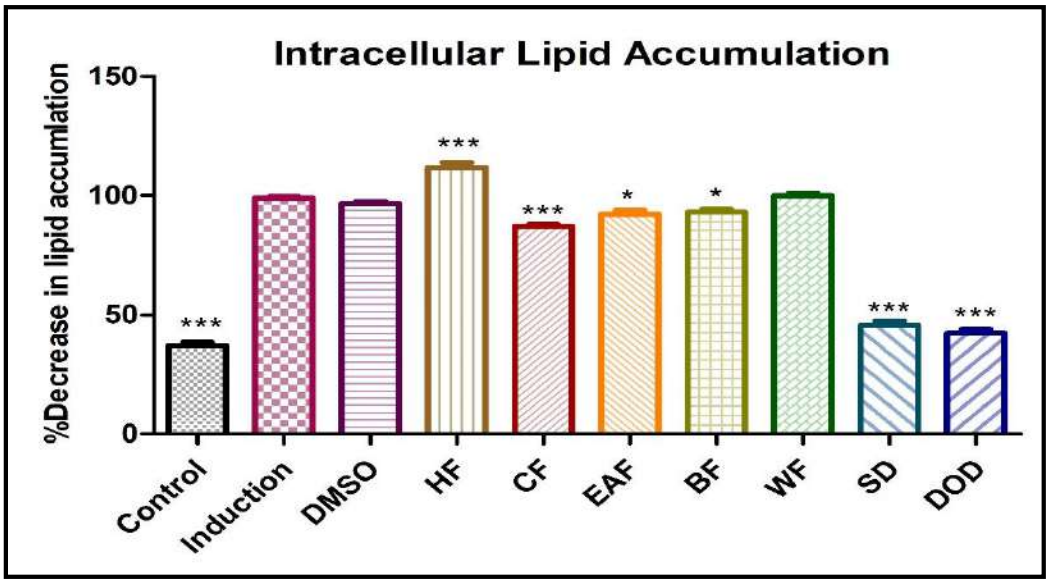


Figure No. 4.21: Intracellular lipid accumulation in 3T3-L1 cells by isopropanol extraction method ****P*< 0.001 and **P*< 0.001 as compare to induction and control using one way ANOVA followed by tukyes test. (HF: hexane fraction, CF: Chloroform fraction, EAF: ethyl acetate fraction, BF: n-butanolic fraction and WF: water fraction, SD: Standard Diosgenin, DOD: *D. oppositifolia* Diosgenin)

4.4.5. Effect of different fractions on mRNA expression of adipogenic genes:

According to the results of the oil red staining, no significant alterations were seen in cells after treatment with all fractions. For that, the influence of all the fractions explored by gene expression studies.

A. Effect of hexane fraction:

The effect of hexane fraction on adipogenic gene regulation is shown in **Figure No. 4.22,** where the expression of the PPARɣ, C/EBPα, SREBP- F1, and FASN genes is compared to the control. Up regulation of the PPARɣ, C/EBPα, SREBP-F1, and FASN genes were seen in cells treated with the hexane fraction at both doses. At 50 µg/ml concentration, PPARɣ and C/EBPα were up regulated ≈35 times and ≈8.7 times, respectively, compared to the control **(Figure No. 4.22 A, B).** SREBP F1 and FASN expression were also up to ≈4.4 and ≈5.6 times higher than in control, respectively **(Figure No. 4.22 C, D).** In the case of CPT-1 gene expression, the hexane fraction inhibits the gene at both doses; at 25 µg/ml, 98% down regulation was seen, and at 50 µg/ml, 53% down regulation was detected as compared to control cells **(Figure No. 4.22 E).**

B. Effect of chloroform fraction:

The adipogenic genes down regulated during the differentiation of 3T3-L1 cells when cells were treated with chloroform fraction. The gene expression of PPARɣ, C/EBPα, and SREBP-F1 were suppressed at both treated concentrations. The chloroform fraction reduced PPAR expression by up to 42% and 72%, respectively **(Figure No. 4.23 A).** The C/EBPα gene was suppressed by up to 74% and 62% at 25µg/ml and 50 µg/ml as compared to the control **(Figure No. 4.23 B).** In the case of 50 µg/ml the expression was slightly increased as compared to the cells treated with the 25 µg/ml concentration. The lower concentration gave better suppression of the C/EBPα gene. The expression of SREBP-F1 was reduced as compared to the control. An increase in the concentration of the chloroform fraction decreases the expression of the gene. Down regulation of the SREBP-F1 gene was observed at 25 µg/ml (7%), and 50 µg/ml (46%) **(Figure No. 4.23 C)**. The fatty acid synthase gene was up regulated at the lower concentration of the chloroform fraction almost 3.1 times at 25µg/ml and the higher 50 µg/ml concentration of the chloroform fraction slightly reduces the FASN gene expression up to 23% **(Figure No. 4.23 D)**. In the case of the CPT-1 gene, up regulation was

observed. At 25 µg /ml and 50 µg /ml, chloroform fraction increases CPT-1 expression by 1.8 and 1.9 times, respectively **(Figure No. 4.23 E).** Over all, the chloroform fraction contains some compounds that can down regulate the adipogenic genes PPARɣ, C/EBPα, and SREBP-F1. While it also contains compounds that stimulate the genes, like CPT-1, which helps to increase the lipolysis activity.

C. Effect of ethyl acetate fraction:

The effect of the ethyl acetate fraction on adipogenesis was examined, and diverse outcomes on adipogenic gene expression were found. The PPARɣ gene was up regulated by the ethyl acetate fraction, which enhanced PPARɣ gene expression about 40 times over the control at 25 µg/ml, and 7 folds over the control at 50 µg/ml **(Figure No. 4.24 A)**. While the expression of the C/EBPα gene was increased by 3 folds at 25 µg/ml compared to the control. However, treatment with a greater dose of 50µg/ml reduces up to 96 % when compared to the control **(Figure No. 4.24 B).** SREBP-F1 was down regulated at both ethyl acetate fraction dosages. Cells treated with 25 µg/ml and 50 µg/ml concentrations reveal 81 and 85 % down regulation of SREBP, respectively **(Figure No. 4.24 C).** The 37% down regulation of fatty acid synthase was shown with a lower dosage of ethyl acetate fraction. The higher dose up-regulates expression 8.6 times more than the control **(Figure No. 4.24 D).** The CPT-1 gene is up regulated 43 times at 25 µg/ml, whereas only 3.5 percent is down regulated at 50 µg/ml **(Figure No. 4.24 E).**

D. Effect of n-butanol fraction:

The expression of adipogenic genes is increased in cells treated with the n-butanolic fraction. The PPARɣ was up-regulated by 3.1 times at 25 µg/ml and 19.9 times at 50 µg/ml when the concentration was raised. The PPARɣ was shown to be up regulated **(Figure No. 4.25 A).** The 25 µg/ml butanolic fraction increases C/EBPα expression 7-fold, but high dosages reduce C/EBPα expression by up to 85 % **(Figure No. 4.25 B).** The butanolic fraction at 25 µg/ml increased SREBP-F1 expression. A tenfold increase was seen, but at 50 µg/ml, a fivefold increase in gene expression was recorded when compared to the control. In this case, a rise in concentration leads to a reduction in SREBP-1 expression **(Figure No. 4.25 C).** At 25 µg/ml and 50 µg/ml, respectively, the butanolic fraction elevated FASN about 199 times and 134 times higher than the control **(Figure No. 4.25 D).** The expression of the CPT-1 gene

increased in a concentration-dependent manner. CPT-1 gene expression was enhanced by 4.2 and 7.8 times at 25 and 50 µg/ml, respectively, as compared to the control **(Figure No. 4.25 E).**

E. Effect of water fraction:

The residual water fraction was also examined for gene expression after separation to determine the influence of the water-soluble chemical on gene expression. The PPARɣ was up regulated in a concentration dependent manner, with a 17-fold and 52-fold increase in gene expression seen at 25 and 50 µg/ml, respectively **(Figure No. 4.26 A).** C/EBPα exhibits a considerable up regulation of expression at both tested doses of 25 µg/ml 2.4 times and 50 µg/ml 1.6 times increase **(Figure No.4.26 B).** However, at 50 µg/ml, the expression of SREBP -F1 was decreased compared to the lower dosage of the water fraction, with only 6.5 times increase in gene expression detected **(Figure No. 4.26 C).** The treatment with the water fraction lowers FASN gene expression. As the concentration rises, so does the expression of the FASN gene 8.8 percent down regulation was observed at 25 µg/ml. The water fraction down regulated up to 70% of the FASN gene at 50 µg/ml **(Figure No. 4.26 D).** At 25 µg/ml, water fraction up regulated the CPT-1 gene 200 times more than the control **(Figure No. 4.26 E).** This increased expression of the CPT-1 gene assists in increasing the rate of the lipolysis process, which supports in the reduction of stored fat in the cells.

While comparing the effects of each fraction, the chloroform fraction inhibits the expression of all examined adipogenic genes more than the other four fractions. It also stimulated the activity of the CPT-1 gene. The PPARɣ, C/EBPα, SREBP-F1, and FASN genes are up regulated by hexane fraction, and down regulates the CPT-1 gene. Treatment with the Ethyl acetate fraction raises PPARɣ, C/EBPα, and FASN levels. SREBP-F1 gene expression was lower than in the control group. The CPT-1 is down regulated at greater concentrations of ethyl acetate and up regulated at lower concentrations. At the studied concentrations, cells treated with n-butanol and water fraction up regulated all adipogenic genes. At greater concentrations of the n-butanol fraction, C/EBPα was down regulated.

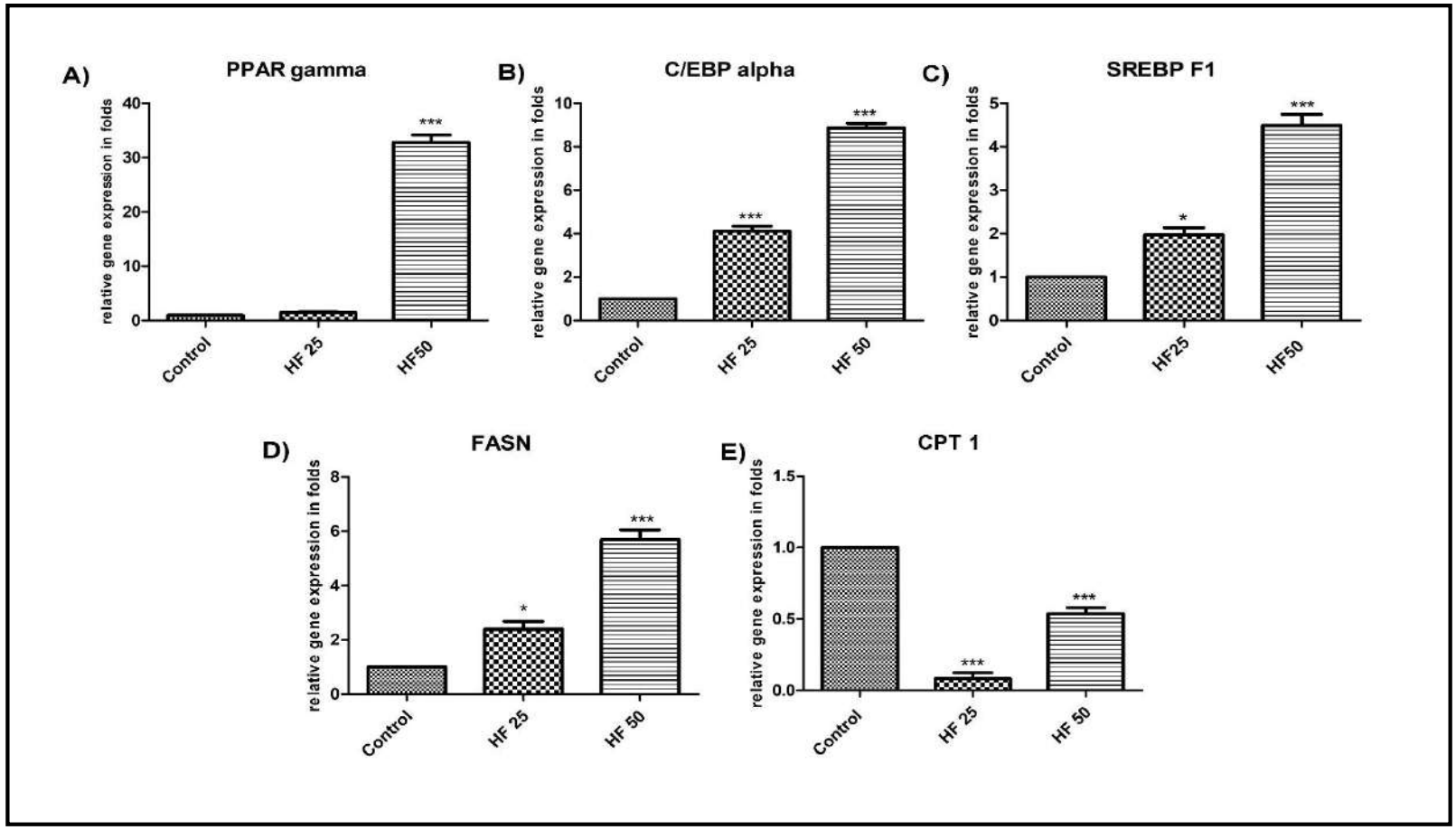


Figure No. 4.22: Effect of hexane fraction (HF) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

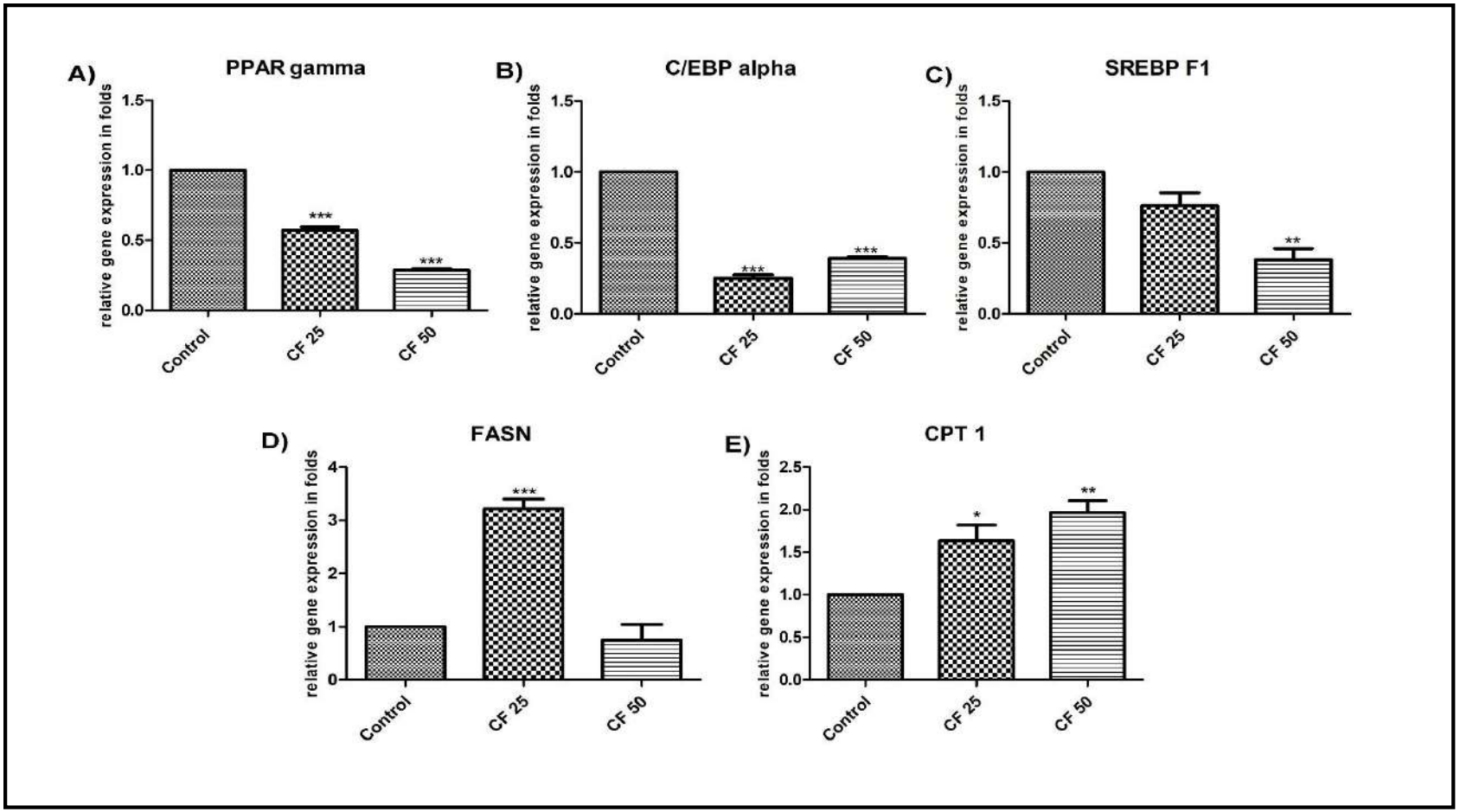


Figure No. 4.23: Effect of Chloroform fraction (CF) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

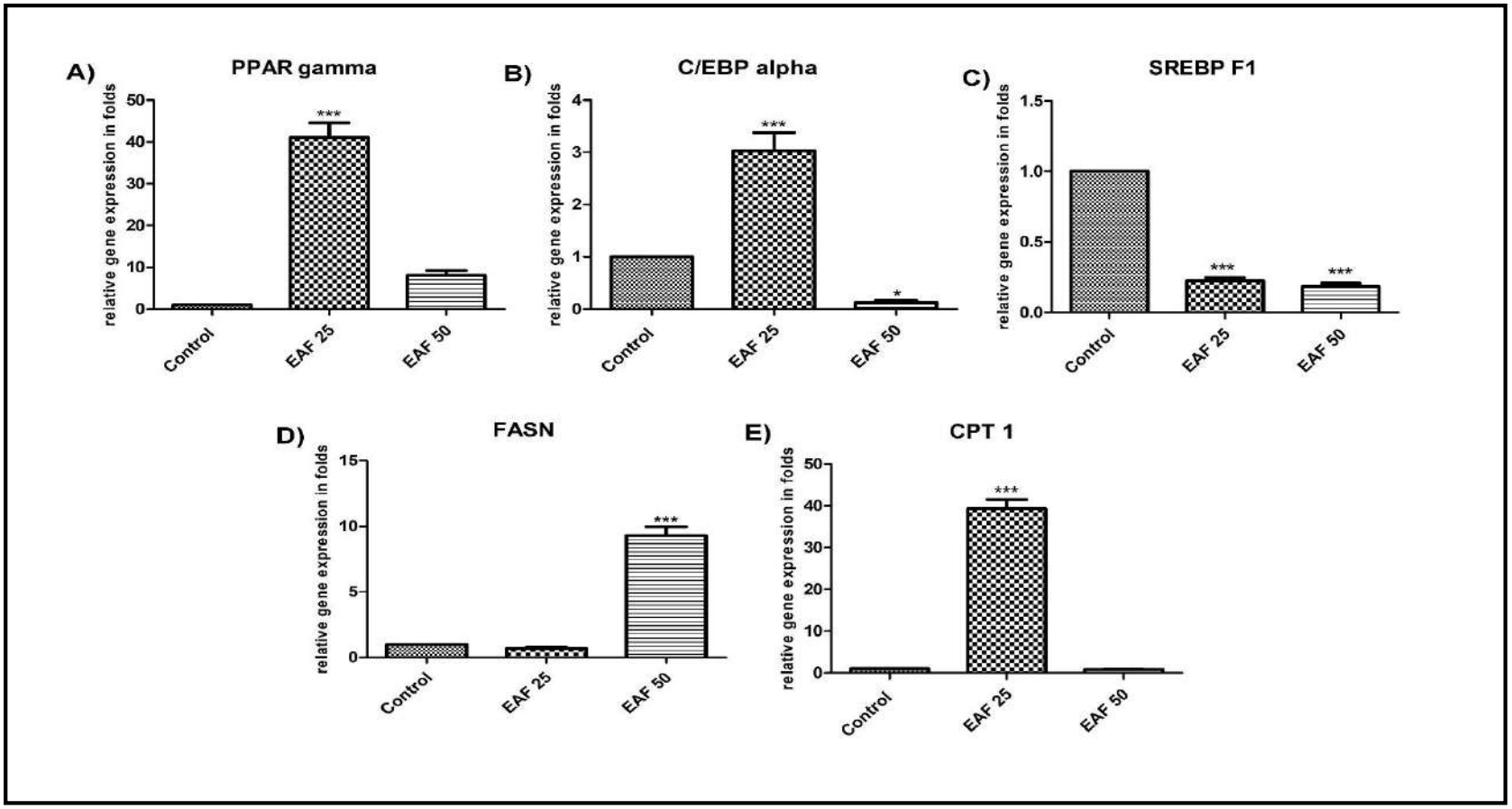


Figure No. 4.24: Effect of ethyl acetate fraction (EAF) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

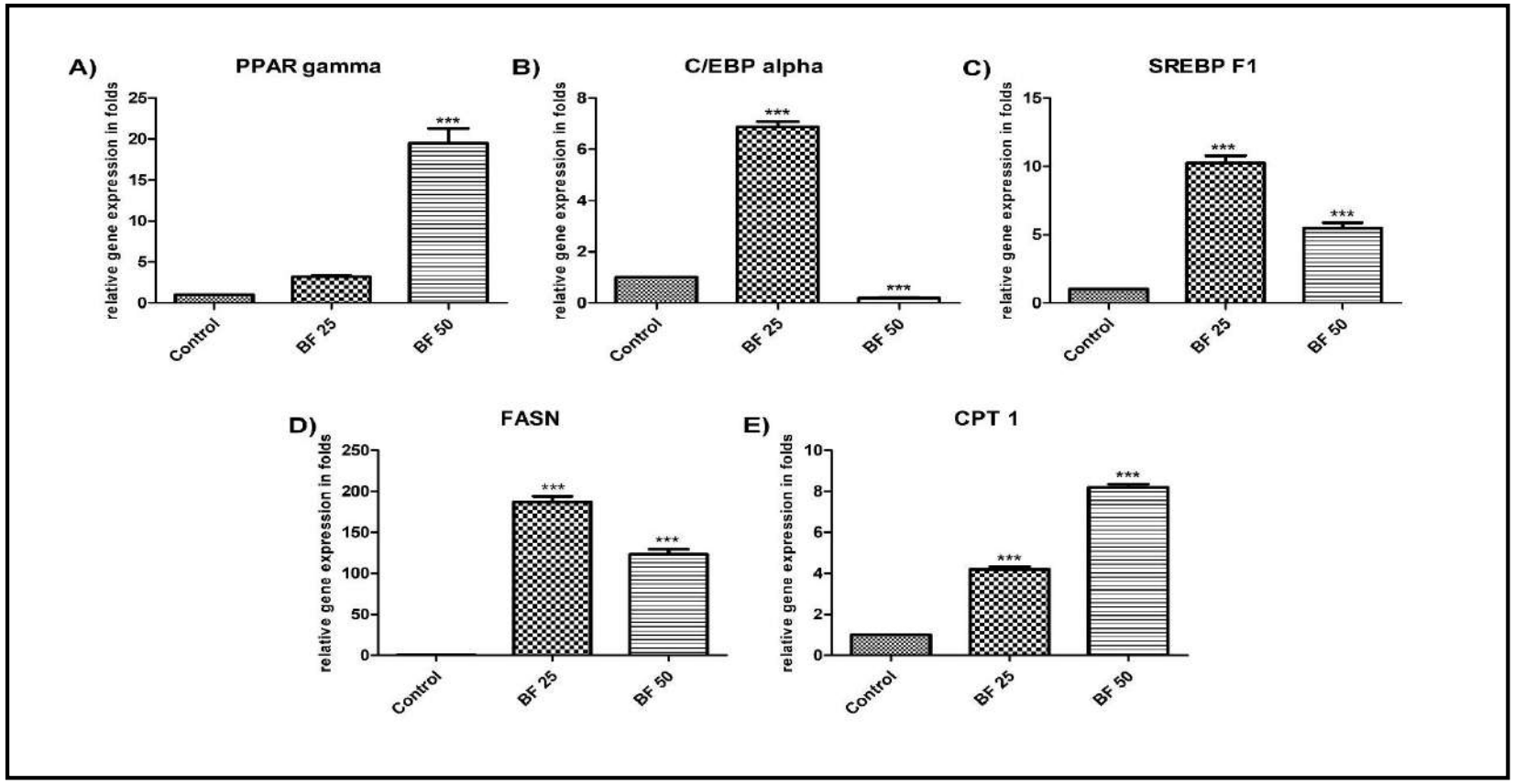


Figure No. 4.25: Effect of n-butanol fraction on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

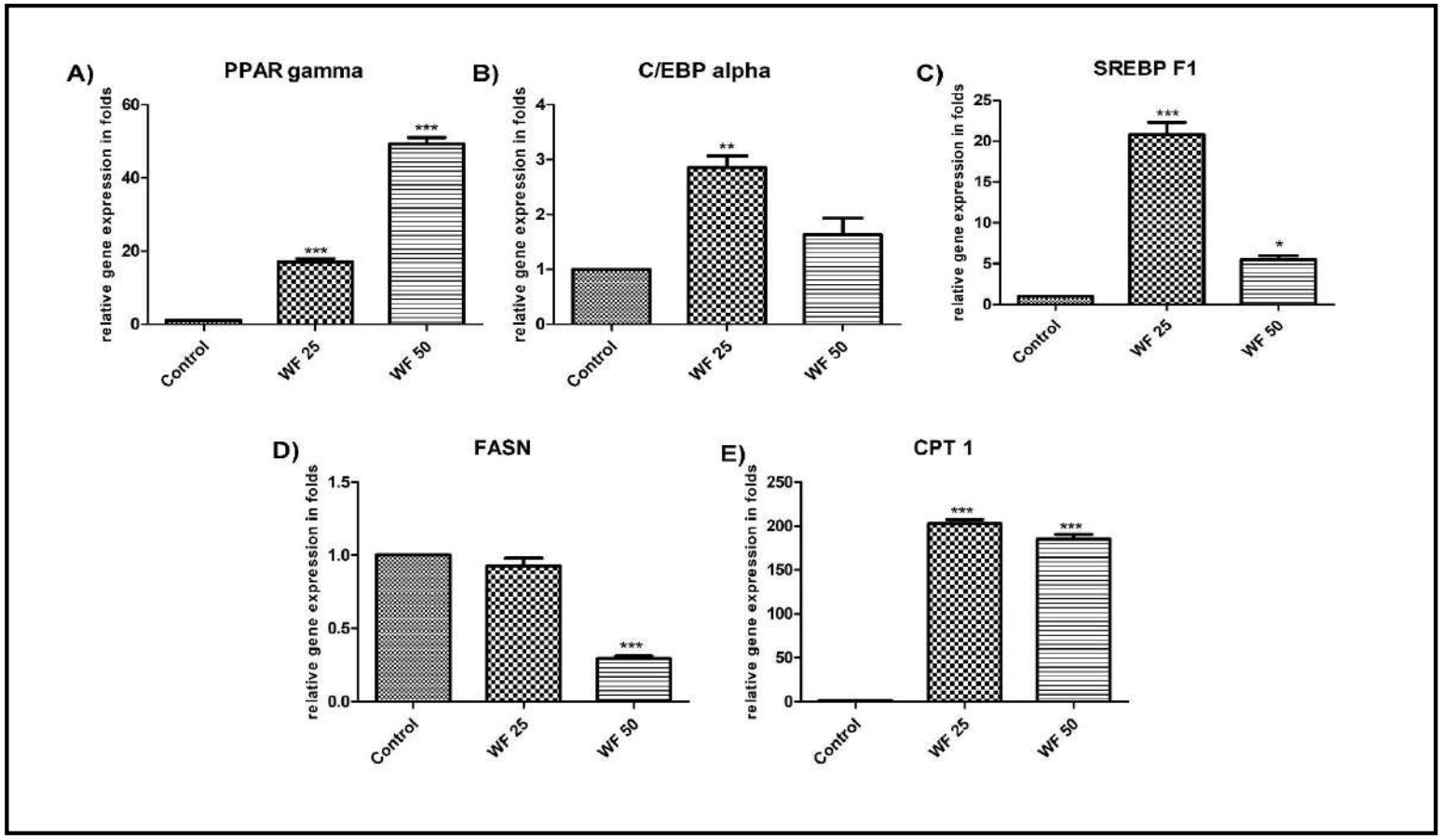


Figure No. 4.26: Effect of water fraction on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

4.4.6. Bioactive compound detection by LCMS:

In LCMS chromatogram of the chloroform fraction of *D. oppositifolia* methanolic whole plant extract shows in **Figure No.4.27**

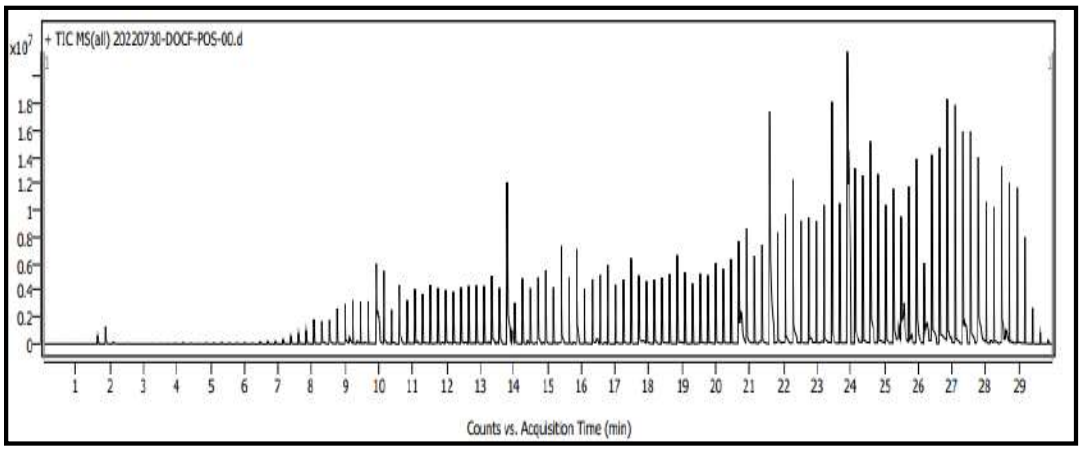


Figure No.4.27. LCMS chromatogram of Chloroform fraction of *D. oppositifolia* methanolic extract

The chloroform fraction contains the 4 anti-obesity metabolites which are identified from the data base and these 4 compounds were previously reported as an anti-obesity effect. Listed below in **Table No. 4.13.**

Table No. 4.13: Anti-obesity metabolites found in chloroform fraction of *D. oppositifolia* methanolic extract

Sr.No.	Compound Name	Class	Formula	RT	m/z	Height
1	Robinetin	Flavonoid	$C_{15}H_{10}O_7$	8.7647	302.0421	21548.5
2	Aloesin	Glycoside	$C_{19}H_{22}O_9$	9.283	417.1153	14347.3
3	Dioscin	Glycoside	$C_{45}H_{72}O_{16}$	19.3088	869.4883	24844.3
4	Oleoyl EthanolAmide	Lipid	$C_{20}H_{39}NO_2$	17.3158	325.2975	2738.5

Chemical structure of all compounds given on page no 90

Batubara *et al.*, (2014) prepared different solvent fractions of *Intsia palembanica* and from the ethyl acetate fraction they extracted probable flavonoids which, including ()-robidanol, (+)-epirobidanol, 4′ dehydroxyrobidanol, fustin, naringenin, robinetin (**Figure No.**

4.28), myricetin, quercetin, and 3,7,3′,5′-tetrahydroxyflavone. These isolated compounds were tested for *In vitro* lipase inhibition activity using substrate 2, 3-dimercapto-1-propanoltributyrate. After screening of compounds seven compounds showed PLE imbibition activity, with IC50 values ranging from 13.7 to 835.0 M. (+)-epirobidanol, robinetin, and naringenin effectively inhibit lipase.

Shin *et al.,* (2011) studied for different *Aloe vera* formulations on Obesity-induced Inflammation in Obese Mice. One of them is the aloesin **(Figure No. 4.29)**. Aloesin inhibited the expression of the adipogenic gene PPARɣ and LXR α genes as compared to control. Obesity-induced inflammatory responses are suppressed by *Aloe* formulations by lowering levels of proinflammatory cytokines, PPARɣ/LXR α, and 11-HSD1, and increasing anti-inflammatory cytokines in WAT and liver, all of which are critical peripheral tissues for insulin responsiveness. The activation of PPARɣ/LXR α has been linked to the therapeutic benefits of *Aloe* formula on obesity-induced insulin resistance and hepatic steatosis.

Kong *et al.,* (2010) studied the effect of processed *Aloe* gel, aloesin, *Aloe* QDM, and an *Aloe* QDM complex on the high fat diet induced mice. The gene expression study exhibited the down regulation of the fatty acid synthase, sterol regulatory element binding protein in liver and white adipose tissue. *Aloe* formula has functional qualities such as regulation of hyperglycemia, hyperlipidemia, blood glucose reduction, and adipogenesis of adipose tissue in HFD-fed mice. Comparison between all the aloe formulas aloesin inhibit the adipogenic genes.

Poudel *et al.,* (2014) examined the effect of marked Dioscin on the 3T3-L1 adipogenesis. Treatment with the dioscin reduce the lipid accumulation and inhibit the adipogenic transcription factors. Increase in the concentration of dioscin down regulates the C/EBPα, β, δ genes. It also reduces the expression of PPARɣ, SREBP1, and FASN genes. They also check the effect of Dioscin on the high fat diet induced mice. They found that dioscin reduces the fat accumulation and weight reduction in the mice by modulating the AMPK/MPK pathway **(Figure No. 4.30)**.

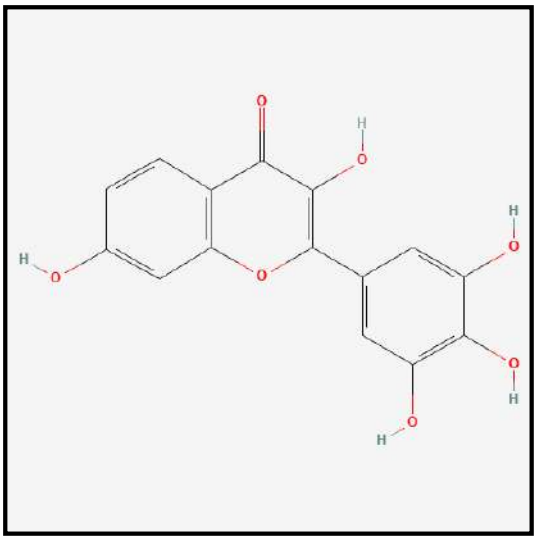


Figure No. 4.28:
Chemical structure of Robinetin

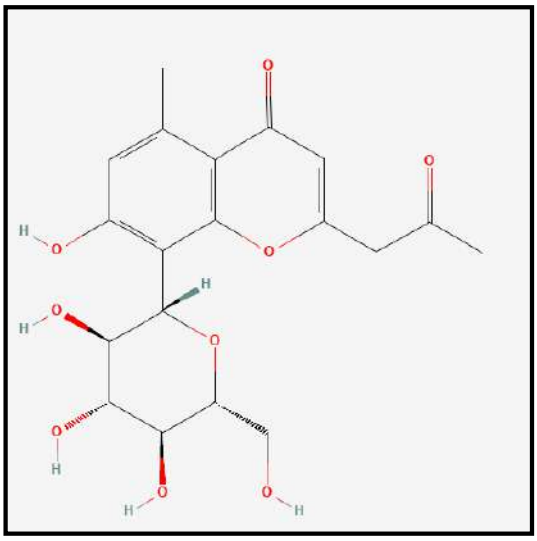


Figure No. 4.29:
Chemical structure of Aleosin

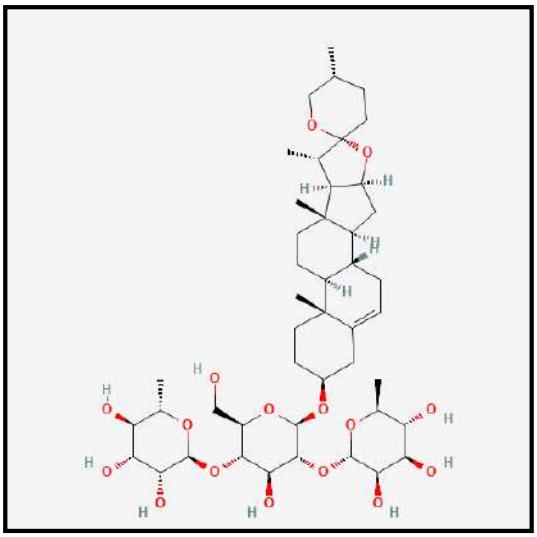


Figure No. 4.30:
Chemical structure of Dioscin

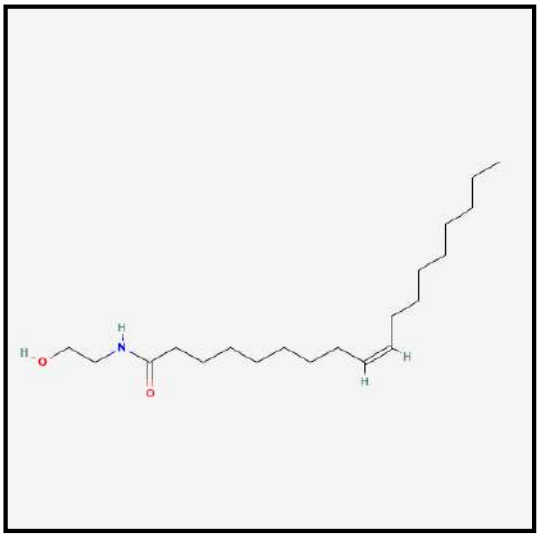


Figure No. 4.31:
Chemical structure of Oleoyl Ethanolamide

Chemical structures acquired from
(https://pubchem.ncbi.nlm.nih.gov)

Dioscin's effects on body weight and serum FFA may aid in the prevention and treatment of cardiovascular disease. As a result, it was suggested that dioscin is useful in controlling the aberrant metabolism of obese mice by restoring the activity of the IRS-1/PI3K/Akt pathway and the PPAR- pathway, therefore reversing the insulin resistance caused by an HFD. Dioscin's lowered body weight and blood FFA levels established it as a viable option for the treatment of various obesity-related illnesses (Li *et al.,* 2019).

Oleoyl Ethanolamide **(Figure No. 4.31)** inhibits food consumption, weight gain and plasma lipid levels in obese Zucker rats which are lacking functioning leptin receptors. Diet-induced obese rats and mice have similar consequences. Subchronic OEA treatment (5 mg kg1, intraperitoneally, i.p., once daily for two weeks) in Zucker rats initiates transcription of PPAR-α and other PPAR-α target genes, including fatty-acid translocase (FAT/CD36), liver fatty-acid binding protein (L-FABP), and uncoupling protein-2 (UCP-2). Furthermore, as measured by Oil red O staining, OEA reduces neutral lipid content in hepatocytes as well as blood cholesterol and triglyceride levels. The findings indicate that OEA modulates lipid metabolism, which may contribute to its anti-obesity benefits (Fu *et al.,* 2005).

Dioscin, aloesin, oleoyl ethanolamide, and robinetin are the four chemicals identified in the chloroform fraction's LCMS MS profile. According to previously published research, these compounds have anti-obesity effect, the presence of these compounds in the chloroform fraction demonstrating anti adipogenic activity.

4.4.7. Effect of extracted Diosgenin on mRNA expression:

Diosgenin was isolated from the dry root powder of *D. oppositifolia* and examined for anti-adipogenic activity on 3T3-L1 pre-adipocytes. Adipogenic genes were down regulated in cells treated with extracted diosgenin. Cells treated with extracted Diosgenin down regulated PPARɣ up to 56% at 25 µg/ml and 69% at 50 µg/ml **(Figure No. 4.32 A),** whereas cells treated with standard Diosgenin PPARɣ down regulated up to 71% and 78% at 25 and 50 µg/ml doses, respectively **(Figure No. 4.33 A).** Wang *et al.,* (2015) investigated the impact of commercial diosgenin on 3T3-L1 adipocytes and mice with a high fat diet. They discovered that increasing the quantities of diosgenin lowers PPARɣ expression and inhibits adipocyte development, as well as reduces adipocyte cell size. In our investigation, the isolated

diosgenin significantly reduced the expression of the PPARϒ gene. C/EBPα expression was moderately up regulated in both cases of diosgenin therapy at 25 µg/ml, 1.6 times **(Figure No. 4.32 B)** and 2.1 **(Figure No. 4.33 B)** times higher than in the control, respectively. However, at greater doses of 50 µg/ml, the C/EBPα gene is down regulated. Standard diosgenin inhibits the gene by up to 47% **(Figure No. 4.32 B),** whereas isolated diosgenin inhibited the gene by 31% **(Figure No. 4.33 B).** When compared to the control, both extracted diosgenin and standard diosgenin diminish the expression of the SREBP 1 gene. Down regulation was detected at 25 and 50 µg/ml of standard diosgenin, with 65 and 76 % down regulation, respectively **(Figure No. 4.33 C).** While isolated diosgenin inhibits the SREBP-1 gene by 85 percent at 25 µg/ml, the effect is reduced to 72 % at 50 µg/ml **(Figure No. 4.32 C).** The expression of the FANS gene was down regulated by Std. Diosgenin up to 67 % at 50 µg/ml, but only 5 percent at 25 µg/ml **(Figure No. 4.33 D).** At 25 µg/ml, isolated diosgenin mildly up regulated the FANS gene by 0.5 times. However, higher concentrations decrease gene expression by up to 40% **(Figure No. 4.32 D).** According to Berndt *et al.*, (2007), increased expression of the fatty acid synthase gene leads to an increase in visceral fat storage in the body. Inhibiting the FASN gene in adipocytes provides a significant increase and may be a viable therapeutic strategy for obesity management. The CPT-1 enzyme is important in the lipolysis of fat. The standard diosgenin promotes CPT-1 gene expression in a concentration-dependent manner. Standard diosgenin at 25 µg/ml increased expression 42 times more than the control. At 50 µg/ml, diosgenin up regulates the gene 103 times more than the control **(Figure No. 4.33 E).**

When cells were treated with extracted diosgenin, the CPT-1 gene level increased up to 223 times higher than the control at 25 µg/ml, but at 50 µg/ml, the CPT-1 level increased up to 147 times higher than the control **(Figure No. 4.32 E).** When compared to the standard diosgenin, the isolated diosgenin provides superior up regulation of CPT-1. The activation of CPT-1 helps in the initiation of fatty acid oxidation on the mitochondrial membrane.

In comparing Standard diosgenin and extracted diosgenin, extracted diosgenin had almost the same impact on adipogenic gene regulation. This difference might be attributed to impurities present in the extracted diosgenin.

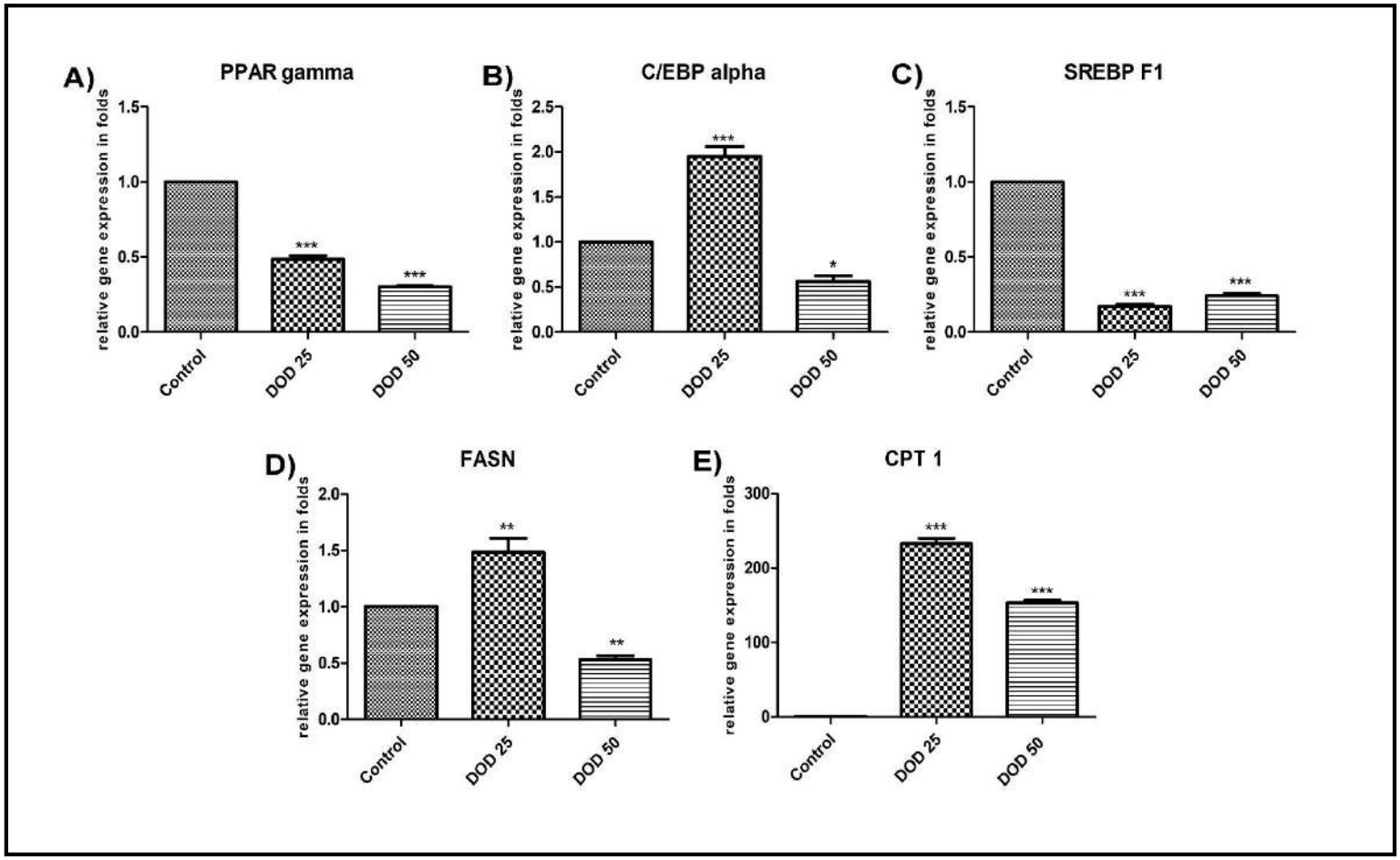


Figure No. 4.32: Effect of extracted Diosgenin (DOD) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

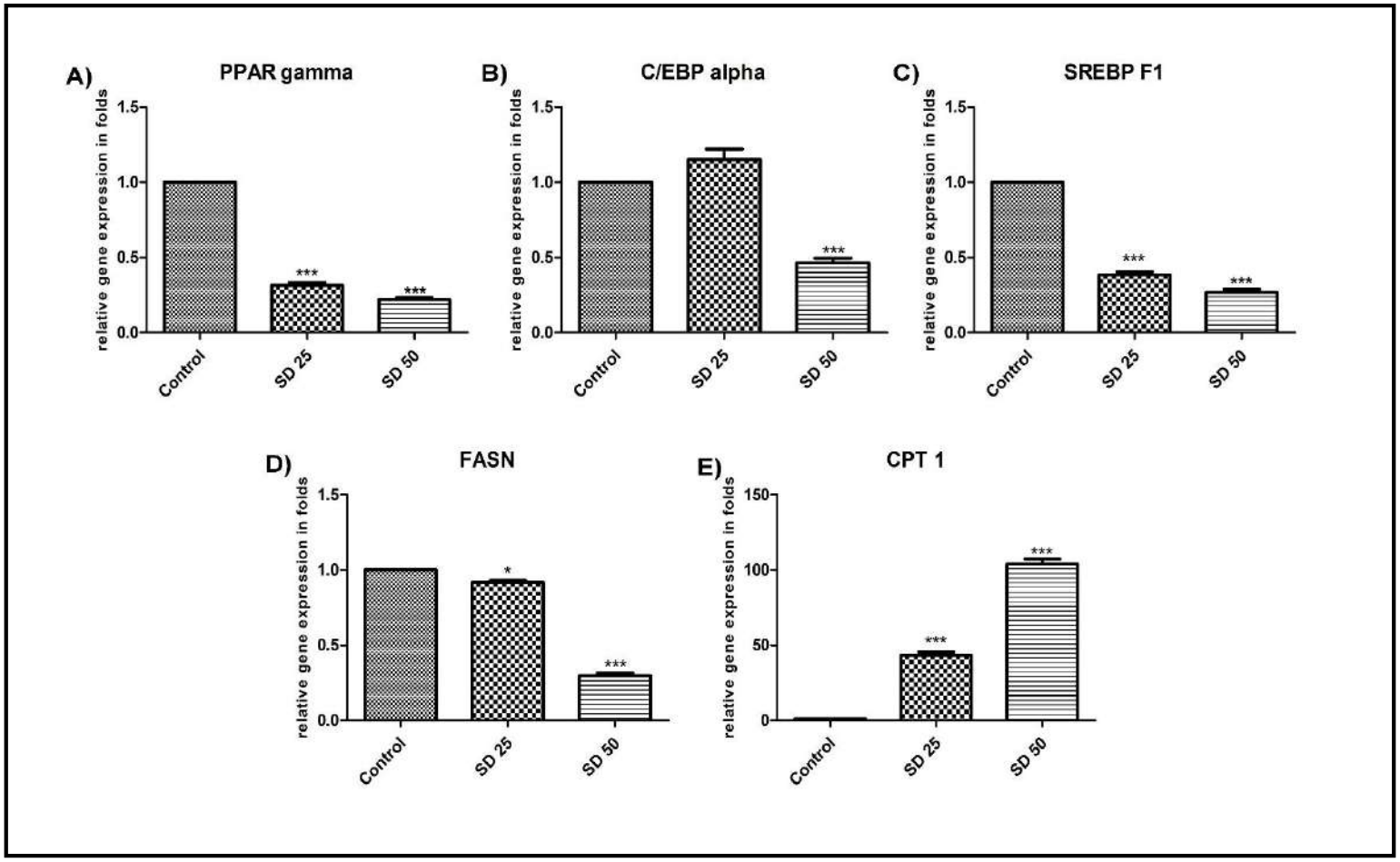


Figure No. 4.33: Effect of standard Diosgenin (SD) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

4.4.8 Detection of extracted diosgenin by HPLC

Diosgenin belongs to the class of steroidal saponins. The presence of diosgenin was identified in the current study utilizing the HPLC technique. Based on the chromatogram obtained from the software, a sharp peck of standard diosgenin was obtained at 3.19 min **(Figure No. 4.34).** The diosgenin was extracted from the *D. oppositifolia* root by acid hydrolysis method **(Figure No 4.35)** and it also showed a sharp peck at the same retention time, confirming that the samples contain diosgenin. The amount of diosgenin was calculated by using standard diosgenin i.e.1.226 mg/100gm of dry root powder.

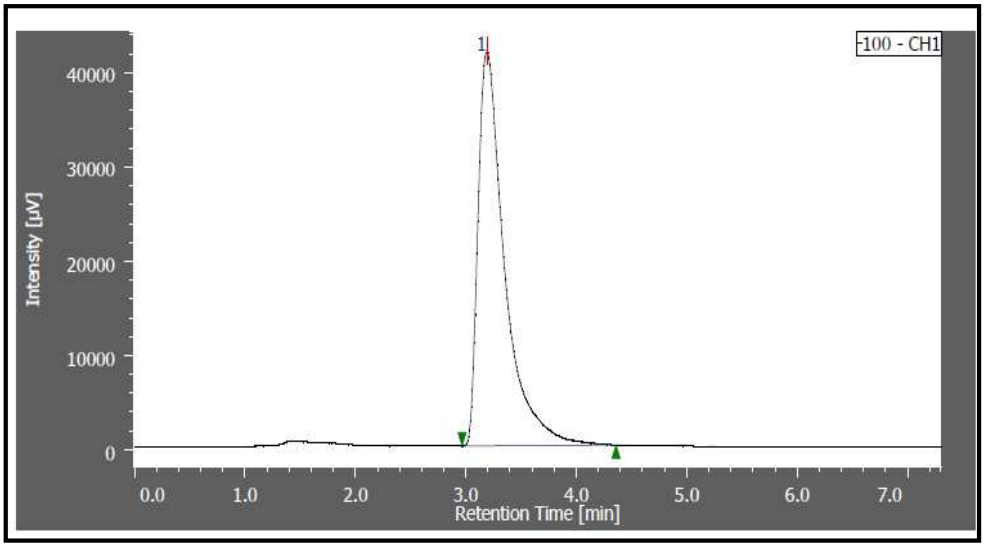


Figure No 4.34 Chromatogram of Standard Diosgenin 100µg/ml

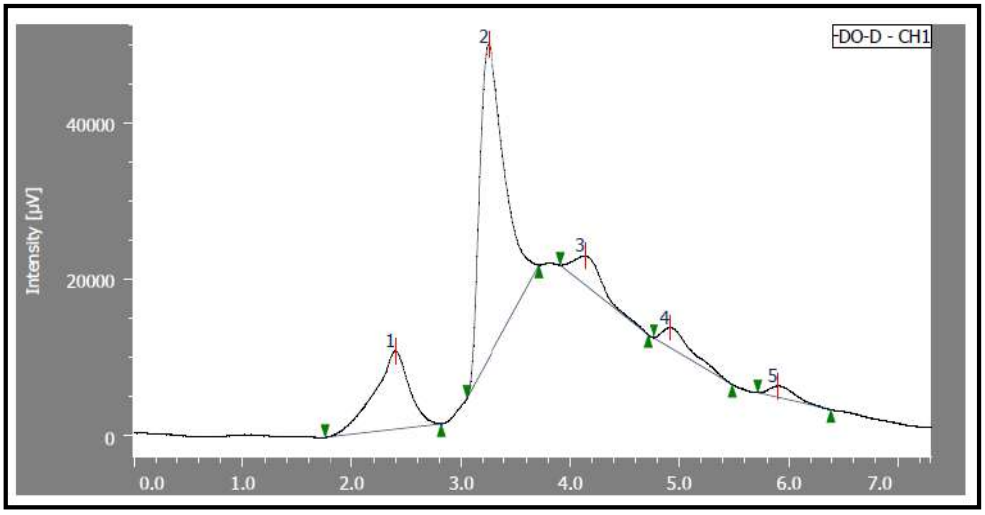


Figure No 4.35 Chromatogram of extracted diosgenin from *D. oppositifolia* Root by using acid hydrolysis

Diosgenin demands special attention because of its biological activity and pharmacological uses like antibacterial, antifungal, anticancer, anti-aging, cardio protective and contraceptive activity. Diosgenin also shows the anti hyperlipidemic activity (Chaudhary *et al.,* 2018). Raina and Misra (2020) estimated the diosgenin content in different *Dioscorea* species by HPTLC method they found that highest content of diosgenin in *D. hispida* and *D. bulbifera* than the other studied species. Niño *et al.*,(2007) collected *D. polygonoides* from different location and diosgenin was extracted in n-hexane by using Soxhlet extractor. Diosgenin was quantified by the HPLC. The concentration of diosgenin was varying 0.02 to 2.64% with the different locations. They suggested that *D. polygonoides* is the good source diosgenin. Yang *et al.,* (2013) extracted diosgenin from *D. Zingiberensis* by using the cellulose enzymolysis and two-phase acid hydrolysis and detected by the RP-HPLC-UV method at 202 nm they have reported that *D. Zingiberensis* root content was 4.46% diosgenin. Edwards *et al.,* (2002) compared diosgenin content in *D. batats* and *D. villosa* the extraction was carried out by homogenizing rhizomes in the methanol followed by overnight stirring in the chloroform: methanol (3:1) mixture after that the diosgenin was detected by HPLC-MS at 210 nm with ortho-phosphoric acid/acetonitrile solvent the observed pick of diosgenin at 28.1 min.

Dioscorea species are a good and natural source of diosgenin. It has multiple biomedical applications. We tested the extracted diosgenin of *D. oppositifolia* root on the 3T3-L1 preadipocytes for antiobesity activity. The extracted diosgenin shows the antiadipogenic activity as exhibited by the standard diosgenin.

4.5 *In vivo* studies:

The effect of *D. oppositifolia* extract on male Wistar rats was studied by creating obesity with a high fat diet. It was shown that consuming plant extracts helps to prevent obesity by lowering body increase in weight and lipid profile

4.5.1 Effect on body weight:

Animals fed a high-fat diet (HFD) have a significant increase in body weight; 22% more body weight than the control group. The animal group feed with the HFD and orlistat exhibited a 24% reduction in body weight as compared to the HFD control group. However, the group

fed with 200mg/kg BW of *D. oppositifolia* whole plant extract along with an HFD showed 23% a reduction in body weight as compared to the HFD group. In case of the animal fed with 400mg/kg BW of *D. oppositifolia* whole plant extracts, exhibited a 40% decrease compared to the HFD control group **(Figure No. 4.36 & 4.37).**

Table No 4.14: Effect of *D. oppositifolia* (DO) extracts on food intake in rats fed along with HFD. Data are expressed as the mean ± SD (n=6), ****p<0.05* vs. HFD control group

Groups	Initial Body weight in gm	Final Body weight in gm
Control	129.33±4.1	261±3.5
HFD	135±3.5	324±4.5
HFD + Orlistat	130.67±2.3	256±3.2
HFD + DO 200mg/kg BW	132.67±3.5	258±2.9
HFD + DO 400 mg/kg BW	131.33±2.9	201±2.6

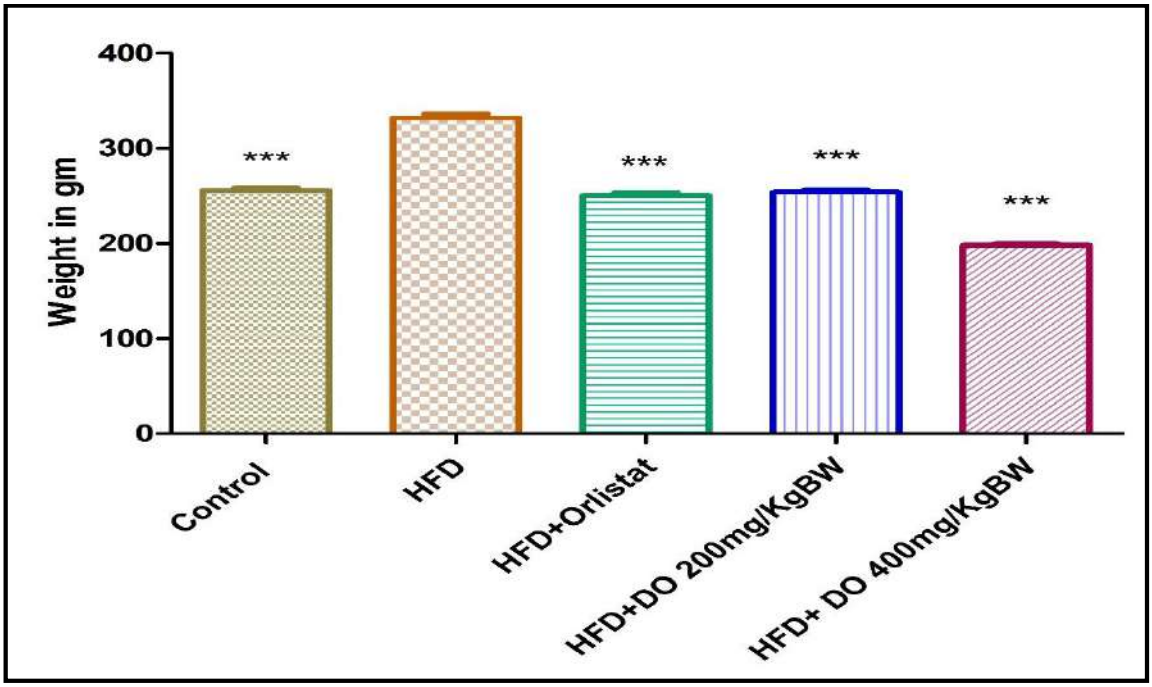


Figure No. 4.36: Effect of *D. oppositifolia* (DO) 200mg/kg/day and *D. oppositifolia* (DO) 400mg/kg/day extracts on food intake in rats fed along with HFD. Data are expressed as the mean ± SD (n=6), ****p<0.05* vs HFD control group

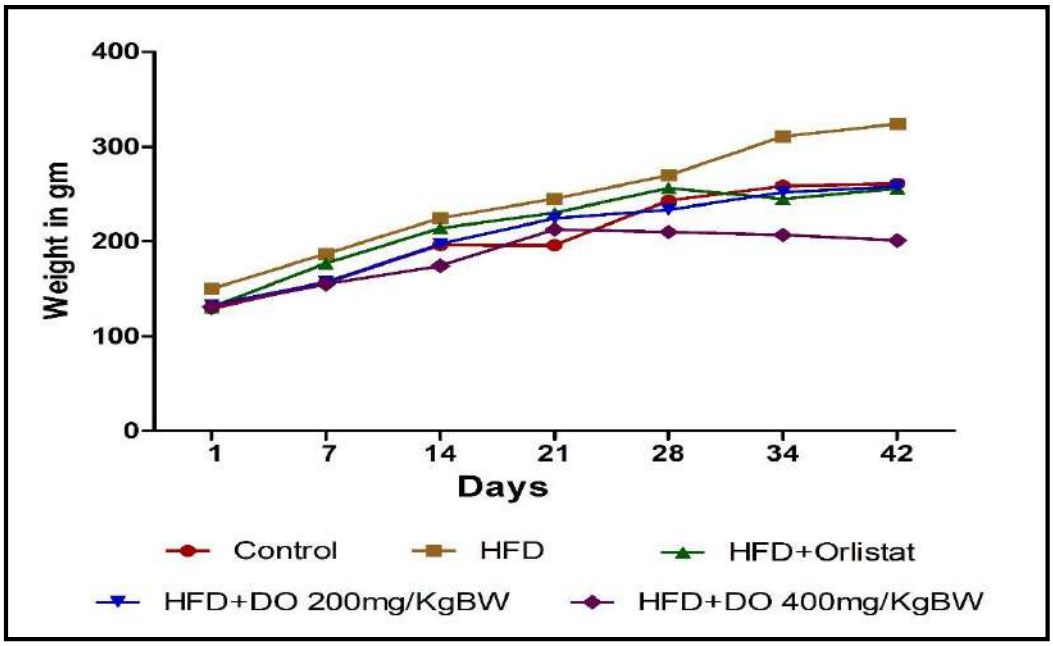


Figure No. 4.37: Effect of High Fat Diet, Orlistat 10mg/kg/day, *D. oppositifolia* (DO) 200mg/kg/day and *D. oppositifolia* (DO) 400mg/kg/day plant extract on weight gain in 6 weeks values are expressed as mean ± SD (n=6)

4.5.2 Effect of *D. oppositifolia* methanolic extract on lipid profile in rat fed with high fat diet:

Serum was separated from the collated blood. Serum lipid content was estimated by commercially available kits. In high fat diet consuming group, the TC, TG, LDL, VLDL levels were elevated than the control group and HDL level was reduced than the control group. The rat group feed with the HFD and 200mg/kg BW of *D. oppositifolia* plant extract the TC, TG, LDL, VLDL levels were reduced as compare to the HFD consuming rat group, but the HDL level was elevated. Whereas the rat group feed with HFD and 400mg/kg BW of *D. oppositifolia* plant extract also exhibit the reduction of TC, TG, LDL and VLDL levels. The orlistat also showing the same effect but plant extracts exhibiting the better results than the orlistat. While comparing in the effect of both the concentration of *D. oppositifolia* extracts, concentration dependent reduction of weight and also the lower the TC, TG, LDL and VLDL levels in the rats were observed. **(Figure No. 4.38A & 4.38B)**

Human studies have revealed that increasing energy intake has been linked with Obesity can be caused by an increase in body weight. This study indicates that the rats treated with

high fat diet had dramatically increased food intake. Whereas the treatment of plant extracts lowered food intake which hypothesises that these plants may have a function in modifying appetite management by reducing food intake.

Dyslipidemia is one of the pathophysiology conditions related with obesity. It results in lower HDL, higher total cholesterol, LDL, and VLDL levels, all of which contribute to the development of cardiovascular disease and atherosclerosis. In this study, *D. oppositifolia* (200 mg/kg b.wt) plant extract administration in conjunction with a high fat diet significantly reduced LDL, VLDL, and total cholesterol levels as compared to the HFD group. *D. oppositifolia* Plant extract supplementation at had superior results than the *D. pentaphylla* plant extracts group.

Jeong *et al.,* (2014) reported that the *D. oppositifolia* n-BuOH extract efficiently reduced fat accumulation in high-fat diet-induced obese mice. This butanolic extracts contains 3,5-dimethoxyphenanthrene-2,7-diol and (3R,5R)-3,5- dihydroxy-1,7-bis(4-hydroxyphenyl)-3,5-heptanediol as major components. This helps to reduce weight in mice body.

Kwon *et al.,* (2003) studied the effect of the methanolic extract of *Dioscorea nipponica* on SD rats. The SD rats feed with the extracts lower the TC, TG, LDL and VLDL and increase the HDL levels in the rats with weight loss. Also, they extracted the Dioscin and Diosgenin and studied the lipase inhibitory activity with IC_{50} 20µg/ml and 28 µg/ml respectively.

Ikete and Chinko (2022) reported that the rat feed with the high fat diet and hydromethanolic extracts of *D. bulbifera* reduces the TC, TG, LDL and VLDL and increase the HDL levels in the rats and lower the weight as compare to the control.

The effects of resistant starch (RS) produced from purple yam (*Dioscorea alata* L.) on lipid metabolism and gut microbiota in hyperlipidemic hamsters were studied. High dosage of RS administration in hamsters was shown to be more efficient in controlling body weight and adipose tissue mass, with increased HDL, concentration and decreased TG, TC, and LDL concentrations. Furthermore, HR changed the makeup of the gut community by increasing the presence and abundance of *Bifidobacteria, Lactobacillus, Coprococcus*, and *Allobaculum* while lowering the relative abundances of Parabacteroides and Dorea. Probiotics like *Bifidobacteria* and *Lactobacillus* were shown to be significantly higher and substantially linked with blood lipid levels. These findings showed that using RS derived from purple yam

might improve lipid metabolism in conjunction with gut microbiota modification, which could give guidelines for future treatment (Li *et al.*, 2019).

The *D. oppositifolia* whole plant extract decreas body weight in a diseased rat model. Treatment with *D. oppositifolia* lowers body weight, TC, TG, LDL, VLDL, and increases HDL levels, suggesting that it may be a viable approach to treating obesity and associated disorders.

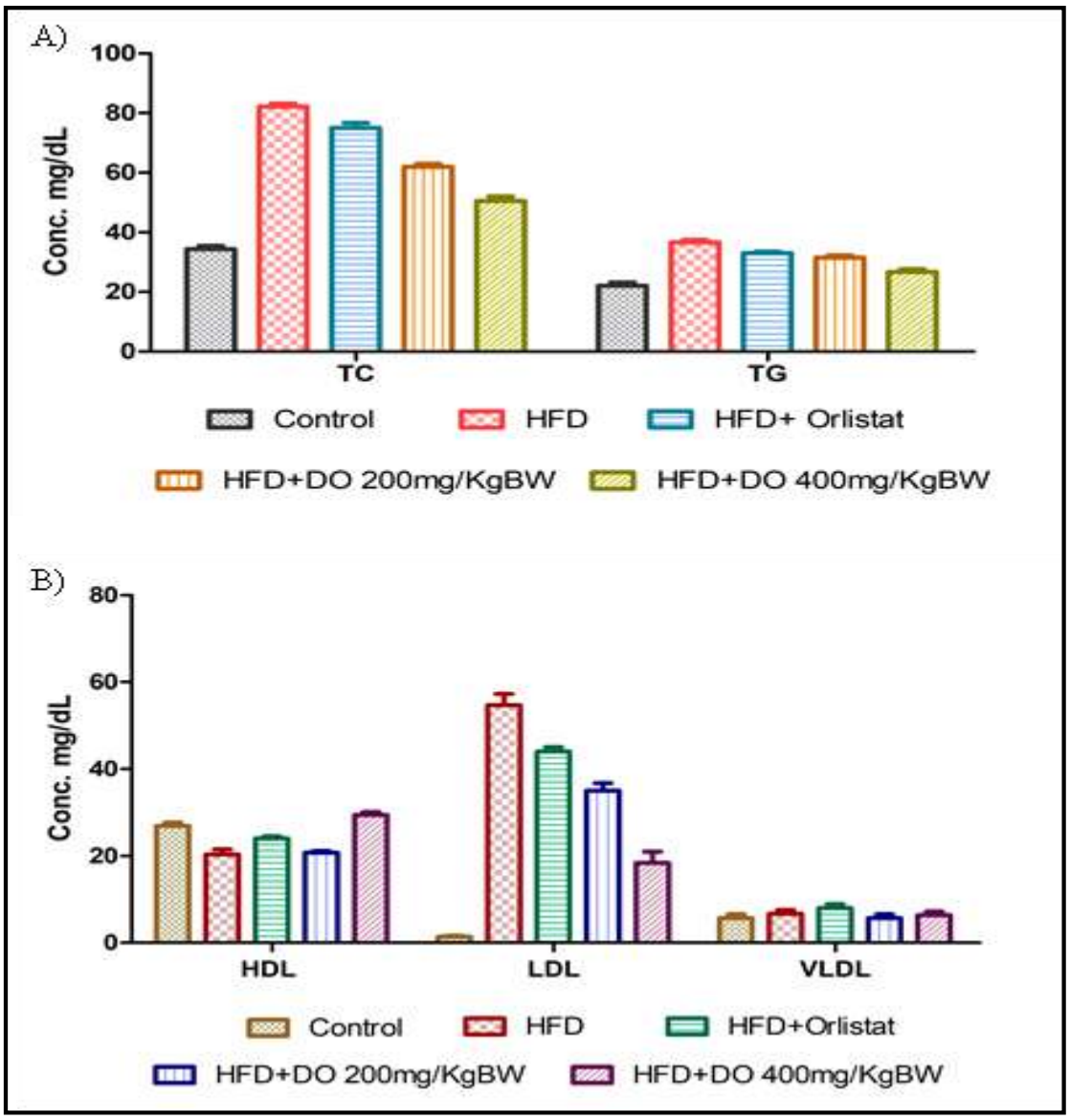


Figure No. 4.38: A and B Effect of Orlistat 10mg/kg/day, *D. oppositifolia* (DO) 200mg/kg/day and *D. oppositifolia* (DO) 400mg/kg/day plant extract on lipid profile in rats fed along with HFD. Data are expressed as the mean ± SD (n=6).


Summary
and
Conclusion

5. SUMMARY AND CONCLUSIONS

Obesity is the major problem now a days. Obesity is linked to many disease like cancer, diabetes, osteoporosis, and cardiovascular disease. Control of the obesity leads to overcome problem of the linked disease. The present study was planned to explore the anti-obesity efficacy of the *Dioscorea* species to avoid increase of obesity. The outcome of present study will possibly help develop a food/beverage with anti-obesity properties.

Collection of *Dioscorea* species:

The four *Dioscorea* species were collected from the Kolhapur district Maharashtra India. Namely *D. alata, D. bulbifera D. pentaphylla* and *D. oppositifolia.* Collected plant germplasm was maintained in botanical garden, and the herbariums of all species deposited to herbarium Department of Botany, Shivaji University Kolhapur. Plant material were dried and used for the preparation of the methanolic and aqueous extracts.

Chemo-profiling of *Dioscorea* extract:

The primary phytochemical analysis was carried out by different colorimetric methods. The total phenolic, total flavonoids, total alkaloids, total terpenoids and total saponin were studied the methanolic extract of *D. oppositifolia* showing the highest content of the all the phytochemicals than the other extracts. The antioxidant activity studied by the free radicals scavenging by DPPH and ABTS assay *D. oppositifolia* methanolic extracts showing the highest % inhibition in both the assay. It also has the ferrous reducing antioxidant power.

Screening of the different *Dioscorea* extract for the anti-obesity potential:

The prepared extracts were screened by using the 2 different methods for the antioxidant activity i.e. lipase inhibition, effect of extracts on the lipid accumulation in the 3T3-L1 cells and regulation of the adipogenic genes.

In lipase inhibition study all the extracts of the *Dioscorea* shows the significant inhibition. The both methanolic and aqueous extracts of the *D. pentaphylla* shows the above 70% inhibition. *D. alata, D. bulbifera,* and *D. oppositifolia* exhibit the above 50% lipase inhibition activity.

In vitro anti-obesity analysis was performed on 3T3-L1 preadipocyte cell line, which serves as an *In vitro* model system to research anti-obesity because it develops from pre-adipocyte to mature adipocyte, which is specialized in lipid droplet formation. The toxicity of

the plant extracts was studied by using the MTT assay and from the IC 50 concentration the dose of treatment was decided for the further study. From the results obtained from the Oil O red staining both aqueous and methanolic extracts of the *D. oppositifolia* reduces by 1.8 and 2.2 times the lipid accumulation in the 3T3-L1 cells as compared with Control induced group.

The 3T3-L1 cells were treated with methanolic and aqueous extracts of *D. oppositifolia* and *D. pentaphylla* during the differentiation. *D. oppositifolia* methanolic extract suppresses expression of PPAR ϒ, C/EBPα, SREBP-1 and FAS concentration dependently. This results in the inhibition of adipogenesis in 3T3-L1 cells. It also increase the expression of the CPT-1 gene that helps to enhances lipolysis of accumulated lipids. Aqueous extract of *D. oppositifolia* down regulate the expression PPARϒ, C/EBPα and SREBP1. At lower concentration of extracts the fatty acid synthase down regulate and at the higher concentration it unregulated the FANS gene. It up regulate the CPT-1 gene that helps in the lipolysis of the accumulated lipids. In case of the *D. pentaphylla* methanolic and aqueous extracts up regulate the PPAR ϒ, C/EBPα, SREBP1, FASN, gene and down regulate the CPT-1 gene. So the *D. oppositifolia* methanolic extract used for the further study.

The LCMS analysis of the *D. pentaphylla* and *D. oppositifolia* methanolic extracts were carried from the obtained data. Group of compound was identified from the human metabolomics database and Pub chem database. The compound having an anti-obesity property previously studied were identified 11 compound shows the anti-obesity activity.

This significant down-regulation of PPARγ gene may be due to the cumulative action of phytochemicals from the extracts. This reduction of adipogenic transcription factors may aid in regulating adipocyte differentiation and lowering adipocyte lipid accumulation.

Screening of anti-obesity metabolites from *D. oppositifolia*:

From the above study the *D. oppositifolia* methanolic extract used for the liquid-liquid extraction by using the hexane, chloroform, ethyl acetate and n-butanol. Fraction were collected and solvents were evaporated reaming reside after evaporation was dissolved in to the DMSO, Also the diosgenin was extracted from the root powder of *D. oppositifolia* by using the acid hydrolysis method.

The effect of all fractions and extracted diosgenin were studied during the differentiation of 3T3-L1 cells. In lipid accumulation study there was no significant changes were seen in cells after treatment with all fractions and extracted diosgenin. For that, the impact of all the fractions explored by gene expression study.

In comparing the effects of each fraction, the chloroform fraction prevents the expression of all examined adipogenic genes more than the other four fractions while also stimulating the activity of the CPT-1 gene. The PPARϒ, C/EBPα, SREBP1, and FASN genes are up regulated by hexane fractions. In contrast, down regulates the CPT 1 gene. Treatment with the Ethyl acetate fraction raises PPARϒ, C/EBPα, and FASN levels. SREBP 1 gene expression was lower than in the control group. The CPT-1 is down regulated at greater concentrations of ethyl acetate and up regulated at lower concentrations. At the studied concentrations, cells treated with n-butanol and water fraction up regulate all adipogenic genes. At greater concentrations of the n-butanol fraction, C/EBPα was down regulated.

LCMS-QTOF analysis was used to profile the metabolites in the chloroform fraction. Robinetin, Aloesin, Dioscin, and Oleoyl Ethanol Amide were identified in the chloroform fraction of *D. oppositifolia.* The inclusion of these substances in the fraction demonstrating antiadipogenic gene regulate.

Dioscorea species are a good and natural source of diosgenin. It has multiple biomedical applications. We tested the extracted diosgenin of *D. oppositifolia* root on the 3T3-L1 preadipocytes for antiobesity activity. The effect of the extracted diosgenin was compared with the effect of standard diosgenin on the adipogenesis were studied. The PPARϒ, and SREBP 1 was down regulated by the extracted diosgenin at tested concentration the expression levels of the C/EBPα, and FASN was slightly up regulated at lower concentration of extracted diosgenin but at higher concentration down regulation were observed. Whereas the CPT -1 gene was up regulated at both the concentration. The extracted diosgenin shows the antiadipogenic activity as exhibited by the standard diosgenin.

The extracted diosgenin was confirmed by using the HPLC with the standard diosgenin. In both sample and standard diosgenin shows the pick at same retention time. Hence it confirmed that the sample contains the diosgenin.

Effect of *D. oppositifolia* on male Wistar rat:

The *D. oppositifolia* whole plant extract decreases body weight in a diseased rat model. Treatment with *D. oppositifolia* lowers body weight, TC, TG, LDL, VLDL, and increases HDL levels as compare to the High fat induced rat group, suggesting that it may be a viable approach to treating obesity and associated disorders.

The key conclusion from the above study is that all the *Dioscorea* species used in the study have lipase inhibition properties. *D. oppositifolia* whole plant extracts have a good source of phytochemicals and antioxidants than other three species. Considering the results obtained from the gene expression study both methanolic and aqueous whole plant extracts of *D. oppositifolia* have an ability to affect adipogenesis at the gene level. Treatment with *D. oppositifolia* whole plant extract to the high-fat diet-induced rats exhibited a lowering of the TC, TG, LDL, VLDL, and body weight as compared to the control group. The chloroform fraction and extracted Diosgenin from the *D. oppositifolia* root shows the virtuous down regulation of the adipogenic genes in 3T3-L1 cells. In LCMS profiling of the chloroform fraction of *D. oppositifolia*, robinetin, aloesin, diocin, and oleoyl ethanol amide were found which has anti-obesity activity, and down regulate the adipogenic genes. So, *D. oppositifolia* whole plant can be a potential candidate for the treatment of obesity and related problems.


Bibliography

BIBLIOGRAPHY

Abdali, D., Samson, S. E., & Grover, A. K. (2015). How effective are antioxidant supplements in obesity and diabetes?. *Medical principles and practice : international journal of the Kuwait University, Health Science Centre*, *24* (3), 201–215.

Abdul, R. H., Saari N., Abas F., Ismail A., Mumtaz M. W., & Abdul H. A. (2016). Anti-obesity and antioxidant activities of selected medicinal plants and phytochemical profiling of bioactive compounds. *International Journal of Food Properties,* 20 (11), 2616–2629.

Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of pharmacy & bioallied sciences*, 12(1), 1–10.

Adeosun, O.M, Arotupin D.J, Toba O.A, & Adebayo A.A. (2016). Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (Air Potatoe) tubers and peels against some pathogenic bacteria. *The Journal of Phytopharmacology* 2016;5(1):15-26.

Ado, M. A., Abas, F., Mohammed, A. S., & Ghazali, H. M. (2013). Anti- and pro-lipase activity of selected medicinal, herbal and aquatic plants, and structure elucidation of an anti-lipase compound. *Molecules (Basel, Switzerland)*, 18(12), 14651–14669.

Aguilar, S. L., Romero, C. O., Gonzalez C. M., & Tortoriello,J., (2012). Effect of *Bursera grandiflora* on body weight and lipemia in obese mice. *Boletín latinoamericano y del Caribe de plantas medicinales y aromáticas ;* 11(2): 138-146.

Ahmad, Q. I., Ahmad, C. B., & Ahmad, S. M. (2010). Childhood obesity. *Indian journal of endocrinology and metabolism*, 14(1), 19–25.

Ahmadian, M., Suh, J. M., Hah, N., Liddle, C., Atkins, A. R., Downes, M., & Evans, R. M. (2013). PPARγ signalling and metabolism: the good, the bad and the future. *Nature medicine*, 19(5), 557–566.

Ahn, E. K., Lee, J. A., Seo, D. W., Hong, S. S., & Oh, J. S. (2012). 1β-Hydroxy-2-oxopomolic acid isolated from *Agrimonia pilosa* extract inhibits adipogenesis in 3T3-L1 cells. *Biological & pharmaceutical bulletin*, *35*(5), 643–649.

Ahn, J. H., Kim, E. S., Lee, C., Kim, S., Cho, S. H., Hwang, B. Y., & Lee, M. K. (2013). Chemical constituents from *Nelumbo nucifera* leaves and their anti-obesity effects. *Bioorganic & medicinal chemistry letters*, *23*(12), 3604–3608.

Ahn, J., Lee, H., Jung, C. H., Choi, W. H., & Ha, T. Y. (2017). Zerumbone ameliorates high-fat diet-induced adiposity by restoring AMPK-regulated lipogenesis and microRNA-146b/SIRT1-mediated adipogenesis. *Oncotarget*, 8(23), 36984–36995.

Alexander, J. and Coursey, D. G. 1969. The domestication of the yams.In: Ucko, P. J. and Dimbleby, G. W. edit. The domestication and exploitation of plants and animals, pp. 405–425. Gerald Duckworth, London.

Ali-Shtayeh, M. S., Jamous, R. M., Abu-Zaitoun, S. Y., Khasati, A. I., & Kalbouneh, S. R. (2019). Biological Properties and Bioactive Components of *Mentha spicata* L. Essential Oil: Focus on Potential Benefits in the Treatment of Obesity, Alzheimer's disease, Dermatophytosis, and Drug-Resistant Infections. *Evidence-based complementary and alternative medicine: eCAM, 2019*, 3834265.

An, S., Han, J. I., Kim, M. J., Park, J. S., Han, J. M., Baek, N. I., Chung, H. G., Choi, M. S., Lee, K. T., & Jeong, T. S. (2010). Ethanolic extracts of *Brassica campestris* spp. rapa roots prevent high-fat diet-induced obesity via beta (3)-adrenergic regulation of white adipocyte lipolytic activity. *Journal of medicinal food*, 13(2), 406–414.

Arçari, D. P., Bartchewsky, W., dos Santos, T. W., Oliveira, K. A., Funck, A., Pedrazzoli, J., de Souza, M. F., Saad, M. J., Bastos, D. H., Gambero, A., Carvalho, P., & Ribeiro, M. L. (2009). Antiobesity effects of yerba maté extract *(Ilex paraguariensis*) in high-fat diet-induced obese mice. *Obesity (Silver Spring, Md.)*, *17*(12), 2127–2133.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants (Basel, Switzerland)*, *8*(4), 96.

Asdaq S. M. B., and C. Malsawmtluangi. (2015). "Evaluation of Anti-Obesity Potential of Cashew Nut (*Anacardium Occidentale*) In Animals" *Indian Journal of Pharmaceutical Science & Research* 5, no. 1: 42-50

Avadhani, M. N. M., Selvaraj, C. I., Rajasekharan, P. E. & Tharachand, C. (2013) The Sweetness and Bitterness of Sweet Flag [*Acorus calamus* L.] – A Review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4: 598-610.

Bansal, V.K., Malaviya, T., & Sharma, P.K. (2014). Phytochemical, Pharmacological Profile and Commercial Utility of Tropically Distributed Plant Bauhinia *Variegata.variegata*. *Global Journal of Pharmacology* 8: 196-205.

Batubara, I., Kuspradini, H., Muddathir, A. M., & Mitsunaga, T. (2014). *Intsia palembanica* wood extracts and its isolated compounds as *Propionibacterium acnes* lipase inhibitor. *Journal of Wood Science*, 60(2), 169–174.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70–76.

Berndt, J., Kovacs, P., Ruschke, K., Klöting, N., Fasshauer, M., Schön, M. R., Körner, A., Stumvoll, M., & Blüher, M. (2007). Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Diabetologia*, 50(7), 1472–1480.

Bhandari, M. R., & Kawabata, J. (2004). Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chemistry*, 88(2), 163–168.

Bhattacharjee R, M Gedil, A Sartie, E Otoo, D Dumet, H Kikuno, P Lava Kumar, and R Asiedu (2011) *Dioscorea.* Wild Crop Relatives: Genomic and Breeding Resources, Industrial Crops (Ed.) Kole C. pp. 71–96.

Blois, M. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* 181, 1199–1200.

Borzelleca JF and Lane RW (2008). The Art, the Science, and the Seduction of Toxicology: an Evolutionary Development. In: Principles and methods of toxicology (5th ed.) (Eds.) Hayes AW. *Taylor and Francis. Philadelphia* pp. 13.

Bounihi, A., Bitam, A., Bouazza, A., Yargui, L., & Koceir, E. A. (2017). Fruit vinegars attenuate cardiac injury via anti-inflammatory and anti-adiposity actions in high-fat diet-induced obese rats. *Pharmaceutical biology*, *55*(1), 43–52.

Bradford P.G., (2013). Curcumin and obesity. *Biofactors* 39: 78-87.

Brown R., (1810). Prodromus Florae Novae. *Hollandiae* pp. 294–295.

Brown, J., (1999). Lipotrim diet in general practice. *The British Journal of General Practice*, *49*(439), 147.

Calderon-Dominguez, M., Sebastián, D., Fucho, R., Weber, M., Mir, J. F., García-Casarrubios, E., Obregón, M. J., Zorzano, A., Valverde, Á. M., Serra, D., & Herrero, L. (2016). Carnitine Palmitoyltransferase 1 Increases Lipolysis, UCP1 Protein Expression and Mitochondrial Activity in Brown Adipocytes. *PloS one*, 11(7), e0159399.

Chang, C. L., Lin, C. S., & Lai, G. H. (2012). Phytochemical characteristics, free radical scavenging activities, and neuroprotection of five medicinal plant extracts. *Evidence-based complementary and alternative medicine:* eCAM, 2012, 984295.

Chaudhary, S. A., Chaudhary, P. S., Syed, B. A., Misra, R., Bagali, P. G., Vitalini, S., & Iriti, M. (2018). Validation of a method for diosgenin extraction from fenugreek (*Trigonella foenum-graecum* L.). *Acta Scientiarum Polonorum Technologia Alimentaria*, 17(4), 377-385.

Choi, J. S., Kim, J. H., Ali, M. Y., Min, B. S., Kim, G. D., & Jung, H. A. (2014). *Coptis chinensis* alkaloids exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBP-α and PPAR-γ. *Fitoterapia*, *98*, 199–208.

Cornago D. F., Rowena Grace O. R., and Inacrist M. G. (2011) Philippine Yam (*Dioscorea* spp.) Tubers Phenolic Content and Antioxidant Capacity Philippine. *Journal of Science* 140 (2): 145-152.

Coursey DG (1967) Yams; An account of the Nature, Origins, Cultivation and Utilisation of the Useful Member of the *Dioscoreaceae*. Longmans, London.

Devlin, M. J., Yanovski, S. Z., & Wilson, G. T. (2000). Obesity: what mental health professionals need to know. *The American journal of psychiatry*, *157*(6), 854–866.

Du H., (2010). Antiobesity and hypolipidemic effects of lotus leaf hot water extract with taurine supplementation in rats fed a high fat diet. *Journal of Biomedical Science* 17: S42.

Dudhia, Z., Louw, J., Muller, C., Joubert, E., de Beer, D., Kinnear, C., & Pheiffer, C. (2013). *Cyclopia maculata* and *Cyclopia subternata (*honeybush tea) inhibits adipogenesis in 3T3-L1 pre-adipocytes. *Phytomedicine: international journal of phytotherapy and phytopharmacology*, *20*(5), 401–408.

Dzomba P, and Musekiwa C. (2014). Anti-obesity and antioxidant activity of dietary flavonoids from *Dioscorea steriscus* tubers., *Journal of Coastal Life Medicine*, Volume 2, Number 6, 2014, pp. 465-470(6)

Edison S., M.Unnikrishnan, B.Vimala, Santha V.Pillai, M.N.Sheela, M.T. Sreekumari and K.Abraham. 2006. Biodiversity of Tropical Tuber Crops in India. NBA Scientific Bulletin Number - 7, National Biodiversity Authority, Chennai, TamilNadu, India, p60.

Edwards, A. L., Jenkins, R. L., Davenport, L. J., & Duke, J. A. (2002). Presence of diosgenin in *Dioscorea batatas* (Dioscoreaceae). *Economic Botany*, 56(2), 204–206.

Ekeleme-Egedigwe, C. A., Ijeh, I. I., & Okafor, P. N. (2017). Modulatory effects of dietary supplementation by *Vernonia amygdalina* on high-fat-diet-induced obesity in Wistar rats. *Acta scientiarum polonorum. Technologia alimentaria*, *16*(4), 431–442.

El Ayed, M., Kadri, S., Smine, S., Elkahoui, S., Limam, F., & Aouani, E. (2017). Protective effects of grape seed and skin extract against high-fat-diet-induced lipotoxicity in rat lung. *Lipids in health and disease*, *16*(1), 174.

Ezeabara, C. A., and Anona, R. O. (2018) Comparative Analyses of Phytochemical and Nutritional Compositions of Four Species of *Dioscorea*. *Acta Scientific Nutritional Health*. Volume 2 Issue 7.

Ezure, T., & Amano, S. (2011). *Rubus suavissimus* S. Lee extract increases early adipogenesis in 3T3-L1 preadipocytes. *Journal of natural medicines*, *65*(2), 247–253.

Farkas, G. J., Gorgey, A. S., Dolbow, D. R., Berg, A. S., & Gater, D. R. (2018). The influence of level of spinal cord injury on adipose tissue and its relationship to inflammatory adipokines and cardiometabolic profiles. *The journal of spinal cord medicine*, *41*(4), 407–415.

Farombi, E. O., Britton, G., & Emerole, G. O. (2000). Evaluation of the antioxidant activity and partial characterisation of extracts from browned yam flour diet. *Food Research International*, *33*(6), 493-499.

Ferré P. (2004). The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes*, 53 Suppl 1, S43–S50.

Forbes A, D Henley and D Henley (2013) 'Pedanius Dioscorides' *In*: Health and Well Being: A Medieval Guide. Chiang Mai, *Cognoscenti Books*, Thailand.

Forman L. and Bridson D. (1989) *The herbarium Handbook*, Royal Botanical Gardens,

Freitas J., Luciano M., and Eduardo B. de Almeida Jr. (2017) "Medicinal plants for the treatment of obesity: ethnopharmacological approach and chemical and biological studies. *American Journal of Translational Research* 9, no. 5 (2017): 2050 -2064.

Freytag, S. O., Paielli, D. L., & Gilbert, J. D. (1994). Ectopic expression of the CCAAT/enhancer-binding protein alpha promotes the adipogenic program in a variety of mouse fibroblastic cells. *Genes & development*, *8*(14), 1654–1663.

Frühbeck, G., Becerril, S., Sáinz, N., Garrastachu, P., & García-Velloso, M. J. (2009). BAT: a new target for human obesity?. *Trends in pharmacological sciences*, *30*(8), 387–396.

Fu, J., Oveisi, F., Gaetani, S., Lin, E., & Piomelli, D. (2005). Oleoylethanolamide, an endogenous PPAR-alpha agonist, lowers body weight and hyperlipidemia in obese rats. *Neuropharmacology*, *48*(8), 1147–1153.

Gadde, K. M., Martin, C. K., Berthoud, H. R., & Heymsfield, S. B. (2018). Obesity: Pathophysiology and Management. *Journal of the American College of Cardiology*, *71*(1), 69–84.

Gao, Q. H., Wu, C. S., & Wang, M. (2013). The jujube (*Ziziphus jujuba* Mill.) fruit: a review of current knowledge of fruit composition and health benefits. *Journal of agricultural and food chemistry*, *61*(14), 3351–3363.

Gesta, S., Tseng, Y. H., & Kahn, C. R. (2007). Developmental origin of fat: tracking obesity to its source. *Cell*, 131(2), 242–256.

Ghosh, S., Derle, A., Ahire, M., More, P., Jagtap, S., Phadatare, S. D., Patil, A. B., Jabgunde, A. M., Sharma, G. K., Shinde, V. S., Pardesi, K., Dhavale, D. D., & Chopade, B. A. (2013). Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. *PloS one*, 8(12), e82529.

Gosmann, G., Barlette, A. G., Dhamer, T., Arçari, D. P., Santos, J. C., de Camargo, E. R., Acedo, S., Gambero, A., Gnoatto, S. C., & Ribeiro, M. L. (2012). Phenolic compounds from maté (*Ilex paraguariensis*) inhibit adipogenesis in 3T3-L1 preadipocytes. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *67*(2), 156–161.

Grasa-López, A., Miliar-García, Á., Quevedo-Corona, L., Paniagua-Castro, N., Escalona-Cardoso, G., Reyes-Maldonado, E., & Jaramillo-Flores, M. E. (2016). *Undaria pinnatifida* and Fucoxanthin Ameliorate Lipogenesis and ers of Both Inflammation and Cardiovascular Dysfunction in an Animal Model of Diet-Induced Obesity. *Marine drugs*, 14(8), 148.

Gupta, P., Goyal, R., Chauhan, Y., & Sharma, P. L. (2013). Possible modulation of FAS and PTP-1B signaling in ameliorative potential of *Bombax ceiba* against high fat diet induced obesity. *BMC complementary and alternative medicine*, *13*, 281.

Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta medica*, *29*(2), 116–122.

Ho, J. N., Son, M. E., Lim, W. C., Lim, S. T., & Cho, H. Y. (2012). Anti-obesity effects of germinated brown rice extract through down-regulation of lipogenic genes in high fat diet-induced obese mice. *Bioscience, biotechnology, and biochemistry*, *76*(6), 1068–1074.

Ho, J.-N., Park, S.-J., Choue, R., & Lee, J. (2013). Standardized Ethanol Extract of *Curcuma longa* L. Fermented by *Aspergillus oryzae* Promotes Lipolysis via Activation of cAMP-Dependent PKA in 3T3-L1 Adipocytes. *Journal of Food Biochemistry,* 37: 595-603.

Hornel J (1934) Indonesian influence on East Afrixcan culture. J R Anthrop. Inst. 64: 3058–3332.

Hsu, C. (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry, 83(1), 85–92.*

Hsu, H.-M., Chen, W.-Y., Pan, P.-H., & Mao, F. C. (2014). *Vitis thunbergii* supplementation demonstrates an anti-obesity effect in developing obese mice. *European Journal of Integrative Medicine, 6(5), 581–587.*

Hua, S., Li, Y., Su, L., & Liu, X. (2016). Diosgenin ameliorates gestational diabetes through inhibition of sterol regulatory element-binding protein-1. *Biomedicine & Pharmacotherapy,* 84, 1460–1465.

Ignjatovic, V., Ogru, E., Heffernan, M., Libinaki, R., Lim, Y., & Ng, F. (2000). Studies on the use of *"slimax",* a chinese herbal mixture, in the treatment of human obesity. *Pharmaceutical Biology, 38(1), 30–35.*

Ikete, P., & Chinko, B. (2022). Inhibition of Serum Lipase as a Mechanism of Action for Anti-Obesity Potentials of *Dioscorea bulbifera* Extracts on Wistar Rats. *Asian Journal of Health Sciences*, *8*(1), ID29.

Ilavenil, S., Kim, d., Vijayakumar, M., Srigopalram, S., Roh, S. G., Arasu, M. V., Lee, J. S., & Choi, K. C. (2016). Potential role of marine algae extract on 3T3-L1 cell proliferation and differentiation: an in vitro approach. *Biological research*, *49*(1), 38.

Jeon, J. R., Lee, J. S., Lee, C. H., Kim, J. Y., Kim, S. D., & Nam, D. H. (2006). Effect of ethanol extract of dried Chinese yam (*Dioscorea batatas*) flour containing dioscin on gastrointestinal function in rat model. *Archives of pharmacal research*, *29*(5), 348–353.

Jeong, E. J., Jegal, J., Ahn, J., Kim, J., & Yang, M. H. (2016). Anti-obesity Effect of *Dioscorea oppositifolia* Extract in High-Fat Diet-Induced Obese Mice and Its Chemical Characterization. *Biological & pharmaceutical bulletin*, *39*(3), 409–414.

Jeong, Y. S., Hong, J. H., Cho, K. H., & Jung, H. K. (2012). Grape skin extract reduces adipogenesis- and lipogenesis-related gene expression in 3T3-L1 adipocytes through the peroxisome proliferator-activated receptor-γ signaling pathway. *Nutrition research (New York, N.Y.)*, *32*(7), 514–521.

Jeong, Y. S., Jung, H. K., Cho, K.-H., Youn, K.-S., & Hong, J.-H. (2011). Anti-obesity effect of grape skin extract in 3T3-L1 adipocytes. *Food Science and Biotechnology, 20(3), 635–642.*

Ju, J. H., Yoon, H. S., Park, H. J., Kim, M. Y., Shin, H. K., Park, K. Y., Yang, J. O., Sohn, M. S., & Do, M. S. (2011). Anti-obesity and antioxidative effects of purple sweet potato extract in 3T3-L1 adipocytes in vitro. *Journal of medicinal food*, *14*(10), 1097–1106.

Juge-Aubry, C. E., Henrichot, E., & Meier, C. A. (2005). Adipose tissue: a regulator of inflammation. *Best practice & research. Clinical endocrinology & metabolism*, *19*(4), 547–566.

Kajimura, S., & Saito, M. (2014). A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. *Annual review of physiology*, *76*, 225–249.

Kang, H. J., Seo, H. A., Go, Y., Oh, C. J., Jeoung, N. H., Park, K. G., & Lee, I. K. (2013). Dimethylfumarate suppresses adipogenic differentiation in 3T3-L1 preadipocytes through inhibition of STAT3 activity. *PloS one*, *8*(4), e61411.

Kang, H., & Koppula, S. (2014). *Houttuynia cordata* Attenuates Lipid Accumulation via Activation of AMP-Activated Protein Kinase Signaling Pathway in HepG2 Cells. *The American Journal of Chinese Medicine, 42(03), 651–664.*

Kang, J. S., Lee, W. K., Lee, C. W., Yoon, W. K., Kim, N., Park, S. K., Lee, H. S., Park, H. K., Han, S. B., Yun, J., Lee, K., Lee, K. H., Park, S. K., & Kim, H. M. (2011). Improvement of high-fat diet-induced obesity by a mixture of red grape extract, soy isoflavone and L-carnitine: implications in cardiovascular and non-alcoholic fatty liver diseases. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *49*(9), 2453–2458.

Kang, Y. H., Lee, Y.-S., Kim, K. K., Kim, D. J., Kim, T. W., & Choe, M. (2013). Study on antioxidative, antidiabetic and antiobesity activity of solvent fractions of *Smilax china L*. leaf extract. *Journal of Nutrition and Health, 46(5), 401.*

Karak, P. (2019). Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research*, *10*(4), 1567-1574.

Karmase, A., Birari, R., & Bhutani, K. K. (2013). Evaluation of anti-obesity effect of *Aegle marmelos* leaves. *Phytomedicine, 20(10), 805–812.*

Karmase, A., Jagtap, S., & Bhutani, K. K. (2013). Anti adipogenic activity of *Aegle marmelos* Correa. *Phytomedicine, 20(14), 1267–1271.*

Kaur, C., & Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*, *37*(2), 153-161.

Kaushal, S. K., Shailesh, K., Brijendra, S., Saurabh, B., Nisha, P., Ishan, P. K., & Prakash, B. S. (2019). Targeting fatty acid synthase protein by molecular docking studies of naturally occurring ganoderic acid analogues acting as anti-obesity molecule. *Research Journal of Biotechnology Vol*, *14*, 7.

Kazemipoor, M., Radzi, C. W., Hajifaraji, M., Haerian, B. S., Mosaddegh, M. H., & Cordell, G. A. (2013). Antiobesity effect of caraway extract on overweight and obese women: a randomized, triple-blind, placebo-controlled clinical trial. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 928582.

Khalid, M., & Siddiqui, H. H. (2012). Evaluation of weight reduction and anti–cholesterol activity of Punarnava root extract against high fat diets induced obesity in experimental rodent. *Asian Pacific Journal of Tropical Biomedicine, 2(3), S1323–S1328.*

Kim, G. S., Park, H. J., Woo, J. H., Kim, M. K., Koh, P. O., Min, W., Ko, Y. G., Kim, C. H., Won, C. K., & Cho, J. H. (2012). *Citrus aurantium* flavonoids inhibit adipogenesis through the Akt signaling pathway in 3T3-L1 cells. *BMC complementary and alternative medicine*, *12*, 31.

Kim, H. R., Kim, J. M., Kim, M. S., Hwang, J. K., Yang, S. H., Kim, H. J., Lee, D. S., Oh, H., Kim, Y. C., Ryu, D. G., Lee, Y. R., & Kwon, K. B. (2014). Inhibitory effects of *Pericarpium zanthoxyli* extract on adipocyte differentiation. *International journal of molecular medicine*, *33*(5), 1140–1146.

Kim, H., Bartley, G. E., Arvik, T., Lipson, R., Nah, S.-Y., Seo, K., & Yokoyama, W. (2014). Dietary Supplementation of Chardonnay Grape Seed Flour Reduces Plasma Cholesterol Concentration, Hepatic Steatosis, and Abdominal Fat Content in High-Fat Diet-Induced Obese Hamsters. *Journal of Agricultural and Food Chemistry*, 62(8), 1919–1925.

Kim, H.J., Hwang, J.T., Kim, M.J., Yang, H.J., Sung, M.J., Kim, S.H., Park, S., Gu, E.J., Park, Y. and Kwon, D.Y., (2014). The inhibitory effect of saponin derived from Cheonggukjang on adipocyte differentiation In vitro. *Food Science and Biotechnology*, 23(4), 1273–1278.

Kim, O. Y., Lee, S. M., Do, H., Moon, J., Lee, K. H., Cha, Y. J., & Shin, M. J. (2012). Influence of quercetin-rich onion peel extracts on adipokine expression in the visceral adipose tissue of rats. *Phytotherapy Research*, 26(3), 432-437.

Kim, Y. M., Lee, E. W., Eom, S. H., & Kim, T. H. (2014). Pancreatic lipase inhibitory stilbenoids from the roots of *Vitis vinifera. International journal of food sciences and nutrition*, *65*(1), 97–100.

Kim, Y. S., Lee, Y. M., Kim, H., Kim, J., Jang, D. S., Kim, J. H., & Kim, J. S. (2010). Anti-obesity effect of *Morus bombycis* root extract: anti-lipase activity and lipolytic effect. *Journal of ethnopharmacology*, *130*(3), 621–624.

Kong, C.-S., & Seo, Y. (2012). Antiadipogenic activity of isohamnetin 3-O-β-D-glucopyranoside from *Salicornia herbacea*. *Immunopharmacology and Immunotoxicology, 34(6), 907–911.*

Kong, H-S., Lee, S-W., Shin, S-M., Kwon, J-H., Jo, T-H.,Shin, E-J., Shim, K-S., Park, Y-I., Lee, C-K., & Kim, K-J., (2010). Down-regulation of adipogenesis and hyperglycemia in diet-induced obesity mouse model by Aloe QDM. *Biomolecules & Therapeutics*, *18*(3), 336-342.

Kubota, H., Morii, R., Kojima-Yuasa, A., Huang, X., Yano, Y., & Matsui-Yuasa, I. (2009). Effect of *Zizyphus jujuba* extract on the inhibition of adipogenesis in 3T3-L1 preadipocytes. *The American journal of Chinese medicine*, *37*(3), 597–608.

Kumar, S., & Alagawadi, K. R. (2013). Anti-obesity effects of galangin, a pancreatic lipase inhibitor in cafeteria diet fed female rats. *Pharmaceutical biology*, *51*(5), 607–613.

Kumar, S., Alagawadi, K. R., & Rao, M. R. (2011). Effect of *Argyreia speciosa* root extract on cafeteria diet-induced obesity in rats. *Indian journal of pharmacology*, *43*(2), 163–167.

Kwon, C. S., Sohn, H. Y., Kim, S. H., Kim, J. H., Son, K. H., Lee, J. S., Lim, J. K., & Kim, J. S. (2003). Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. *Bioscience, biotechnology, and biochemistry*, *67*(7), 1451–1456.

Laliotis, G. P., Bizelis, I., & Rogdakis, E. (2010). Comparative Approach of the de novo Fatty Acid Synthesis (Lipogenesis) between Ruminant and Non Ruminant Mammalian Species: From Biochemical Level to the Main Regulatory Lipogenic Genes. *Current genomics*, *11*(3), 168–183.

Lee, J. J., Park, M. R., Kim, A. R., & Lee, M. Y. (2011). Effects of ramie leaves on improvement of lipid metabolism and antiobesity effect in rats fed a high fat/high cholesterol diet. *Korean Journal of Food Science and Technology*, *43*(1), 83-90.

Lee, J., Kim, D., Choi, J., Choi, H., Ryu, J. H., Jeong, J., Park, E. J., Kim, S. H., & Kim, S. (2012). Dehydrodiconiferyl alcohol isolated from *Cucurbita moschata* shows anti-adipogenic and anti-lipogenic effects in 3T3-L1 cells and primary mouse embryonic fibroblasts. *The Journal of biological chemistry*, *287*(12), 8839–8851.

Lee, M., Song, J. Y., Chin, Y.-W., & Sung, S. H. (2013). Anti-adipogenic diarylheptanoids from *Alnus hirsuta F*. sibirica on 3T3-L1 cells. *Bioorganic & Medicinal Chemistry Letters, 23(7), 2069–2073.*

Lei, F., Zhang, X. N., Wang, W., Xing, D. M., Xie, W. D., Su, H., & Du, L. J. (2007). Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *International journal of obesity (2005)*, *31*(6), 1023–1029.

Li, H., Yu, L., & Zhao, C. (2019). Dioscin attenuates high-fat diet-induced insulin resistance of adipose tissue through the IRS-1/PI3K/Akt signaling pathway. *Molecular medicine reports*, *19*(2), 1230–1237.

Li, T., Teng, H., , An, F., , Huang, Q., , Chen, L., , & Song, H., (2019). The beneficial effects of purple yam (*Dioscorea alata* L.) resistant starch on hyperlipidemia in high-fat-fed hamsters. *Food & function*, *10*(5), 2642–2650.

Liang, C. H., Chan, L. P., Chou, T. H., Chiang, F. Y., Yen, C. M., Chen, P. J., Ding, H. Y., & Lin, R. J. (2013). Brazilein from *Caesalpinia sappan* L. Antioxidant Inhibits Adipocyte Differentiation and Induces Apoptosis through Caspase-3 Activity and Anthelmintic Activities against *Hymenolepis nana* and *Anisakis simplex*. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 864892.

Lieberman, S. (2004). A New Potential Weapon for Fighting Obesity: Forskolin—the Active Diterpene in Coleus. *Alternative and Complementary Therapies, 10(6), 330–333.*

Lindley, J. (1853). The vegetable kingdom, or, The structure, classification, and uses of plant*s* (3rd ed., with corrections and additional genera.). Bradbury and Evans.

Linnaeus C (1737) Flora Zeylanica. Laurentius Salvius, Stockholm 170–172.

Liu, R.H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American journal of clinical nutrition, 78 3 Suppl*, 517S-520S .

Liu, Y., Li, H., Fan, Y., Man, S., Liu, Z., Gao, W., & Wang, T. (2016). Antioxidant and Antitumor Activities of the Extracts from Chinese Yam (*Dioscorea opposite* Thunb.) Flesh and Peel and the Effective Compounds. *Journal of food science*, *81*(6), H1553–H1564.

Louise, E.O., Marju, O.M., Lena, W.O., Kajsa, S.H., Lars, S., Leif, G., Bjorn, C., Lena, M.S. C., Bob, O., (2008). CCAAT/enhancer binding protein (C/EBP) in adipose tissue regulates genes in lipid and glucose metabolism and a genetic variation in C/EBP is associated with serum levels of triglycerides. *The Journal of Clinical Endocrinology & Metabolism*. 93 (12), 4880–4886.

Lowe, M. E. (1997). Structure and Function of Pancreatic Lipase and Colipase. *Annual Review of Nutrition, 17(1), 141–158.*

Luximon-Ramma, A., Bahorun, T., Soobrattee, M. A., & Aruoma, O. I. (2002). Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of Cassia fistula. *Journal of agricultural and food chemistry*, 50(18), 5042-5047.

Mabberley DJ (2008) Mabberly's Plant Book – A Portable Dictionary of Plants, Their Classification and Uses. Third Edition, *Cambridge University Press* pp 276–277.

Manna, P., & Jain, S. K. (2015). Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metabolic syndrome and related disorders*, *13*(10), 423–444.

Marrelli, M., Loizzo, M. R., Nicoletti, M., Menichini, F., & Conforti, F. (2013). Inhibition of key enzymes linked to obesity by preparations from Mediterranean dietary plants: effects on α-amylase and pancreatic lipase activities. *Plant foods for human nutrition*, *68*(4), 340-346.

Matsusue, K., Peters, J. M., & Gonzalez, F. J. (2004). PPARβ/δ potentiates PPARγ-stimulated adipocyte differentiation. *The FASEB Journal, 18(12), 1477–1479.*

Merola, N., Castillo, J., Benavente-García, O., Ros, G., & Nieto, G. (2017). The Effect of Consumption of Citrus Fruit and Olive Leaf Extract on Lipid Metabolism. *Nutrients*, *9*(10), 1062.

Miyata, M., Koyama, T., & Yazawa, K. (2010). Water extract of *Houttuynia cordata* Thunb. leaves exerts anti-obesity effects by inhibiting fatty acid and glycerol absorption. *Journal of nutritional science and vitaminology*, *56*(2), 150–156.

Mohamed, G. A., Ibrahim, S. R. M., Elkhayat, E. S., & El Dine, R. S. (2014). Natural anti-obesity agents. *Bulletin of Faculty of Pharmacy, Cairo University, 52(2), 269–284.*

Moon, J., Do, H.-J., Kim, O. Y., & Shin, M.-J. (2013). Antiobesity effects of quercetin-rich onion peel extract on the differentiation of 3T3-L1 preadipocytes and the adipogenesis in high fat-fed rats. *Food and Chemical Toxicology, 58, 347–354.*

Moro, C. O., & Basile, G. (2000). Obesity and medicinal plants. *Fitoterapia, 71 Suppl 1*, S73–S82.

Morton, G. J., Meek, T. H., & Schwartz, M. W. (2014). Neurobiology of food intake in health and disease. *Nature reviews. Neuroscience, 15*(6), 367–378.

Mostafa, U. E., & Labban, L. (2013). The effect of *Zizyphus jujube* on serum lipid profile and some anthropometric measurements. *Pakistan Journal of Nutrition, 12*(6), 538-543.

Mukherjee, M. (2003). Human digestive and metabolic lipases—a brief review. *Journal of Molecular Catalysis B: Enzymatic, 22(5-6), 369–376.*

Munshi, R. P., Joshi, S. G., & Rane, B. N. (2014). Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. *Indian journal of pharmacology, 46*(3), 270–276.

Narayana, D. B. A., Katayar, C. K., & Brindavanam, N. B. (1998). Original system: search, research or re-search. *IDMA Bulletin, 29*(17), 413-416.

Ngondi, J. L., Etoundi, B. C., Nyangono, C. B., Mbofung, C. M., & Oben, J. E. (2009). IGOB131, a novel seed extract of the West African plant *Irvingia gabonensis*, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. *Lipids in health and disease, 8*, 7.

Niño, J., Jiménez, D. A., Mosquera, O. M., & Correa, Y. M. (2007). Diosgenin quantification by HPLC in a *Dioscorea polygonoides* tuber collection from colombian flora. *Journal of the Brazilian Chemical Society, 18(5), 1073–1076.*

Nugara, R. N., Inafuku, M., Iwasaki, H., & Oku, H. (2014). Partially purified *Peucedanum japonicum* Thunb extracts exert anti-obesity effects in vitro. *Nutrition (Burbank, Los Angeles County, Calif.), 30*(5), 575–583.

Nugara, R. N., Inafuku, M., Takara, K., Iwasaki, H., & Oku, H. (2014). Pteryxin: A coumarin in *Peucedanum japonicum* Thunb leaves exerts antiobesity activity through modulation of adipogenic gene network. *Nutrition, 30(10), 1177–1184.*

O'Neill, H. M., Holloway, G. P., & Steinberg, G. R. (2013). AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: Implications for obesity. *Molecular and Cellular Endocrinology, 366(2), 135–151.*

Odimegwu, J. I., Odukoya, O., Yadav, R. K., Chanotiya, C. S., Ogbonnia, S., & Sangwan, N. S. (2013*).* A New Source of Elemol Rich Essential Oil and Existence of Multicellular Oil Glands in Leaves of the *Dioscorea* Species. *The Scientific World Journal, 2013, 1–6.*

Ogbuagu, M.N., (2008). Nutritive and Anti-Nutritive Composition of the Wild (In-Edible) Species of *Dioscorea bulbifera* (Potato Yam) and *Dioscorea dumentorum* (Bitter Yam). *Journal of Food Technology*, 6: 224-226.

Oh, J., JUNG, S. R., LEE, Y. J., Park, K. W., Kim, S. Y., & Han, J. (2013). Antioxidant and antiobesity activities of seed extract from campbell early grape as a functional ingredient. *Journal of Food Processing and Preservation*, 37(4), 291-298.

Oh, S.D., Kim, M., Min, B.I., Choi, G.S., Kim, S.K., Bae, H., Kang, C., Kim, D.G., Park, B.J. and Kim, C.K., (2014). Effect of *Achyranthes bidentata* Blume on 3T3-L1 adipogenesis and rats fed with a high-fat diet. *Evidence-Based Complementary and Alternative Medicine,* 2014.

Osbaldeston, T. A., & Wood, R. P. (2000). Dioscorides: De materia medica.

Park, H. J., Chung, B. Y., Lee, M. K., Song, Y., Lee, S. S., Chu, G. M., Kang, S. N., Song, Y. M., Kim, G. S., & Cho, J. H. (2012). Centipede grass exerts anti-adipogenic activity through inhibition of C/EBPβ, C/EBPα, and PPARγ expression and the AKT signaling pathway in 3T3-L1 adipocytes. *BMC complementary and alternative medicine*, *12*, 230.

Park, J., Jeon, Y. D., Kim, H. L., Lim, H., Jung, Y., Youn, D. H., Jeong, M. Y., Kim, H. J., Kim, S. H., Kim, S. J., Hong, S. H., & Um, J. Y. (2013). Interaction of *Veratrum nigrum* with *Panax ginseng* against Obesity: A Sang-ban Relationship. *Evidence-based complementary and alternative medicine: eCAM*, *2013*, 732126.

Park, J., Jin, K. S., Lee, J. Y., Kwon, H. J., & Kim, B. W. (2013). Anti-oxidative and anti-obesity activities of *Tetrapanax papyriferus* and *Siegesbeckia pubescens* extracts and their synergistic anti-obesity effects. *Microbiology and Biotechnology Letters*, *41*(3), 341-349.

Park, J., Jin, K. S., Lee, J. Y., Kwon, H. J., & Kim, B. W. (2014). Anti-oxidative and anti-obesity effects of *Amomum Cardamomum* L. extract. *Microbiology and Biotechnology Letters*, *42*(3), 249-257.

Park, M., Sharma, A., & Lee, H. J. (2019). Anti-adipogenic effects of delphinidin-3-O-β-glucoside in 3T3-L1 preadipocytes and primary white adipocytes. *Molecules*, *24*(10), 1848.

Patil, D. N., Yadav, S. R., Patil, S., Bapat, V. A., & Jadhav, J. P. (2020). Multidimensional studies of *Pancratium parvum* Dalzell against acetylcholinesterase: a potential enzyme for Alzheimer's management. *Journal of the American College of Nutrition*, *39*(7), 601-618.

Patra, S., Nithya, S., Srinithya, B., & Meenakshi, S. M. (2015). Review of medicinal plants for anti-obesity activity. *Translational Biomedicine*, *6*(3), 0-0.

Poornima, G. N., & Ravishankar, R. V. (2009). Evaluation of phytonutrients and vitamin contents in a wild yam, *Dioscorea belophylla* (Prain) Haines. *African Journal of Biotechnology*, *8*(6).

Poudel, B., Lim, S. W., Ki, H. H., Nepali, S., Lee, Y. M., & Kim, D. K. (2014). Dioscin inhibits adipogenesis through the AMPK/MAPK pathway in 3T3-L1 cells and modulates fat accumulation in obese mice. *International Journal of Molecular Medicine*, *34*(5), 1401-1408.

Rahim, A. T. M., Takahashi, Y., & Yamaki, K. (2015). Mode of pancreatic lipase inhibition activity in vitro by some flavonoids and non-flavonoid polyphenols. *Food Research International*, *75*, 289-294.

Raina, A. P., & Misra, R. (2019). Evaluation of diosgenin, a bioactive compound from natural source of *Dioscorea* species: A wild edible tuber plant. *Journal of Pharmacognosy and Phytochemistry*, *9*(1), 1120-1124.

Ramírez, N.M., Toledo, R.C.L., Moreira, M.E.C., Martino, H.S.D., dos Anjos Benjamin, L., de Queiroz, J.H., Ribeiro, A.Q. and Ribeiro, S.M.R., (2017). Anti-obesity effects of tea from *Mangifera indica* L. leaves of the Ubá variety in high-fat diet-induced obese rats. *Biomedicine & Pharmacotherapy*, *91*, 938-945.

Rayalam, S., Della-Fera, M. A., & Baile, C. A. (2008). Phytochemicals and regulation of the adipocyte life cycle. *The Journal of nutritional biochemistry*, *19*(11), 717-726.

Redinger, R. N. (2009). Fat storage and the biology of energy expenditure. *Translational Research*, *154*(2), 52-60.

Resende, P. E., Verza, S. G., Kaiser, S., Gomes, L. F., Kucharski, L. C., & Ortega, G. G. (2012). The activity of mate saponins (*Ilex paraguariensis*) in intra-abdominal and epididymal fat, and glucose oxidation in male Wistar rats. *Journal of ethnopharmacology*, *144*(3), 735–740.

Rezaee, F., & Dashty, M. (2013). Role of adipose tissue in metabolic system disorders adipose tissue is the initiator of metabolic diseases. *Journal of Diabetes and Metabolic Disorders*, *13*(2).

Roh, C., & Jung, U. (2012). *Nepeta japonica* Maximowicz extract from natural products inhibits lipid accumulation. *Journal of the Science of Food and Agriculture*, *92*(10), 2195-2199.

Romano, A., Coccurello, R., Giacovazzo, G., Bedse, G., Moles, A., & Gaetani, S. (2014). Oleoylethanolamide: a novel potential pharmacological alternative to cannabinoid antagonists for the control of appetite. *BioMed Research International*, *2014*.

Rosidah, Yam, M., Sadikun, A., & Asmawi, M. (2008). Antioxidant potential of Gynura procumbens. *Pharmaceutical Biology*, *46*(9), 616-625.

Sakthidevi, G., & Mohan, V. R. (2013). Total phenolic, flavonoid contents and in vitro antioxidant activity of Dioscorea alata L. tuber. *Journal of Pharmaceutical Sciences and Research*, *5*(5), 115.

Sam, S., & Mazzone, T. (2014). Adipose tissue changes in obesity and the impact on metabolic function. *Translational Research*, *164*(4), 284-292.

Sari, D. R. T., Safitri, A., Cairns, J. R. K., & Fatchiyah, F. (2020, October). Virtual screening of black rice anthocyanins as antiobesity through inhibiting TLR4 and JNK pathway. *Journal of Physics: Conference Series* (Vol. 1665, No. 1, p. 012024).

Sarker, S. D., & Nahar, L. (2012). An introduction to natural products isolation. *Natural products isolation*, 1-25.

Shah, H. J., & Lele, S. S. (2012). Extraction of diosgenin, a bioactive compound from natural source Dioscorea alata Var purpurae. *Journal of Analytical & Bioanalytical Techniues*, *3*(4), 1-3.

Shikov, A. N., Pozharitskaya, O. N., Makarova, M. N., Makarov, V. G., & Wagner, H. (2014). *Bergenia crassifolia* (L.) Fritsch–Pharmacology and phytochemistry. *Phytomedicine*, *21*(12), 1534-1542.

Shin, E., Shim, K. S., Kong, H., Lee, S., Shin, S., Kwon, J., Jo, T. H., Park, Y. I., Lee, C. K., & Kim, K. (2011). Dietary aloe improves insulin sensitivity via the suppression of obesity-induced inflammation in obese mice. *Immune Network*, *11*(1), 59-67.

Singh, A. K., Patki, P. S., & Mitra, S. K. (2008). Evaluation of clinical efficacy of ayurslim on body weight, body mass index, lipid profile and skin fold thickness: a phase IV clinical trial. *The Antiseptic*, *105*(5), 241-243.

Singh, B. P., Vij, S., & Hati, S. (2014). Functional significance of bioactive peptides derived from soybean. *Peptides*, *54*, 171-179.

Singh, D. K., Srivastava, B., & Sahu, A. (2004). Spectrophotometric determination of Rauwolfia alkaloids: estimation of reserpine in pharmaceuticals. *Analytical sciences*, 20(3), 571-573.

Slanc, P., Doljak, B., Kreft, S., Lunder, M., Janeš, D., & Štrukelj, B. (2009). Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *23*(6), 874-877.

Son, I. S., Kim, J. H., Sohn, H. Y., Son, K. H., Kim, J. S., & Kwon, C. S. (2007). Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea spp*.), on high-cholesterol fed rats. *Bioscience, biotechnology, and biochemistry*, *71*(12), 3063-3071.

Song, H., Shen, X., Chu, Q., & Zheng, X. (2021). *Vaccinium bracteatum* Thunb. fruit extract reduces high-fat diet-induced obesity with modulation of the gut microbiota in obese mice. *Journal of Food Biochemistry*, *45*(7), e13808.

Song, M. Y., Lv, N., Kim, E. K., Kwon, K. S., Yoo, Y. B., Kim, J. H., ... & Kwon, K. B. (2009). Antiobesity activity of aqueous extracts of Rhizoma *Dioscoreae Tokoronis* on high-fat diet-induced obesity in mice. *Journal of medicinal food*, *12*(2), 304-309.

Song, M., Yang, G., Hoa, T. Q., Hieu, H. D., Amin, A., Choe, W., Kang, I., Kim, S. S., & Ha, J. (2020). Anti-obesity Effect of Fermented Persimmon Extracts via Activation of AMP-Activated Protein Kinase. *Biological & pharmaceutical bulletin*, *43*(3), 440–449.

Sonibare, M. A., & Abegunde, R. B. (2012). In vitro antimicrobial and antioxidant analysis of *Dioscorea dumetorum* (Kunth) Pax and *Dioscorea hirtiflora* (Linn.) and their bioactive metabolites from Nigeria. *Journal of Applied Biosciences*, *51*, 3583-3590.

Stoeckman, A. K., & Towle, H. C. (2002). The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. *Journal of Biological Chemistry*, *277*(30), 27029-27035.

Stohs, S. J., Preuss, H. G., & Shara, M. (2012). A review of the human clinical studies involving *Citrus aurantium* (bitter orange) extract and its primary protoalkaloid p-synephrine. *International journal of medical sciences*, *9*(7), 527.

Swinburn, B. A., Sacks, G., Hall, K. D., McPherson, K., Finegood, D. T., Moodie, M. L., & Gortmaker, S. L. (2011). The global obesity pandemic: shaped by global drivers and local environments. *The Lancet*, *378*(9793), 804-814.

Tamaru, S., Ohmachi, K., Miyata, Y., Tanaka, T., Kubayasi, T., Nagata, Y., & Tanaka, K. (2013). Hypotriglyceridemic potential of fermented mixed tea made with third-crop green tea leaves and Camellia (*Camellia japonica*) leaves in Sprague–Dawley rats. *Journal of agricultural and food chemistry*, *61*(24), 5817-5823.

Thapyai, C. (2004). *Taxonomic Revision of Dioscoreaceae in Thailand*. Kasetsart University, Graduate SchoolThesis

Toromanyan, E., Aslanyan, G., Amroyan, E., Gabrielyan, E., & Panossian, A. (2007). Efficacy of Slim339® in reducing body weight of overweight and obese human subjects. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(12), 1177-1181.

Totani, N., Tateishi, S., Takimoto, T., Maeda, Y., & Sasaki, H. (2011). Gallic acid glycerol ester promotes weight-loss in rats. *Journal of oleo science*, *60*(9), 457-462.

Trayhurn P. (2007). Adipocyte biology. *Obesity reviews: an official journal of the International Association for the Study of Obesity*, 8 Suppl 1, 41–44.

Tzeng, T. F., Lu, H. J., Liou, S. S., Chang, C. J., & Liu, I. M. (2014). Lipid-lowering effects of zerumbone, a natural cyclic sesquiterpene of Zingiber zerumbet Smith, in high-fat diet-induced hyperlipidemic hamsters. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, *69*, 132–139.

Udani, J., Hardy, M., & Madsen, D. C. (2004). Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2™ brand proprietary fractionated white bean extract. *Alternative medicine review*, *9*(1), 63-69.

Uemura, T., Goto, T., Kang, M. S., Mizoguchi, N., Hirai, S., Lee, J. Y., Nakano, Y., Shono, J., Hoshino, S., Taketani, K., Tsuge, N., Narukami, T., Makishima, M., Takahashi, N., & Kawada, T. (2011). Diosgenin, the main aglycon of fenugreek, inhibits LXRα activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice. *The Journal of nutrition*, *141*(1), 17–23.

Velusami, C. C., Agarwal, A., & Mookambeswaran, V. (2013). Effect of Nelumbo nucifera petal extracts on lipase, adipogenesis, adipolysis, and central receptors of obesity. *Evidence-Based Complementary and Alternative Medicine, 2013*.

Wafer, R., Tandon, P., & Minchin, J. E. (2017). The role of peroxisome proliferator-activated receptor gamma (PPARG) in adipogenesis: Applying knowledge from the fish aquaculture industry to biomedical research. *Frontiers in endocrinology*, *8*, 102..

Wang, X., Liu, J., Long, Z., Sun, Q., Liu, Y., Wang, L., Zhang, X., & Hai, C. (2015). Effect of diosgenin on metabolic dysfunction: Role of ERβ in the regulation of PPARγ. *Toxicology and applied pharmacology*, *289*(2), 286–296.

Watanabe, T., Hata, K., Hiwatashi, K., Hori, K., Suzuki, N., & Itoh, H. (2010). Suppression of murine preadipocyte differentiation and reduction of visceral fat accumulation by a *Petasites japonicus* ethanol extract in mice fed a high-fat diet. *Bioscience, biotechnology, and biochemistry*, *74*(3), 499-503.

Wolfe, K. L., & Liu, R. H. (2003). Apple peels as a value-added food ingredient. *Journal of Agricultural and Food Chemistry*, *51*(6), 1676-1683.

Wozniak, S. E., Gee, L. L., Wachtel, M. S., & Frezza, E. E. (2009). Adipose tissue: the new endocrine organ? A review article. *Digestive diseases and sciences*, *54*(9), 1847-1856.

Yamamoto, Y., & Oue, E. (2006). Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. *Bioscience, biotechnology, and biochemistry*, *70*(4), 933-939.

Yang, H., Chen, B., Wang, X., Chue, P., Shen, Y., Xia, G., & Jia, X. (2013). Rapid quantitative analysis of diosgenin in the tubers of *Dioscorea zingiberensis* C.H. Wright by coupling cellulose enzymolysis and two-phase acid hydrolysis in tandem with HPLC-UV. *Natural Product Research,* 27(20), 1933–1935.

Yang, J., Martinson, T. E., & Liu, R. H. (2009). Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry*, *116*(1), 332-339.

Yang, M. H., Chin, Y. W., Chae, H. S., Yoon, K. D., & Kim, J. (2014). Anti-adipogenic constituents from *Dioscorea opposita* in 3T3-L1 cells. *Biological and Pharmaceutical Bulletin*, *37*(10), 1683-1688.

Yazici-Tutunis, S., Gurel-Gurevin, E., Ustunova, S., Demirci-Tansel, C., & Mericli, F. (2016). Possible effects of *Phillyrea latifolia* on weight loss in rats fed a high-energy diet. *Pharmaceutical Biology*, *54*(10), 1991-1997.

You, J. S., Lee, Y. J., Kim, K. S., Kim, S. H., & Chang, K. J. (2014). Anti-obesity and hypolipidaemic effects of *Nelumbo nucifera* seed ethanol extract in human pre-adipocytes and rats fed a high-fat diet. *Journal of the Science of Food and Agriculture*, *94*(3), 568-575.

Zhang, X. H., Huang, B., Choi, S. K., & Seo, J. S. (2012). Anti-obesity effect of resveratrol-amplified grape skin extracts on 3T3-L1 adipocytes differentiation. *Nutrition research and practice*, *6*(4), 286-293.

Zheng, C. D., Duan, Y. Q., Gao, J. M., & Ruan, Z. G. (2010). Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs. *Journal of the Chinese Medical Association*, *73*(6), 319-324.

Zhu, J., Jin, J., Ding, J., Li, S., Cen, P., Wang, K., Wang, H., & Xia, J. (2018). Ganoderic Acid A improves high fat diet-induced obesity, lipid accumulation and insulin sensitivity through regulating SREBP pathway. *Chemico-biological interactions*, *290*, 77–87.

Phytomedicine Plus 2 (2022) 100293

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Dioscorea oppositifolia plant extract reduces adipogenesis by down-regulating PPARγ, C/EBPα, SREBP-1, and FASN in 3T3L1 pro-adipocytes

Ruturaj S. Patil [a], Mahesh P. Mane [a], Aditya B. Magdum [b], Mansingraj S. Nimbalkar [b,*]

[a] Department of Biotechnology, Shivaji University, Kolhapur, Maharashtra, India
[b] Department of Botany, Shivaji University, Vidya Nagar, Kolhapur, Maharashtra 416004, India

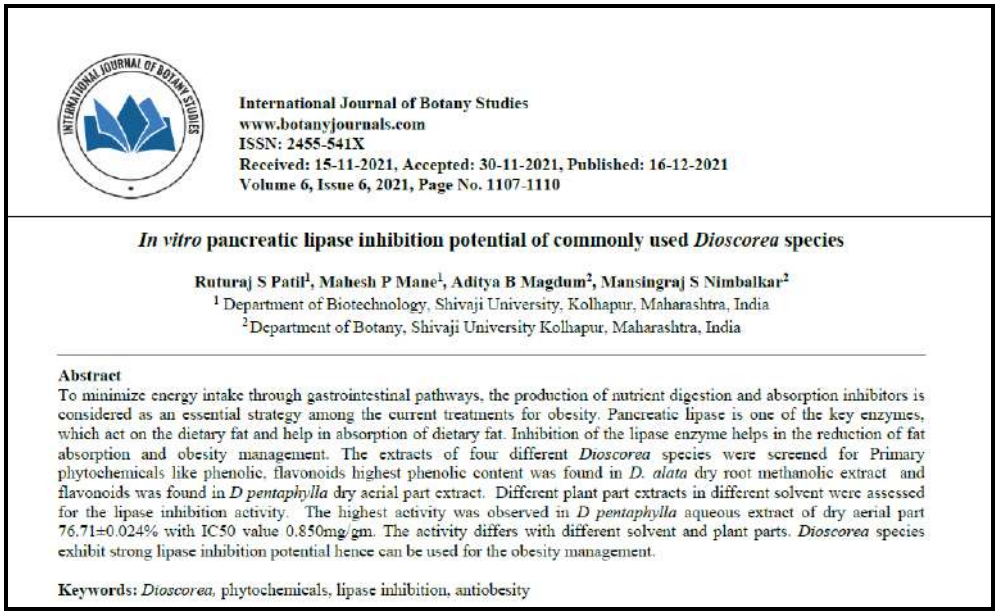
ARTICLE INFO

Keywords:
Antiadipogenic
Anti-obesity
Antilipase
Lipase inhibition

ABSTRACT

Background: Obesity is a major problem nowadays. It is a serious chronic disease associated with increased risk of insulin resistance, type 2 diabetes, cardiovascular disease, cancer, gallstones, fatty liver disease, osteoarthritis, oxidative stress, and inflammation-based pathologies in the human population. Throughout the Tropics, *Dioscorea* has strong ethnobotanical values. There are several accounts on the local claim on *Dioscorea* species worldwide. Some *Dioscorea* species have been investigated for their anti-obesity potential.

Publications



International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 15-11-2021, Accepted: 30-11-2021, Published: 16-12-2021
Volume 6, Issue 6, 2021, Page No. 1107-1110

***In vitro* pancreatic lipase inhibition potential of commonly used *Dioscorea* species**

Ruturaj S Patil[1], Mahesh P Mane[1], Aditya B Magdum[2], Mansingraj S Nimbalkar[2]
[1] Department of Biotechnology, Shivaji University, Kolhapur, Maharashtra, India
[2]Department of Botany, Shivaji University Kolhapur, Maharashtra, India

Abstract
To minimize energy intake through gastrointestinal pathways, the production of nutrient digestion and absorption inhibitors is considered as an essential strategy among the current treatments for obesity. Pancreatic lipase is one of the key enzymes, which act on the dietary fat and help in absorption of dietary fat. Inhibition of the lipase enzyme helps in the reduction of fat absorption and obesity management. The extracts of four different *Dioscorea* species were screened for Primary phytochemicals like phenolic, flavonoids highest phenolic content was found in *D. alata* dry root methanolic extract and flavonoids was found in *D pentaphylla* dry aerial part extract. Different plant part extracts in different solvent were assessed for the lipase inhibition activity. The highest activity was observed in *D pentaphylla* aqueous extract of dry aerial part 76.71±0.024% with IC50 value 0.850mg/gm. The activity differs with different solvent and plant parts. *Dioscorea* species exhibit strong lipase inhibition potential hence can be used for the obesity management.

Keywords: *Dioscorea,* phytochemicals, lipase inhibition, antiobesity

Ruturaj S. Patil, Mahesh P. Mane, Aditya B. Magdum, Mansingraj S. Nimbalkar, **(2022)** *Dioscorea oppositifolia* plant extract reduces adipogenesis by down-regulating PPARγ, C/EBPα, SREBP-1, and FASN in 3T3L1 pro-adipocytes, *Phytomedicine Plus,* Volume 2, Issue 3, 100293.

Ruturaj S. Patil, Mahesh P. Mane, Aditya B. Magdum, Mansingraj S. Nimbalkar, **(2021)** In vitro pancreatic lipase inhibition potential of commonly used *Dioscorea* speciess *International Journal of Botany Studies* volume 6 Issue 6, pages 1259-1264.

Mahesh P. Mane, **Ruturaj S. Patil,** Aditya B. Magdum, Samidha S. Kakade, Devashree N. Patil, Mansingraj S. Nimbalkar, **(2022)** Chemo-profiling by UPLC-QTOF MS analysis and in vitro assessment of Anti-inflammatory activity of Field Milkwort (*Polygala arvensis* Willd.), *South African Journal of Botany*, Volume 149, Pages 49-59.

Mahesh P. Mane, **Ruturaj S. Patil,** Aditya B. Magdum, Mansingraj S. Nimbalkar, **(2021)** Evaluation of antioxidant and pancreatic lipase inhibitory potential of Polygala glaucoides L. and Polygala erioptera DC *International Journal of Botany Studies* volume 6 Issue 6, pages 1107-1110.

Chirag U. Narayankar, M. Mane, **R. Patil,** A. Magdum M. D. Satpute, S. Gaikwad, D. K. Gaikwad **(2021)** "Inhibition of proliferation of K-562 Human Blood Cancer Cell due to Opuntia elatior fruit extract", *Journal of Science and Technology,* Vol. 06, Issue 04, July-August 2021, pp32-38.

STATEMENT I

The present investigation deal with **"Studies on Antiobesity Metabolites from *Dioscorea* Species"**. In the present investigation an attempt has been made to study screening of *Dioscorea* species against the obesity and find the biological active metabolites showing antiobesity activity. Further this work has not been submitted for the award of any degree or diploma in any institute.

Dr. M. S. Nimbalkar **Mr. Ruturaj Sudhakar Patil**

Guide **Candidate**

STATEMENT II

The present investigation embodies **"Studies on Antiobesity Metabolites from *Dioscorea* Species".** The work reports a new investigation. The source from which information is gathered have been listed in the last part of the thesis- "Bibliography". The current issue of Journal, review articles, textbook and monograph have been extensively referred and correlated. Every attempt has been made to keep the reference work as updated as possible.

Dr. M. S. Nimbalkar **Mr. Ruturaj Sudhakar Patil**

Guide **Candidate**

Plate-1 : Morphology of *Dioscorea alata* L.

A., B. Habit, **C.** Tuber, **D.** Bulbils, **E.** Quadrangular stem, **F.** Germplasm

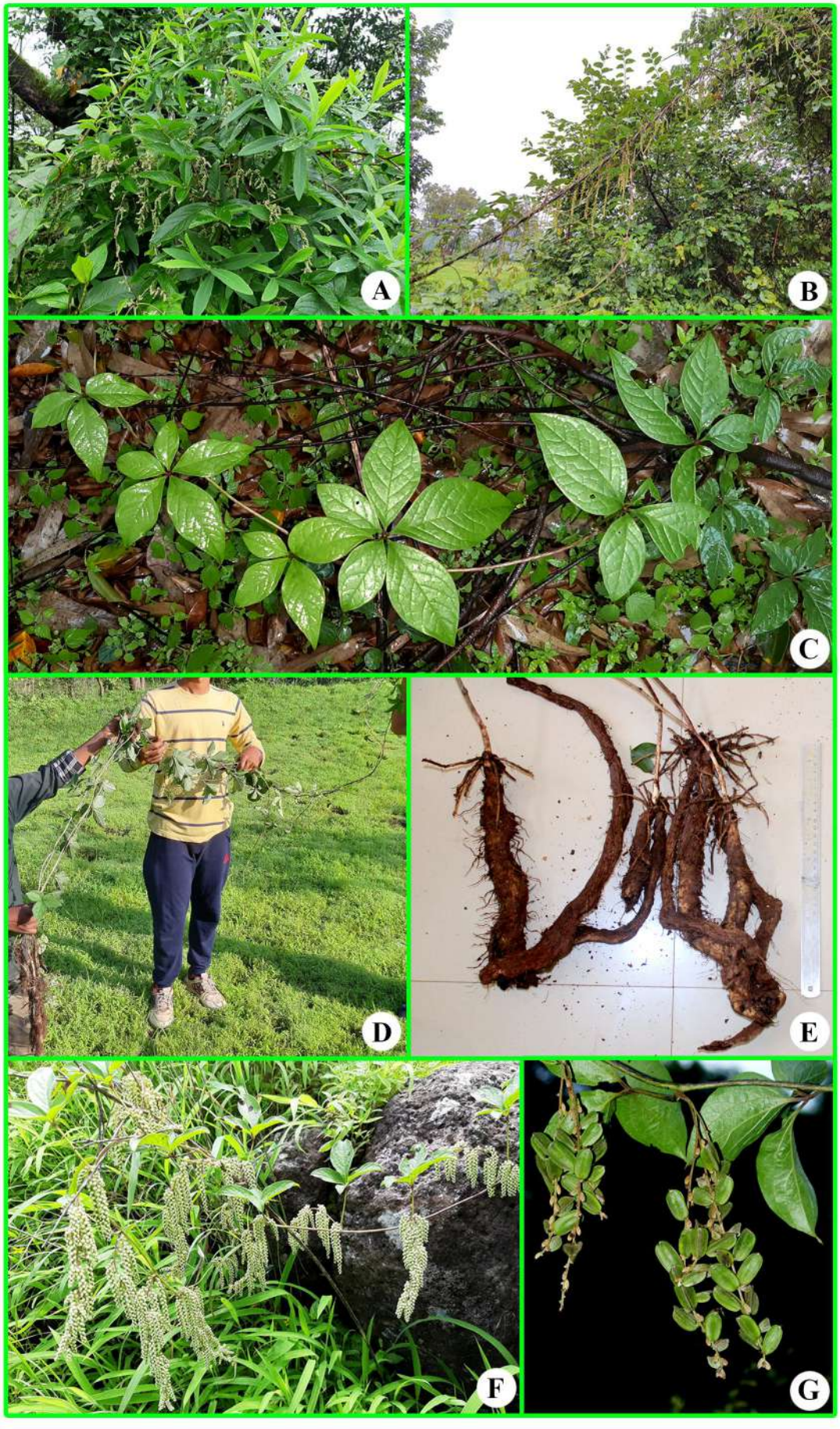
Plate-2 : Morphology of *Dioscorea bulbifera* L.

A., B. Habit, **C.** Flowering, **D.** Tuber, **E.** Inflorescence and Bulbils, **F.** Fruiting, **G.** Germplasm

Plate -3: Morphology of *Dioscorea oppositifolia* L.

A. B. Habit with fruiting, **C. D.** Tuber

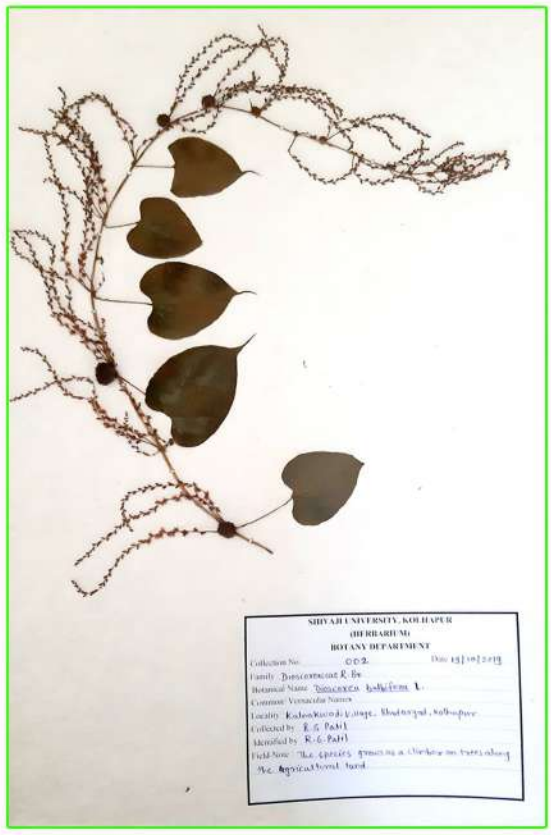
Plate -4: Morphology of *Dioscorea pentaphylla* L.



A-D. Habit, **E.** Tubers, **F.** Flowering, **G.** Fruiting

Plate-5: Harberium

***Dioscorea oppositifolia* L.**

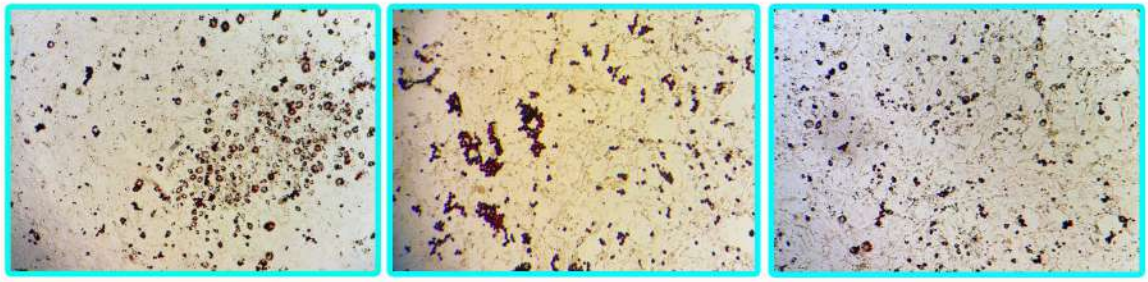


***Dioscorea bulbifera* L.**

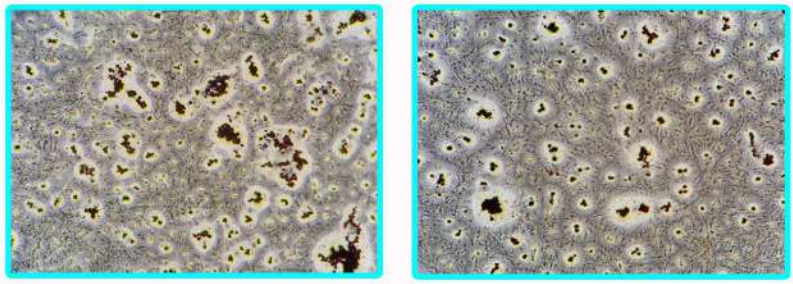
***Dioscorea alata* L.**

***Dioscorea pentaphylla* L.**

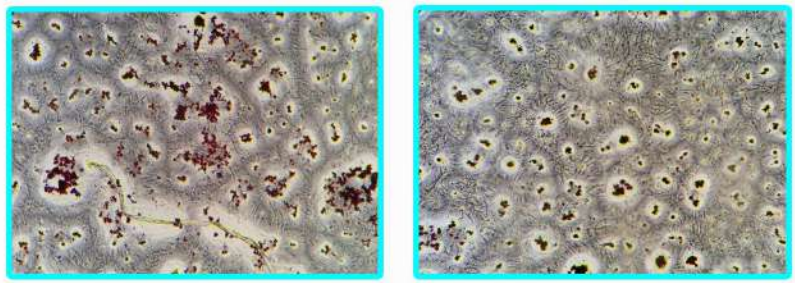
Plate-6: Screening of whole plant extract of *Dioscorea* sp. on the basis of oil red O staining



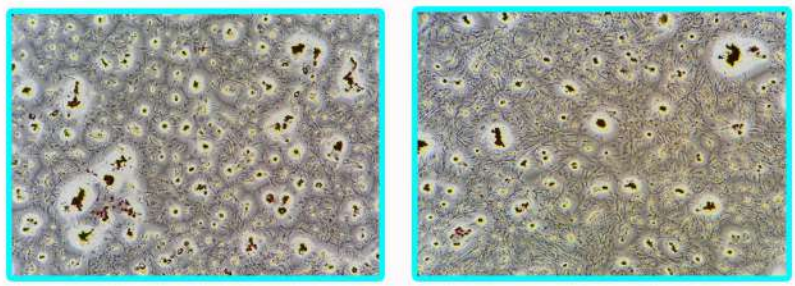
Control Water Methanol



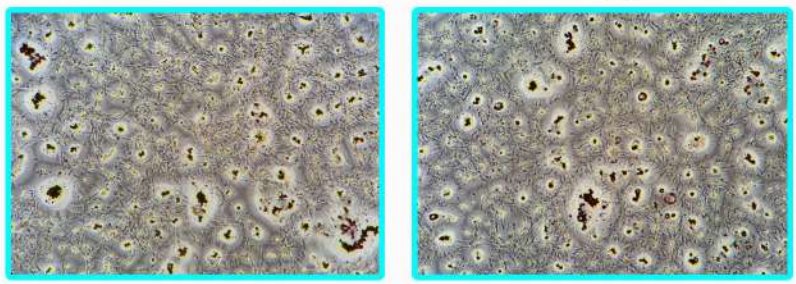
DAM 25 µg/ml DBM 25 µg/ml



DAM 75 µg/ml DBM 75 µg/ml



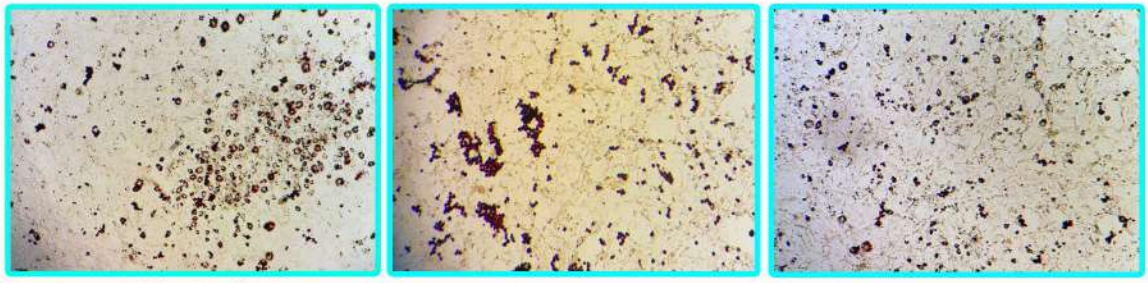
DAA 25 µg/ml DBA 25 µg/ml



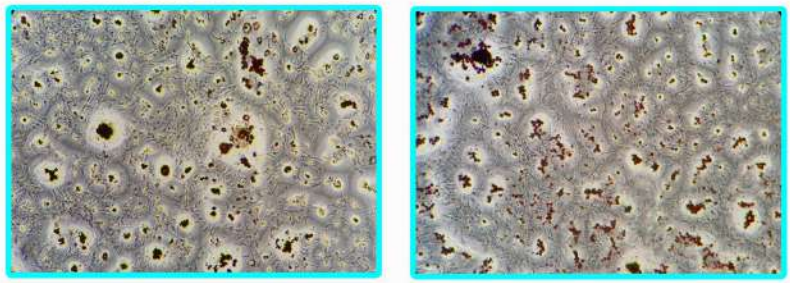
DAA 75 µg/ml DBA 75 µg/ml

DAM - *Dioscorea alata* methanolic extract
DAA - *Dioscorea alata* aqueous extract
DBM - *Dioscorea bulbifera* methanolic extract
DBA - *Dioscorea bulbifera* aqueous extract

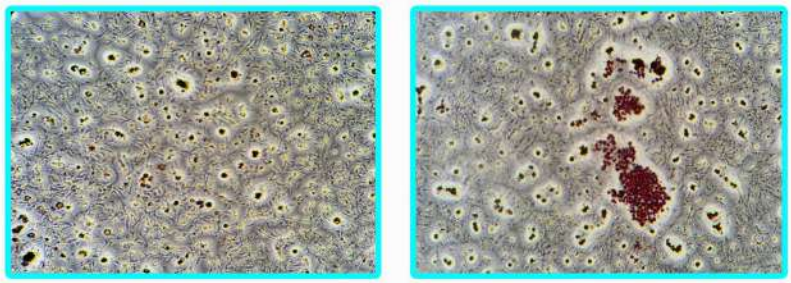
Plate-7: Screening of whole plant extract of *Dioscorea* sp. on the basis of oil red O staining



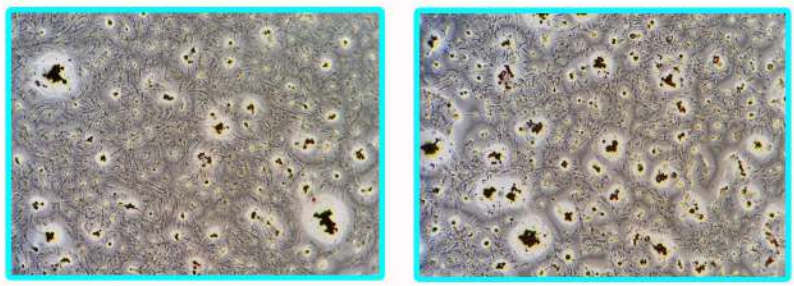
Control | Water | Methanol



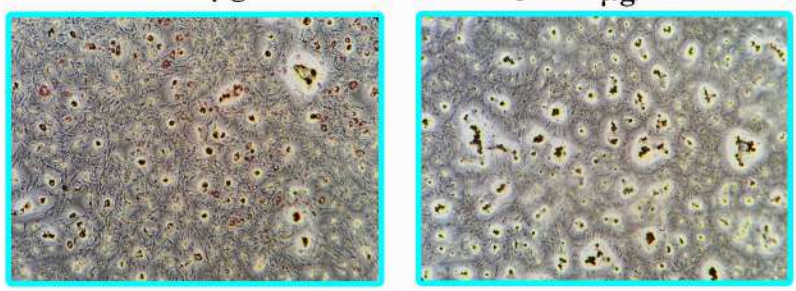
DPM 25 µg/ml | DOM 25 µg/ml



DPM 75 µg/ml | DOM 75 µg/ml



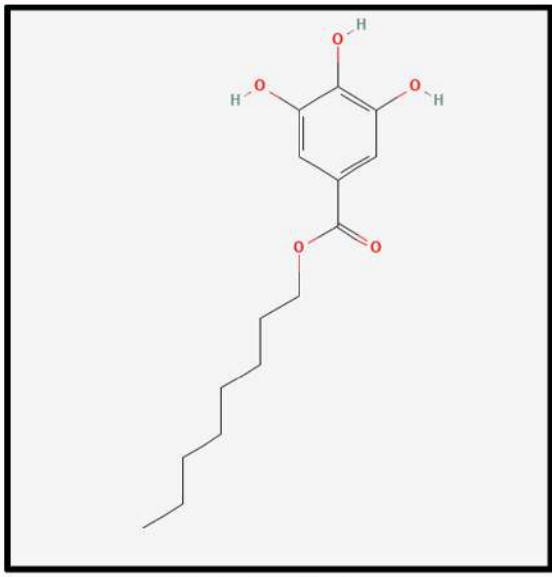
DPA 25 µg/ml | DOA 25 µg/ml



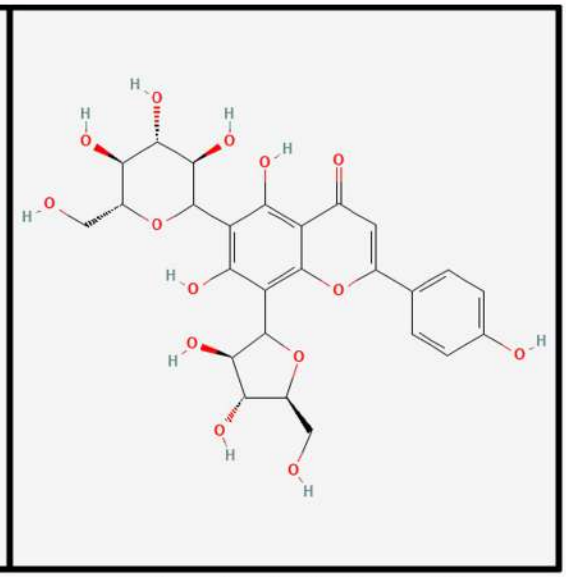
DPA 75 µg/ml | DOA 75 µg/ml

DPM - *Dioscorea pentaphylla* methanolic extract
DPA - *Dioscorea pentaphylla* aqueous extract
DOM - *Dioscorea oppositifolia* methanolic extract
DOA - *Dioscorea oppositifolia* aqueous extract

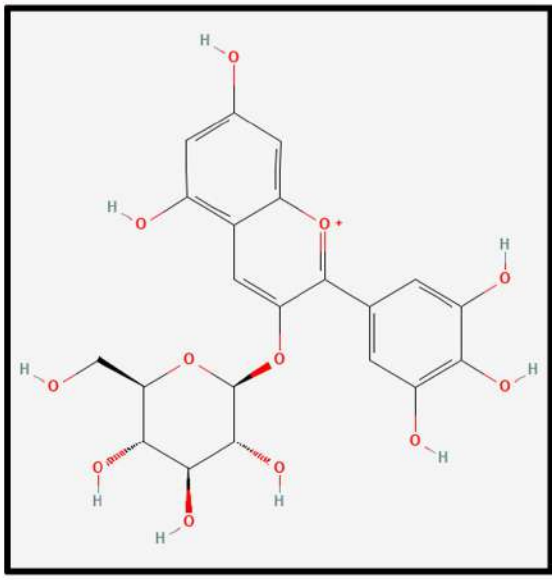
Plate-8: Antiobesity Compounds from *Dioscorea* sp.



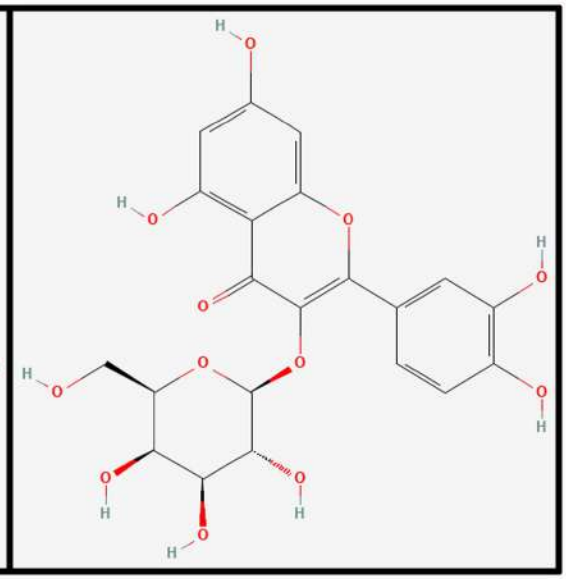
Octyl gallate



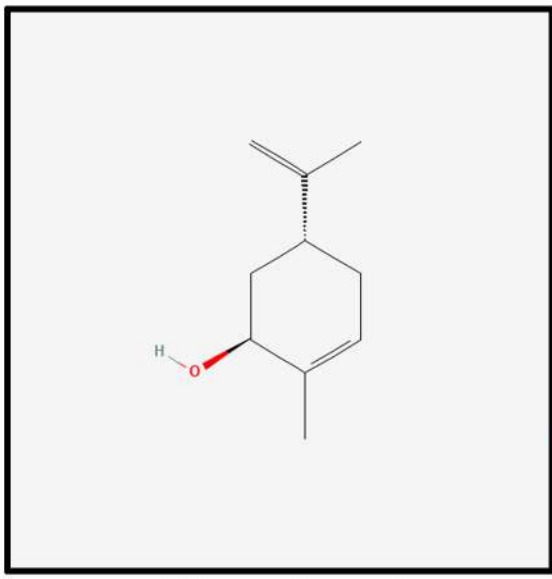
Apigenin 6-C-glucoside



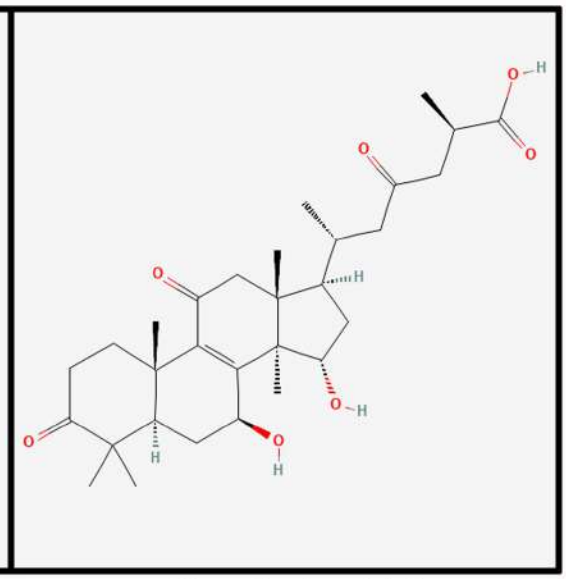
Delphinidin 3-O-beta-D-glucoside



Quercetin 3-galactoside

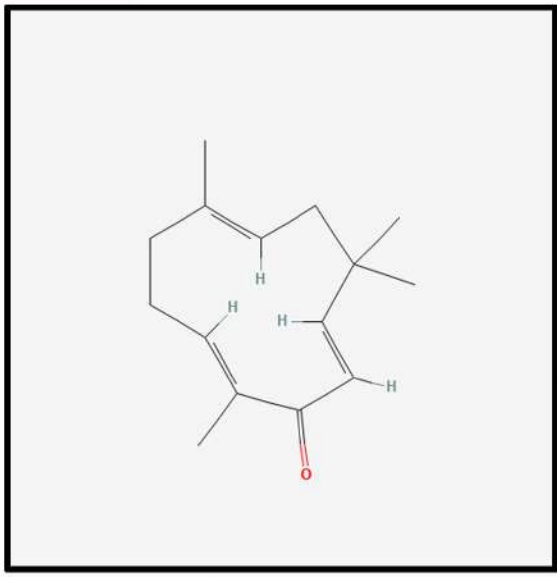


(-)-trans-Carveol

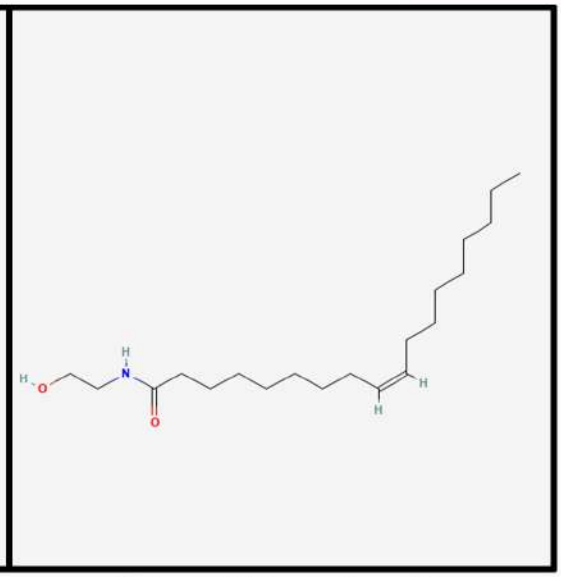


Ganoderic acid A

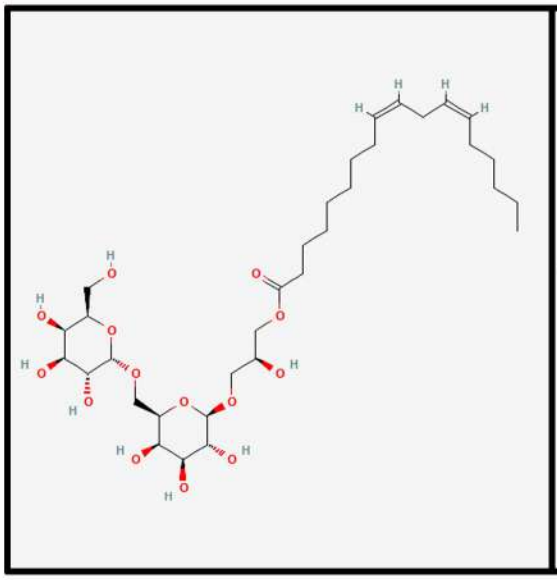
Plate-9: Antiobesity compunds from *Dioscorea* Sp.



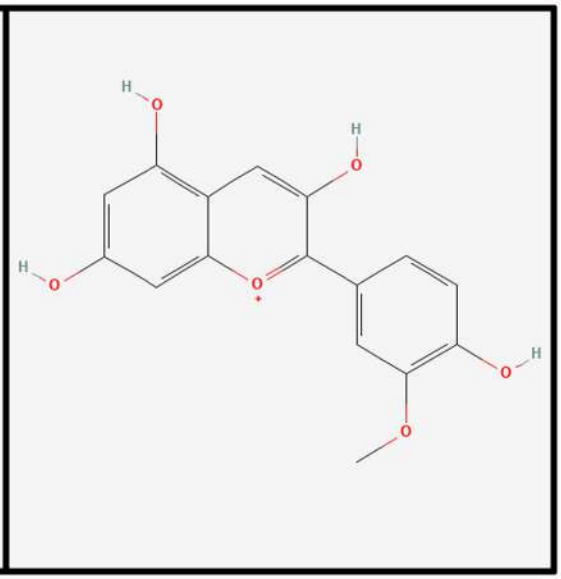
Zerumbone



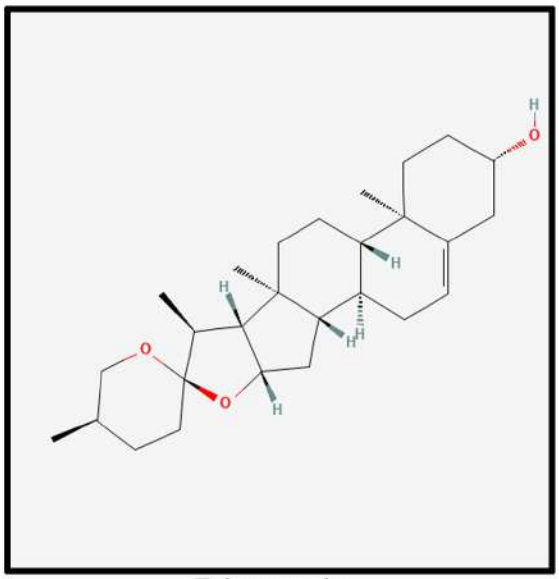
Oleoyl Ethanolamide



Gingerglycolipid B

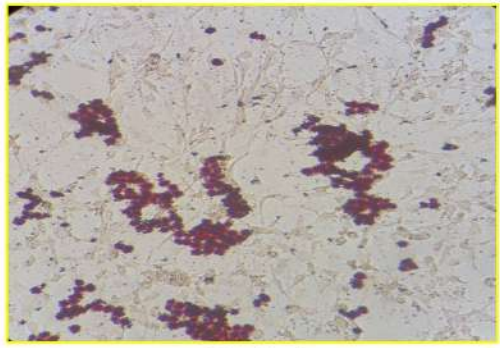


Peonidin

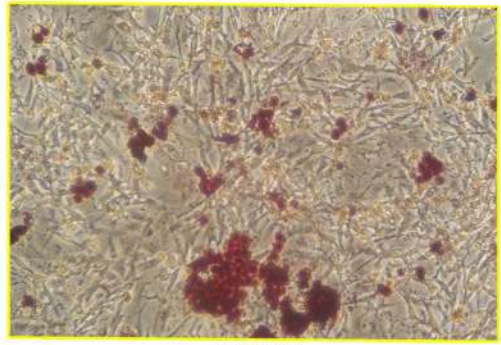


Diosgenin

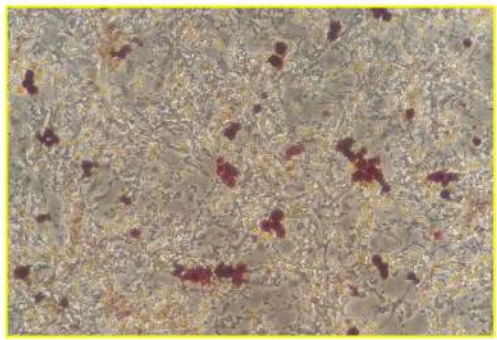
Plate-10: Screening of diffrent solvent fractions of *D.oppositifolia* extracts on the basis of Oil O red staining .



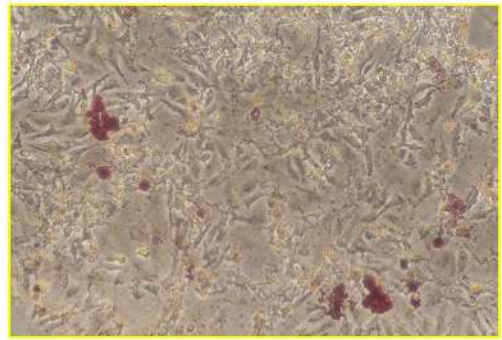
Control



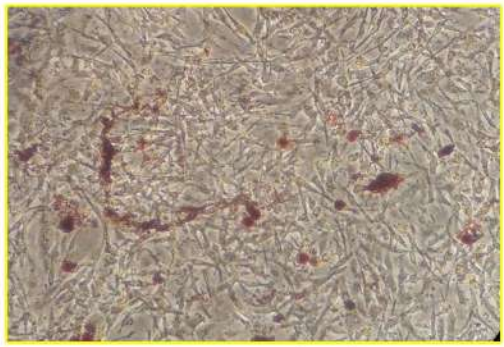
DMSO



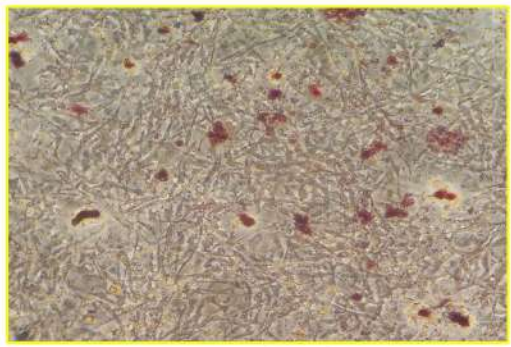
Hexane fraction



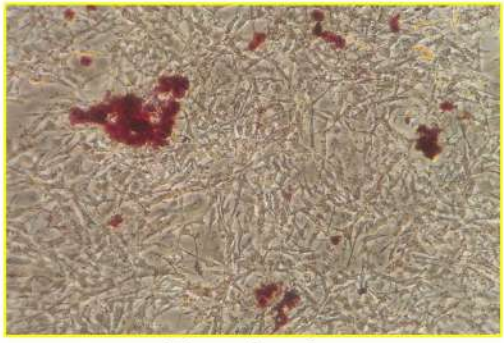
Chloroform fraction



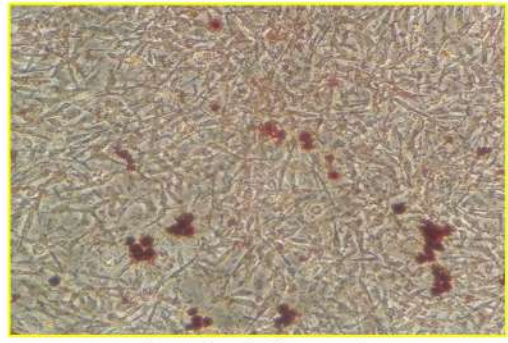
Ethyl acetate fraction



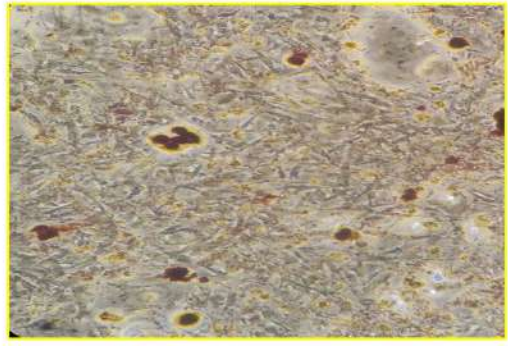
Butanol fraction



Water fraction



Standerd diosgenin



***Dioscorea oppositifolia* root extracted diosgenin**

Part II

1. University Name : **Shivaji University, Vidyanagar, Kolhapur**

2. Department **:** **Department of Biotechnology**

3. Name of Researcher **:** **Mr. Ruturaj Sudhakar Patil**

4. Name of Guide **:** **Dr. M.S. Nimbalkar**

5. Type of Degree **:** **Ph.D.**

6. Registration Date **:** **1 July 2018**

7. Completed date **:** **28 September 2022**

8. Thesis title **:** **Studies on Anti-Obesity Metabolites From *Dioscorea* Species**

9. Size of thesis **:** **21.2 MB (161 pages)**

STUDIES ON ANTI-OBESITY METABOLITES FROM *DIOSCOREA* SPECIES

A

THESIS SUBMITTED TO

SHIVAJI UNIVERSITY, KOLHAPUR

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

UNDER THE FACULTY OF SCIENCE AND TECHNOLOGY

BY

MR. PATIL RUTURAJ SUDHAKAR
M. Sc.

UNDER THE GUIDANCE OF

DR. MANSINGRAJ S. NIMBALKAR
M. Sc., Ph. D.

DEPARTMENT OF BOTANY
SHIVAJI UNIVERSITY,
KOLHAPUR - 416 004
MAHARASHTRA, INDIA

2022

DECLARATION

I hereby declare that the thesis entitled **"Studies on Anti-obesity Metabolites from *Dioscorea* species"** completed and written by me has not previously formed the basis for the award of any degree or diploma or other similar title of this or any other university or examining body. Further, I declare that I have not violated any of the provisions under Copyright and Piracy/ Cyber/ IPR Act amended from time to time.

In view of University Grants Commissions (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulations, 2018 dated 31st July 2018, I hereby submit an undertaking that this thesis is my original work and it is free of any plagiarism. Further, it is also to state that this thesis has been duly checked through a plagiarism detection tool approved by Shivaji University.

Place: Kolhapur

Date: **Mr. Ruturaj Sudhakar Patil**

Department of Biotechnology
Shivaji University, Kolhapur

CERTIFICATE

This is to certify that the thesis entitled **"Studies on Anti-obesity Metabolites from *Dioscorea* species"** is being submitted herewith for the award of the degree of doctor of philosophy in Biotechnology to Shivaji University, Kolhapur. The work reported in this thesis is based upon the results of original experimental work carried out by **Mr. Ruturaj Sudhakar Patil** under my supervision and guidance. To the best of my knowledge and belief the work embodied in this thesis has not formed earlier the basis for the award of any Degree or Diploma or similar title of this or any other university or examining body.

In view of University Grants Commissions (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulations, 2018 dated 31st July 2018, this is also to certify that the work done by **Mr. Ruturaj Sudhakar Patil** is plagiarism free.

Place: Kolhapur

Date:

Dr. Mansingraj S. Nimbalkar
(Research Guide)

Prof. J. P. Jadhav
Head (I/C)
Department of Biotechnology,
Shivaji University, Kolhapur

ACKNOWLEDGMENTS

First and foremost, I would like to convey my deep sense of gratefulness to my research guide Dr. Mansingraj S. Nimbalkar This work would not have been possible without his guidance, unceasing support and inspiration.

I gratefully acknowledge to Prof. Dr. (Mrs.) J. P. Jadhav head of the department and Prof. Dr. V. A. Bapat for their valuable help, cognizance, and inspiration towards my research work which has provided root for the completion of Ph. D. work.

I gratefully acknowledge to Chhatrapati Shahu Maharaj Research Training and Human Development Institute (SARTHI), Pune, for financial assistance.

I wish to express my wholehearted gratitude to Prof. Dr. R. V. Gurav (Head, Department of Botany), Prof. Dr. (Mrs.) V. D. Jadhav and Prof. Dr. D. K. Gaikwad (Ex- Head Department of Botany), for granting me the opportunity to utilize excellent facilities available at the department.

It is my pleasant duty to offer my sincere thanks to Prof. Dr. G. B. Dixit, Prof. Dr. S. S. Kamble, Prof. Dr. N. S. Chavan, Prof. Dr. N. B. Gaikwad, Dr. (Smt.) K. B. Pawar, Dr. Nilesh Powar, Dr. V. B. Shimple, Dr. (Mrs.) S. A. Patil, Dr. M. M. Lekhak, and Dr. S. G. Ghane for encouragement and valuable suggestions.

It is my pleasant duty to offer my sincere thanks to Dr Ravikiran S. Pagare Sandip University, Nashik, and Maharashtra for helping in the plant identification and valuable suggestions.

I would like to thank teaching faculty of Vivekananda College, Kolhapur Mr. S. G. Kulkarni, Mrs. Salma Nadaf, Mr. Satish Parte, Mrs. Sarita Mankar who taught me how interesting and lively field of Science and Biotechnology is.

I am also grateful to Mr. Amol Jadhav, Nirav BioSolutions Pvt. Ltd. - A Biotechnology Resource Center Pune for providing the cell culture facility and his constant suggestions and timely help.

I would like to thanks teaching and non-teaching of staff Dr. Onkar Apinee, Dr. (Mrs.) Sushma Apinee Mrs. Supriya Sathe Dr. Sachin Otari for their valuable suggestions and encouragement throughout tenure.

During this work I have worked with many seniors and colleagues for whom I have great regard. I wish to extend my warmest thanks to Miss. Devashree Patil, Miss. Manali Rane, Mr. Chetan Aware, Mr. Suresh Suryawashi, Miss. Samidha Kakade, Miss. Partibha Mali, Mr. Subham Sutar, Mr. Rahul Jadhav from department of Biotechnology, Dr. Pramod Lawand, Dr. Chirag Narayankar, Mr. Aditya Magadum, Mr. Sudarshan Charpale, Dr. Indarjit Samant, Mr. Suraj Patil, Mr. Pradeep Deshmukh, Miss. Komal Walevekar colleagues from the department of Botany who have provided great time and company during the course of this work.

I am forever grateful to all non-teaching staff and gardeners from department of botany for their help. I sincerely thank Dr. (Smt.) S. S. Fernandes, Mrs. Seema Kumbhar, Mr. Gavade Mr. Harshal Vhatkar, Mr. Krishnat Patil, Mr. Mukund Kamble, Mr. Shambhuraje Warke, Mr. Vishal Tambekar, Mr. Vishal Nalawade, Mr. Sandeep Kesarkar, Mr. Santosh Kumbhar, Mr. Ramjan Pathan, Mr. Mahesh Powar and Mr. Abhijeet Powar.

I specially thank to Mr. Mahesh Mane for his support and encouragement from my B.Sc. till my Ph.D. work.

The technical difficulties in this time of technological development are painstaking. I am very much thankful to Mr. Ganesh Salokhe for their technical support.

The friends are the friends indeed useful in need too. I specially thanks to Mr. Pushrottam Jamdar, Miss. Varsharani Jamdar and Jamdar family, Mr. Omkar Patil, Mr. Auvdhut Medhe, Mr. Nikhil Patil, Mr. Abhijeet Shelake, Mr. Tejas Shaha, Mr. Nabarun Saha, Miss. Sasha Porenca, Miss. Sagarika Karmakar, Miss. Sayantani Chakraborty, Miss. Parjoti Palani, Mr. Chetan Urunkar, Mr. Yuvraj Kashid, Mr. Uttam More, Mr. Rohit Salunkhe, Mr. Rohit Nagtile, and Mr. Rahul Dongare.

I would also like to extend hug, warm thanks to my beloved brother Dr. Ajay Nalawade his generous support, constant encouragement, for making the photographic representation more attractive and accomplishing the work.

My family deserves a special mention for their inseparable support and prayers. My grandparents Mr. Dyandev Salokhe and Mrs. Sushila Salokhe and grandmother Late Sushila Dinkar Patil who are constant inspiration for me ever since I was child. I would like to express my thanks to in-laws Mr. and Mrs. Vijay Bhandari and all members of Bhandari family. I must express my appreciation for my father Mr. Sudhakar and mother Geeta. I also thanks to my brother Mr. Karan, sister-in-law Shivani.

In the end I express my deep hearted gratitude to my wife Amruta and my little champ Advait for their inspiration, love, and support constant encouragement during the work.

Thanks to all those who helped me knowingly and unknowingly for the fruitful realization of thesis, as well as expressing my apology to them whom I could not mention personally.

Place: Kolhapur

Date:

Mr. Ruturaj Sudhakar Patil

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

%	:	Percentage
µl	:	microliter
µg	:	microgram
^{0}C	:	Degree Celsius
ABTS	:	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	:	Analysis of variance
ASA	:	Acetylsalicylic acid
C/EBP α	:	CCAAT/Enhancer binding protein
CE	:	Colchicine equivalent
CO_2	:	Carbon dioxide
CPT 1	:	Carnitine palmitoyltransferase 1
DE	:	Diosgenin equivalent
DMSO	:	Dimethyl sulfoxide
DPPH	:	2,2-diphenylpicrylhydrazyl
EDTA	:	Ethylenediamine tetraacetic acid
ESI+	:	Electrospray ionization positive mode
FASN	:	Fatty acid synthase
FC	:	Folin–Ciocalteu *reagent*
$FeCl_3$	:	Ferrous chloride
g/l	:	Gram per litre
GAE	:	Gallic acid equivalent
GAPDH	:	Glyceraldehyde-3-phosphate dehydrogenase.
gm	:	Gram
H_2SO_4	:	Sulphuric acid
HMDB	:	Human metabolic database
HPLC	:	High pressure liquid chromatography
hrs	:	Hours
IC_{50}	:	Half-maximal inhibitory concentration
KEGG	:	Kyoto Encyclopaedia of Genes and Genomes

LC-MS	:	Liquid chromatography-mass spectroscopy
M	:	Molar
m/z	:	Mass charge ratio
mg / ml	:	Milligram per millilitre
mg/gm	:	Milligram per grams
mg/ml	:	Milligram per microlitre
mins	:	minutes
ml	:	Millilitre
mM	:	Millimolar
MS	:	Murashige and Skoog
MTT	:	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide
NaCl	:	Sodium chloride
NCBI	:	*National Centre for Biotechnology Information*
NLRP3	:	NLR family pyrin domain containing 3
OD	:	Optical density
PCR	:	Polymerase chain reaction
PPAR ɣ	:	Peroxisome proliferator-activated receptor-gamma
PSI	:	pound per square inch
QE	:	Quercetin equivalent
Q-TOF MS	:	Quadrupole time-of-flight mass spectrometry
rpm	:	Revolutions Per Minute
RT	:	Retention time
SREEBP F1	:	Sterol regulatory element binding protein 1
Temp.	:	Temperature
UAE	:	Ursolic acid equivalent
v/v	:	Volume by volume
w/v	:	Weight by volume
µg/ml	:	Microgram per microlitre
µm	:	Micrometre
µM	:	Micromolar

1. INTRODUCTION

1. Obesity:

Obesity is rapidly expanding around the world. It is estimated that there are more than 300 million obese people in the world today (Ahmad *et al.,* 2010). It is one of the foremost health problems reflected by the accumulation of an excessive amount of body fat. Obesity, also known as adiposity, is the "New World Syndrome," and its prevalence is increasing rapidly in both developed and underdeveloped world. Swinburn *et al.,* (2011) found that obesity is characterized by an increase in adipose cell size, which is determined by the amount of fat accumulated in the cytoplasm of adipocytes. It is a serious and chronic disease with an associated increased risk of insulin resistance, type 2 diabetes, cardiovascular disease, cancer, gallstones, fatty liver disease, osteoarthritis, and oxidative stress and inflammation-based pathologies in the human population (Devlin *et al.,* 2000).

Obesity and being overweight are described as abnormal or excessive fat accumulations in adipose tissue that lead to health problems. Obesity or overweight is measured in the form of a body mass index (BMI) on the basis of the BMI. Obesity is categorized into six different classes. These are as follows:

Table No 1.1 Categories of obesity

Category	**BMI (kg/m^2)**
Underweight	< 18.5
Normal weight	18.5 – 24.9
Overweight	25.0 – 29.9
Obese (Class I)	30.0 – 34.9
Obese (Class II)	35.0 – 39.9
Obese (Class III)	≥ 40.0

A BMI of 25 or above is considered overweight, while a BMI of 30 or more is considered obese. According to the global burden of illness, the issue has reached epidemic proportions, with over 4 million people dying each year as a result of being overweight or obese in 2017.

According to the WHO (2016), there are slightly less than 2 billion overweight individuals worldwide. Over 600 million of them are classified as obese. In 2016, almost 40% of adults were overweight, and slightly under 15% were obese. Between 1975 - 2016, the global prevalence of obesity climbed threefold. Many of the obesity-related comorbidities are represented in metabolic syndrome, which was originally defined arbitrarily by WHO on the basis of insulin resistance along with other obesity-related problems. Obesity is also linked with malnutrition the change in the diet and lifestyle leads to obesity and related problem.

Obesity and being overweight are substantial risk factors for a variety of chronic illnesses, including cardiovascular disorders like heart disease and stroke, which are the leading causes of mortality worldwide. Obesity can also lead to diabetes and its complications, such as blindness, limb amputations, and the need for dialysis. Diabetes prevalence has doubled worldwide since 1980. Excess weight can cause musculoskeletal diseases such as osteoarthritis. Obesity has also been associated with endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon cancers.

2. Management of obesity:

Obesity's complicated pathophysiology necessitates the discovery and development of novel medications and alternatives for its prevention and treatment. Natural goods, particularly medicinal herbs, are thought to be promising anti-obesity agents as conventional medicines fail to provide long-term remedies. Different natural product combinations operate synergistically on multiple molecular targets in diverse ways, either boosting weight reduction or avoiding weight gain (Liu *et al.,* 2003).

Medicinal plants use mechanisms such as inhibition of lipid hydrolyzing and metabolizing enzymes, disruption and modification of adipogenic factors, and appetite suppressants to battle obesity (Rayalam *et al.,* 2008; Freitas *et al.,* 2017).

There are two approaches to treating obesity. One of these is the inhibition of pancreatic lipase. Enzyme lipase act on the dietary lipids in the intestine and helps to absorb fat in the body. Inhibition of the lipase reduces the excess absorption of the free fatty acid in the body. In the market, drugs like Orlistat reduce fat absorption through inhibition of pancreatic lipase, are available. Scientists have reported that some plant extracts inhibit pancreatic lipase, (Lei *et al.,* 2007, Ado *et al.,* 2013, Roh and Jung 2012).

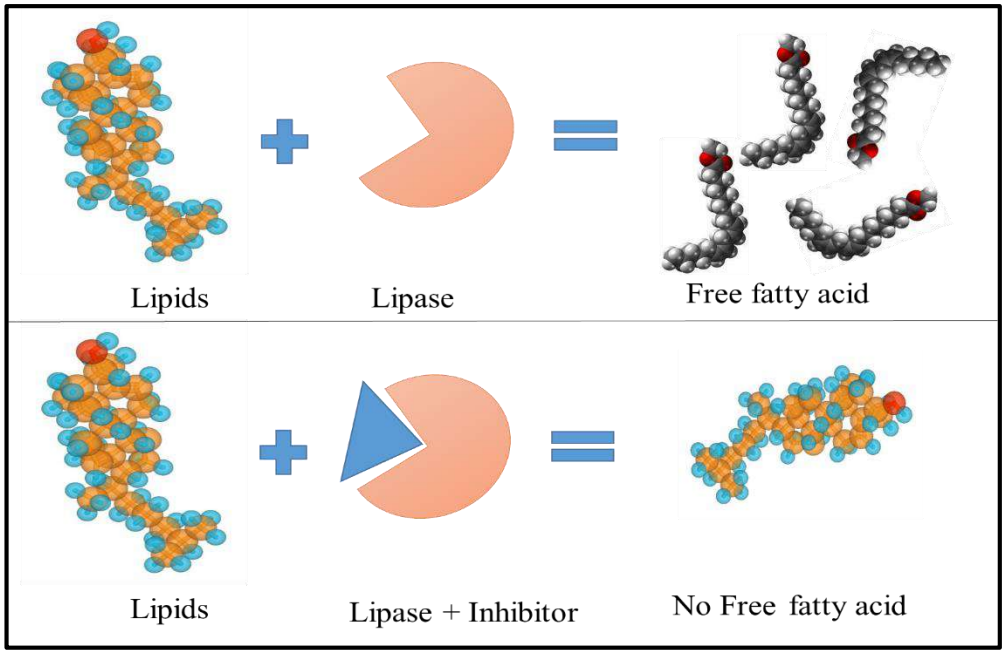


Fig 1.1 Activity of Lipase

Another approach to reducing obesity is the degradation of accumulated fat. Adipose tissues are the major part of the body that stores excess fat. Some medicines and herbal products are available in the market that down regulates adipogenic genes or by stimulating lipolysis of the stored fat. There are some plant extracts that stimulate gene transcription that activates the lipolytic pathway and restricts enzymes like 5'AMP activated protein kinase (AMPK), which inactivates Acetyl-CoA carboxylase (ACC) and enhances fatty acid oxidation by up-regulating the expression of CPT-1, PPARϒ, and uncoupling protein.

Adipose tissue, an endocrine organ, regulates metabolism and homeostasis through the release of many physiologically active adipokines. Three key transcription factors are peroxisome proliferator-activated receptor (PPAR) ϒ, CCAAT/enhancer-binding protein (C/EBP) α, and sterol regulatory element-binding protein (SREBP) 1c control the expression of these lipid-metabolizing enzymes during adipose tissue development. 5' AMP-activated protein kinase (AMPK) plays a major role in glucose and lipid metabolism by inactivating acetyl-CoA carboxylase (ACC) and stimulating fatty acid oxidation by up-regulating the expression of carnitine palmitoyltransferase-1 (CPT-1), PPARα, and uncoupling protein (Patra *et al.*, 2015).

Plant extracts are known to have multiple effects that can be used for obesity management. It is worthwhile to explore the potential of unexplored medicinal or endemic plants. Saponins, flavonoids, phenols, and alkaloids are plant components having anti-obesity potential. The existence of numerous phytochemical combinations in plant

medications may result in synergistic effects by acting on many molecular targets, providing advantages over therapies that typically employ a single ingredient.

India has tremendous sources of medicinal plants in the region of the Western Ghats. Some of these plants are used as a traditional medicine to treat obesity problems. These medicinal plants are easily available and are non-harmful compared to modern allopathic drugs. Thus, various combinations of the active components of these plants after isolation and identification can be made and have to be further assessed for their effects. The preparation of standardized doses and dosage regimes may play a critical role in the management of obesity. It seems to be an effective effort for the good health of humans. There is a broad scope to derive potent anti-obesity bioactive natural compounds from medicinal plants.

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Monocots
Order: Dioscoreales
Family: Dioscoreaceae
Genus: *Dioscorea* L.

3. Plant *Dioscorea* L.:

There are around 630 species in the genus *Dioscorea* (Mabberley, 2008). Yams are recognized as one of the most important basic foods. It is mostly found in the tropical and subtropical regions of Africa, Central and South America, and Asia (Coursey 1967). 50 *Dioscorea* species have been cultivated for food. Thirty of them are cultivated for medically relevant chemicals such as steroids, sapogenins, and dioscorin (Bhattacharjee *et al.,* 2011).

Dioscorea species are herbaceous vines with starchy tubers. Tubers include steroids, saponins, and sapogenins that are significant in the manufacture of anti-inflammatory, androgenic, estrogenic, and contraceptive medicines. Cytotoxic, anticancer, antifungal, immunoregulatory, hypoglycemic, and cardiovascular effects are also demonstrated by the active substances. They are used in folk medicine to relieve menstrual cramps as well as to alleviate labor discomfort.

Tubers also include monacolin-K (a cholesterol-lowering agent) and monascin (an anti-inflammatory substance). The majority of the species have commercial importance and are also significant ethnically as secondary foods. (Edison *et al.*, 2006).

Dioscorea is important in food and medicine, these species should be explored more for their medicinal properties, which include anti-obesity properties. In this study, four *Dioscorea* species from Kolhapur, Maharashtra, India were screened for their anti-obesity potential.

2. REVIEW OF LITERATURE

2.1 The *Dioscorea*:

Yam is the popular name for the monocotyledonous family Dioscoreaceae. Brown (1810) established the family name '*Dioscoreae*,' which was later altered to Dioscoreaceae by Lindley in 1836 (Coursey, 1967). The biggest genus within the family is *Dioscorea* L. Linnaeus (1737) created it with eight species in his Species Plantarum. The genus name was chosen to honor Pedenios Dioscorides, a Greek physician who served in the army and gathered medicinal plants throughout the Roman Empire during his lengthy travels (Borzelleca and Lane, 2008). His five-volume work, "De Materia Medica (On Medical Material)," in native Greek, served as the foundation for all current pharmacopeias (Forbes *et al.*, 2013). Charles Plumer named the genus of edible yams after Pedenios Dioscorides in 1703. (Osbaldeston, 2000). *Dioscorea* evolved in the tropical regions of Africa, Southeast Asia, and South America (Edison *et al.,* 2006).

The Dioscoreaceae family is a pantropical group that is found in natural environments. However, members of this family are uncommon in arid places (Thapyai, 2004). Though rainfall patterns dominate the distribution, certain members have adapted to biologically severe environments such as rainforests and savannahs. Some members live on coastal plains, while others live in mountains at high elevations of several thousand meters. According to Coursey (1967), *Dioscorea* species were discovered in the desert near the tropical area, where rainfall averages 40/50 cm per year, as well as high altitude mountains with significant frost. Although *Dioscorea* is widespread, the other members of the family are not.

As a staple meal, *Dioscorea* tubers have had an essential influence on the current distribution of several edible species. The tubers were transferred from area to region by migrating people. Climate change and environmental whims appear to had a significant effect on *Dioscorea* migration and distribution (Thapyai, 2004). According to Coursey (1967), yams were chosen on ships owing to their abundant energy supply in the form of stored food and their long shelf life, which greatly aided global distribution. Furthermore, because of the commercial importance of yams, they were transported at an international level during the sixteenth century, and the rise of sea transportation greatly assisted this (Coursey, 1967). Hornel (1934) published a thorough examination of the evidence for a theory based on societal cultural aspects, linguistic and physical characteristics in its dispersion. By the end of the Cretaceous epoch, around 75 million years ago, *Dioscorea*

had spread all across the earth (Alexander and Coursey, 1969). It was widely distributed in the southern world, and its first expansion appears to have occurred via the Antarctic Continent in Asia (Coursey, 1967).

2.2 Obesity:

Obesity is connected with higher triglyceride accumulation and a slower rate of lipid breakdown. Obesity-related lipid metabolism is mostly due to decreased catecholamine-stimulated lipolysis. Blunted catecholamine-stimulated fatty acid metabolism in obesity appears to be irrespective of fat mass since it is evident in obese participants' first-degree nonobese relatives and after weight reduction in obese insulin-resistant people. Hormone sensitive lipase (HSL) and Adipose triglyceride lipase (ATGL) expression levels were shown to be lower in separated adipocytes from obese subjects, as well as in insulin-resistant states unrelated to obesity (Sam & Mazzone 2014). Obese and insulin-resistant individuals have lower expression of lipolytic b2-adrenoceptors70 and higher antilipolytic properties of a2-adrenoceptors77, both of which contribute to a decrease in catecholamine-induced lipolysis. Other variables, such as leptin, may also influence ATGL and HSL expression in obese people. Despite the fact that catecholamine-induced lipolysis is inhibited in obese patients, FAA levels are enhanced due to greater basal lipolysis associated with larger adipose tissue mass (Sam & Mazzone 2014).

2.3 Adipose Tissue:

Adipose tissue (AT) is a wide and highly specialized connective tissue made of various cell types. It serves as a significant energy reservoir in the body, storing surplus energy as lipids and releasing it as needed, as well as providing temperature control. AT is regarded as a significant endocrine organ that mediates biological effects on metabolism, hence contributing to the maintenance of energy balance and the prevention of obesity-related metabolic disorders (Wozniak *et al.,* 2009).

AT is divided into two categories based on its position and colour. AT is classified as brown adipose tissue (BAT) and white adipose tissue (WAT) based on colour, with major variations in shape and function. AT-tissues are characterised as visceral or subcutaneous based on their location (Rezaee and Dashty, 2013; Farkas *et al.*, 2018).

2.3.1 BAT-Adipocytes feature many tiny lipid droplets in their cytoplasm and are multilocular. As a result, energy stored in the form of triglycerides in lipid droplets is

available for fast hydrolysis and oxidation of fatty acids. BAT generates energy through non-oxidative phosphorylation by uncoupling protein 1. BAT possesses an abundance of mitochondrial chromogens, which are responsible for the brown colour. They have a high vascular supply and respond to the sympathetic nervous system to induce lipolysis, which results in the release of free fatty acids (FFA) and an increase in energy expenditure (Redinger, 2009; Frühbeck *et al.*, 2009).

2.3.2 WAT- Adipocytes are unilocular and have a distinct lipid droplet capable of storing triglycerides with high energy density (Trayhurn 2007). They are key factors of adipose tissue and play a role in metabolic control via energy balance, adipocyte development, and insulin sensitivity. Mature WAT adipocytes differentiate into a range of cells (pre-adipocytes, macrophages, fibroblasts, and endothelial cells) that drain FFA and inflammatory mediators in portal circulation and influence metabolism (Juge-Aubry *et al.*, 2005; Gesta *et al.*, 2007).

2.4 Role of the transcription factor in the adipocyte differentiation:

2.4.1 Peroxisome proliferator-activated receptor gamma (PPARɣ): It is both required and sufficient for WAT adipogenesis in animals, and is regarded as an adipogenesis "master regulator." PPARɣ is necessary for adipocyte differentiation both in vitro and in vivo in mice and plays a key role in placental vascularization, monocyte differentiation, and heart development. PPARɣ loss-of-function (LOF), severe lipodystrophy, insulin resistance, and hyperglycaemia can all result from naturally occurring mutations within the PPARɣ coding sequence in humans. Furthermore, in mice, adipocyte-specific deletion of PPARɣ leads to the complete lack of WAT. Surprisingly, PPARɣ expression, in conjunction with the presence of an activating ligand, is sufficient to launch an adipogenic programme and maintain an adipocyte phenotype in previously non-adipogenic cells. As a result, PPARɣ plays an important role in mammalian adipogenesis, as seen by PPARɣ LOF in humans, which is linked to severe lipodystrophy, metabolic disorders, and illness. (Wafer *et al.*, 2017).

2.4.2 CCAAT/enhancer-binding protein alpha (C/EBPα): The C/EBPα has been linked to adipoblast differentiation regulation. In this work, they have studied C/EBPα ability to induce the adipogenic pathway in a range of fibroblastic cells. The C/EBPα gene is transduced into eight mouse fibroblastic cell lines using retroviruses and DNA transfection, resulting in adipocyte colonies at varying frequencies. When the C/EBPα gene is

transduced by retroviruses, the percentage of G418-resistant colonies with an adipocyte shape is reproducibly greater than 50% in NIH-3T3 cells. The capacity to boost the adipogenic programme needs C/EBP alpha's powerful transcriptional activation domain, which C/EBPβ lacks. Despite its antimitogenic properties, clonal cell lines expressing high levels of C/EBPα can be easily generated. Stable expression of C/EBP α in BALB/c-3T3 cells improves their potential to develop into adipocytes. They have reported that C/EBPα may successfully enhance the adipogenic programme in a range of mouse fibroblastic cells, including those with little or no spontaneous adipogenic potential. (Freytag *et al.,* 1994).

2.4.3 Sterol regulatory element binding protein 1 (SREBP 1): When the mammalian liver cells are treated with a high carbohydrate diet, it increases the transcription of triglyceride production (lipogenesis) enzymes. The treatment activates hepatic insulin signalling, which helps in sterol regulatory element-binding protein-1c transcription (SREBP-1C). They have investigated the role of SREBP-1c in the expression of lipogenic genes in glucose- and insulin-treated primary rat hepatocytes by using an inducible adenovirus system. They found that overexpression of fatty acid synthase, S (14), and acetyl-CoA carboxylase mRNAs results in a mild elevation of fatty acid synthase, S (14), and acetyl-CoA carboxylase mRNAs in response to insulin therapy, but not lipogenic enzyme genes. Restoring insulin overexpression in cells did not result in an increase in mRNA levels. This study indicates that SREBP 1 regulates the expression of the fatty acid synthase and related genes. This is the essential gene for adipocyte development (Stoeckman and Towle 2002). Downregulation of these genes helps in the treatment of obesity.

2.4.4 Fatty acid synthase (FASN): It helps to catalyse the synthesis of palmitate (C16:0, a long-chain saturated fatty acid) from acetyl-CoA and malonyl-CoA in the presence of NADPH, and produces the fatty acid chain in the adipose tissue. Extra energy is converted to the stored energy in adipose tissue. Berndt *et al.,* (2007) stated that enhanced FASN mRNA expression in fatty tissue has been connected to visceral fat accumulation, impaired insulin sensitivity, increased circulating fasting insulin, IL-6, leptin, and RBP4, indicating that lipogenic pathways play an important role in the causal association between the consequences of excessive caloric intake and the development of obesity and type 2 diabetes. While considering the above statement, we need to search for a compound that controls or downregulates the fatty acid synthase gene expression to develop an anti-obesity compound.

2.4.5 Carnitine palmitoyl transferase (CPT-1): The presence of brown adipose tissue (BAT) in mature humans, as well as its reduction in obese and diabetic individuals, has highlighted this tissue as a major actor in obesity-induced metabolic problems. Because BAT controls energy expenditure via thermogenesis, boosting its thermogenic fat-burning capacity is an appealing therapeutic strategy. For this, they try to increase BAT fat burning by enhancing the rate of fatty acid oxidation (FAO). For the study, they used adenoviral infection to produce carnitine palmitoyl transferase 1 AM (CPT1AM), a permanently active mutant version in a rat brown adipocyte (rBA) cell line. CPT1AM-expressing rBA had higher FAO, lipolysis, UCP1 protein levels, and mitochondrial activity. Furthermore, increased FAO decreased the palmitate-induced rise in triglyceride level as well as the production of obesity and inflammatory markers. As a result, CPT1AM-expressing rBA exhibited increased fat-burning ability and reduced lipid-induced dysregulation. This suggests that CPT1AM-mediated increases in brown adipocytes FAO might be a novel method for treating obesity-related diseases. (Calderon-Dominguez *et al.*, 2016).

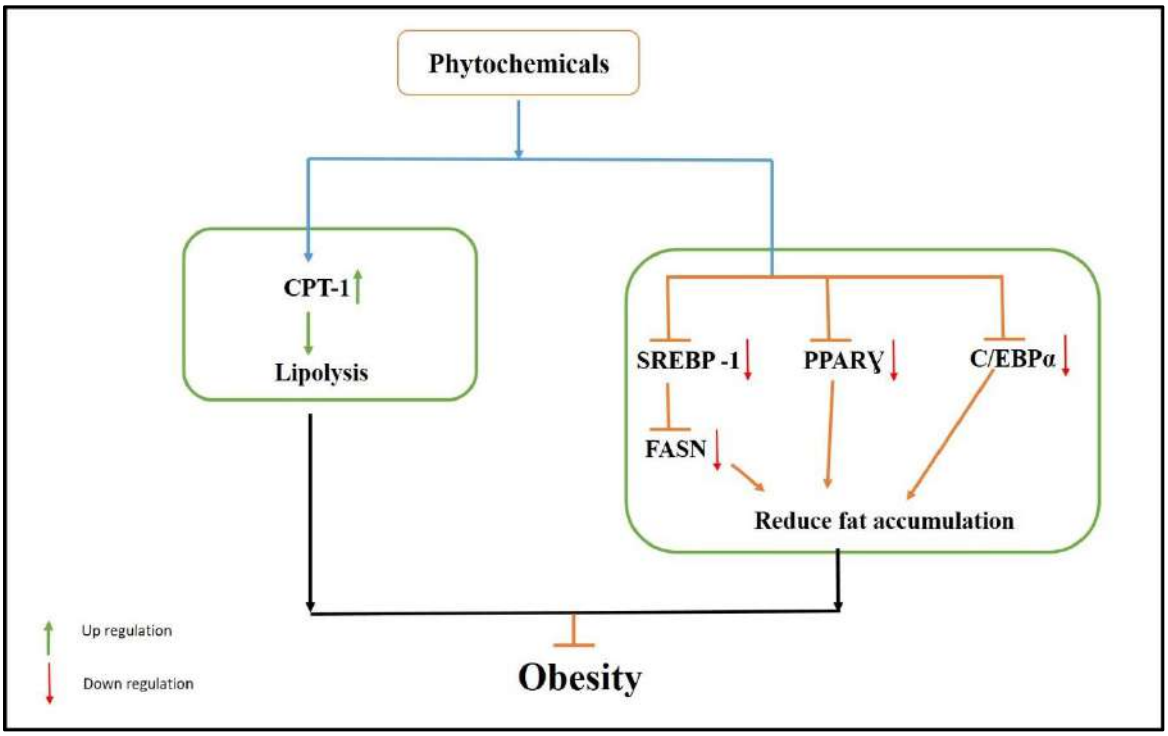


Figure No. 2.1: Over view of the gene involved in the obesity

2.5 Oxidative Stress and Obesity:

Oxidative stress is linked to the development of obesity-related co-morbidities. Hyperglycaemia, higher tissue lipid levels, vitamin and mineral deficiencies, and food type are all potential factors for oxidative stress in obesity. Antioxidant enzymes Cu-Zn superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity were shown to be decreased in obese participants' erythrocytes compared to non-obese controls. Insulin resistance and diabetes, as well as cardiovascular issues, sleep disorders, asthma, oncological problems, reproductive, rheumatological problems, and liver failure, are all caused by obesity-induced oxidative stress. (Manna and Jain, 2015).

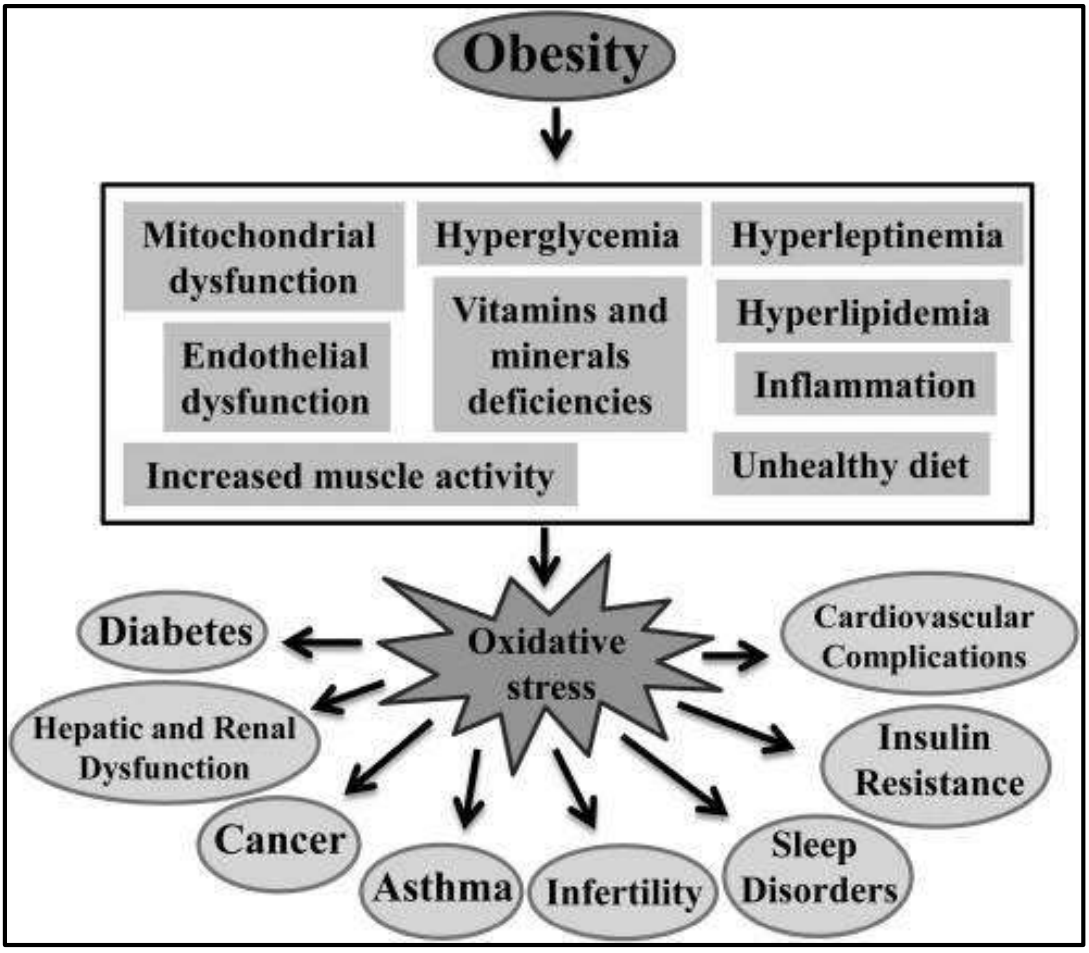


Figure No. 2.2: The role of oxidative stress in the development of obesity-related health risks and the conditions that cause oxidative stress in the pathogenesis of obesity (Manna and Jain, 2015)

To lower oxidative stress, possible antioxidant molecules from natural resources must be identified; plants are an excellent source of antioxidants. Due to their ability to donate hydrogen atoms to free radicals, phenolic and flavonoid molecules are key antioxidant

components that are responsible for deactivating free radicals. They also have excellent structural properties for free radical scavenging. Several studies have found a linear relationship between total phenolic and flavonoid concentration and antioxidant capability. (Aryal *et al.,* 2019).

Table No.2.1: Role of antioxidants in oxidative stress and obesity (Abdali *et al.,* 2015)

Antioxidant	Plant sources	Supplementation's effects
Polyphenols and carotenoids		
Procyanidins	*Cinnamomum cassia*, *Malus pumila*, Cranberries, Avocado, Red beans, Almonds, Peanuts	*Cinnamomum cassia* is the most studied plant. The extract has very little coumarin, making it safer; it may reduce FBG and HbA1c, oxidative stress, and perhaps fat mass.
Catechins	Green tea (*C. sinensis*), Apples, Blackberries	Increased thermogenesis and fat oxidation may result in weight loss; green tea also includes caffeine, which may be required for diabetic benefits; excessive caffeine (>300 mg/day) produces tolerance
Chlorogenic Acid	Green Coffee, Plums, Peaches, Dates	Fruits have the ability to help you lose weight; they also include ferulic acid, which is a potentially useful antioxidant.
Resveratrol	Red wine, Japanese knot wood, Acai, Blueberry, Bilberry, Cranberry, Pomegranate, *Ziziphus*	Reduces oxidative stress
Lycopene	Guava, Papaya, Watermelon, *Asparagus*, Tomato, Eggplant, Potato	There were minor impacts on oxidative stress state, IL-6, TNF-, or CRP; no effects on weight, FBG, HbA1c, or glucose tolerance impairment.

2.6 Current available anti-obesity drugs:

2.6.1 Pharmacotherapy:

Anti-obesity medications or weight reduction pharmaceuticals of pharmacological substances lower or regulate weight by affecting essential human body processes such as regulation, appetite regulation, neuroendocrine regulation, or calorie absorption. (Mohamed *et al.,* 2014)

Table No. 2.2: Available synthetic drugs available in market for long term and short-term treatment

Drugs	Action
Orlistat	Inhibits pancreatic lipase
Cetilistat	Inhibits pancreatic lipase
Rimonabant	Inverse agonist f or the cannabinoid receptor CB1. Its primary mode of action is appetite suppression.
Diethyl propionfluxetine	Reduce food intake through selective inhibition serotonin re uptake
Phentermine	Works on the central nervous system to suppress appetite.
Lorcaserin	Reduces appetite by activating a type of serotonin receptor
Sibutramine	A selective serotonin and noradrenaline re-uptake inhibitor

The synthetic medications that are now accessible have several adverse effects. Drugs that imitate the sympathetic nervous system induce the body to become stressed or anxious, resulting in headaches, sleeplessness, irritability, breathlessness, and agitation. Drugs that inhibit enzyme function induce diarrhoea, bloating, and stomach discomfort (Gadde *et al.,* 2018). As a result, there is an urgent need for the development of novel medications with low or no long-term negative effects.

2.6.2 Herbal approaches to treating the obesity:

Herbal extracts or compounds help to reduce obesity by inducing different pathways such as inhibiting the breakdown and absorption of dietary lipids, appetite suppressants, adipocyte differentiation as well as increasing intestinal transportation, β-oxidation and metabolic rate. Plant extracts have anti-obesity properties through several mechanisms; possibly the suggested method for obesity therapy might be achieved by the synergism of

different plant extracts with varied activities. As a result, natural plant products provide a great prospect and promise for new preventative and therapeutic approaches to obesity (Rayalam *et al.,* 2008). Some of the drugs are listed below in **Table No. 2.4**

Table No. 2.3: Herbal products available in the market

Drug	Content	Mode of action	Reference
Lipotrim	*Garcinia cambogia* extract 50 mg. Chromium 100 mcg	Diet	Brown 1999
Slimax	Aqueous extract of *H. vulgare, P. multiflorum, D. longan, L. sinense, L. brownii* and *Z. officinale*	Modification of lipid metabolism, with accumulation and the release of lipid from adipose tissue, increase lipid metabolism	Ignjatovic *et al.,* 2000
Phase 2	Fractionated *Phaseolus vulgaris* extract	Decrease carbohydrate absorption by inhibiting Alpha amylase.	Udani *et al.,* 2004
Forslean	Extract of *Coleus forskohlii* forskolin (25 mg)	Increase camp activates the hormone sensitive lipase results in lipolysis	Lieberman 2004
Slim339	Combination of *G. cambogia* extract with calcium pantothenate and extracts of *M. chamomilla, R. damascena, L. officinalis and Canangaodorata*	Appetite suppression, inhibition of pancreatic alpha amylase and intestinal alpha glycosidase	Toromanyan *et al.,* 2007
Ayurslim	*G. cambogia, B. mukul, G. sylvestre, T. chebula and T. graecum*	Reducing the consumption of fats and carbohydrates	Singh *et al.,* 2008 Asdaq *et al.,* 2015;

2.7 Mechanism of herbal products for anti-obesity:

2.7.1 Gene Regulation during adipocyte differentiation:

Adipocytes are required for lipid regulation and energy balance. Adipose tissue stores triglycerides and produces free fatty acids in response to energy needs. As a result, herbal extract expected at suppressing adipogenesis is being developed. Many transcription factors, including C/EBPα and PPARϒ, are inhibited, resulting in the suppression of adipocyte development. (Kang *et al.,* 2013).

2.7.2 Lipase inhibition effect:

Pancreatic lipase, a major enzyme in dietary triglyceride absorption, breaks down fat enzymatically; pancreatic lipase hydrolyses triglyceride to monoglyceride and FA (Marrelli *et al.,* 2013). Lipase inhibition, which interferes with fat absorption in the Gastrointestinal (GI) tract, is a potential approach for obesity. Lipase inhibitors are therefore commonly considered to be a successful treatment for obesity.

2.7.3 Rise in energy expenditure:

Some anti-obesity herbal drugs manage body weight by increasing energy consumption through the activity of BAT, which facilitates in the conversion of energy from food into heat by uncoupling protein1 (UCP1/thermogenin) As a result, plant extracts that can up-regulate UCP1 gene expression may be a promising technique for delivering an anti-obesity impact by boosting energy expenditure. (Kajimura *et al.,* 2014).

2.7.4 Appetite suppressant:

Many plant extracts stimulate the sympathetic nervous system, increasing satiety and energy expenditure by suppressing hunger, as well as increasing fat oxidation via neural signal peptides such as serotonin, histamine, and dopamine, and their associated receptor activities, which are associated with satiety regulation. These brain signal peptides and their receptors might be prospective targets for the development of anti-obesity dietary items by reducing calorie intake while improving satiety (Morton *et al.,* 2014).

2.7.5 Stimulating lipid metabolism:

Accelerated fat oxidation promotes triglyceride hydrolysis, which reduces fat storage and combats obesity by upregulating adrenergic receptors, which trigger lipolysis

via activation of adenosine monophosphate-activated protein kinase (AMPK), resulting in increased fatty acid oxidation (O'Neill *et al.*, 2013). In a conclusion, plant extracts that overexpress lipolysis can be explored in the development of anti-obesity therapies**.**

Obesity has become a serious global concern, necessitating the development of effective anti-obesity natural products. Several plant extracts and bioactive molecules have been studied in order to reduce obesity and related problems. Some of the plant extract and active components having anti-obesity potential are listed below in **Table No. 2.4.**

2.8 Indian scenario about Medicinal plants:

Herbal plant extracts are a type of traditional medicine that has been utilised by humans since the beginning of civilization and is still widely used today. According to the WHO, almost 80% of the population in undeveloped countries depend on traditional medicines for their primary health concern. India was a pioneer in the creation and recorded usage of traditional medicine, namely Ayurveda, Siddha, and Unani. More than 2000 medications of natural origin originating from various folkloric traditions are used in Indian medicine (Narayana *et al.*, 1998). In past decades, there has been a surge in the use of alternative medications all over the world to modify obesity, prevent obesity, and obesity-related illnesses. Several plant products, vegetables, herbs, and fruits are habitually consumed as part of a diet that provides significant health advantages by providing valuable nutritional and therapeutic qualities. Allopathic drugs' use has been limited due to their negative side effects.

Table No. 2.4: List of the plants reported for the anti-obesity properties

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
1	*Acorus calamus* Linn (Araceae)	Rhizome, roots and leaves	Ethyl acetate extract of *A. calamus* inhibits α-glucosidase activity.	HTT-T15 cell line	Avadhani (2013)
2	*Aegle marmelos* Linn (Rutaceae)	Leaves	The active ingredients Umbelliferone and Esculetin reduce Hyperlipidemia in obese rats fed a high-fat diet by depleting lipid content in adipocytes.	3T3-L1 preadipocytes	Karmase *et al.*, (2013)
3	*Agrimonia pilosa* Ledeb (Rosaceae)	Aerial parts	Active substance 1beta-hydroxy-2-oxopomolic acid suppresses adipocyte development and the expression of adipogenic marker genes such as PPAR-Ɣ, C/EBP alpha, GLUT4, adiponectin, aP2, ADD1/SREBP1c, resistin, and fatty acid synthase. By inhibiting PPAR-Ɣ and C/ EBP alpha expression, it also suppresses adipocyte development by downregulating certain adipocytokines.	3T3-L1 preadipocytes	Ahn *et al.*, (2012)
4	*Alnus hirsuta* (Spach) Rupr. (Betulaceae)	Leaves	Platyphyllonol-5-O-β-d-xylopyranoside suppresses the induction of PPARγ and C/EBPα protein expression, and inhibits adipocyte differentiation.	3T3-L1 preadipocyte	Lee *et al.*, (2013)
5	*Amomum cardamomum* L. (Zingiberaceae)	Seeds	C/EBPα, C/EBPβ, and PPARƔ gene and protein expression were regulated by seed extract.	3T3-L1 preadipocyte	Park (2014)
6	*Bauhinia variegate* L. (Fabaceae)	Flowers, flower buds, stem, roots, stem bark, seeds, leaves	It lowers elevated levels of total cholesterol, triglycerides, and LDLP while increasing levels of HDLP and brain serotonin. β-sitosterol in the stem causes serotonin release in the brain, which has anti-obesity properties.	Human neutrophils.	Bansal (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
7	*Brassica rapa* L. (Brassicaceae)	Root	Lipolysis-related genes, such as 3-adrenergic receptor, hormone-sensitive lipase, adipotriglyceride lipase, and uncoupling protein, are increased in white adipocytes of rats treated with B. campestris extract. In EBR-treated 3T3-L1 cells, cyclic AMPK, HSL, and extracellular signal-regulated kinase are activated.	3T3-L1 preadipocytes	An *et al.,* (2010)
8	*Caesalpinia sappan* L. (Leguminosae)	Heartwood	Brazilein decreases the induction of peroxisome PPAR-Ɣ (PPAR) and inhibits intracellular lipid accumulation during adipocyte development in 3T3-L1 cells.	3T3-L1 preadipocytes	Liang *et al.,* (2013)
9	*Citrus aurantium* L. (Rutaceae)	Fruits, leaves	It prevents AKT activation and GSK3 phosphorylation, which causes lipid build up and lipid metabolising genes to be down-regulated, preventing adipocyte development.	3T3-L1 preadipocytes	Moro and Basile (2000) Kim *et al,* (2012) Stohs *et al.,* (2012)
10	*Coptis chinensis* Franch. (Ranunculaceae)	Rhizome	In 3T3-L1 cells, it prevents lipid build up. Several adipocyte marker genes, such as proliferator activated receptor and CCAAT/ enhancer-binding protein, are drastically reduced by the five alkaloids found in this plant. Adipogenesis was shown to be inhibited by isolated alkaloids.	3T3-L1 preadipocytes	Choi *et al.,* (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
11	*Cucurbita moschata* Duchesne (Cucurbitaceae)	Stems	Decrease lipid accumulation by inhibiting peroxisome PPAR-Ɣ, CCAAT/ enhancer-binding protein α, fatty acid-binding protein 4, sterol response element-binding protein-1c, and stearoyl-coenzyme A desaturase-1 expression.	3T3-L1 preadipocytes	Lee *et al.*, (2012)
12	*Curcuma longa* L. (Zingiberaceae)	Rhizomes	AMPK causes lipolysis by increasing hormone-sensitive lipase and adipose triglyceride lipase mRNA levels and decreasing perilipin mRNA levels. Curcumin inhibits macrophage infiltration and nuclear factor kB activation produced by inflammatory agents in adipose tissue.	3T3-L1 preadipocytes	Bradford (2013) Ho (2013)
13	*Cyclopia falcata* (Harv.) Kies (Leguminosae)	Stem	Phloretin-3′,5′-di-C-glucoside, a flavonoid, decreases intracellular triglyceride and suppresses adipogenesis in vitro.	33T3-L1 preadipocytes	Dudhia *et al.*, (2013)
14	*Cyclopia maculata* (Andrews) Kies (Leguminosae)	Stems	Hesperidin and Mangiferin suppress intracellular triglyceride and fat accumulation, as well as PPAR2 expression, and can prevent adipogenesis in vitro.	3T3-L1 preadipocytes	Dudhia *et al.*, (2013)
15	*Eremochloa ophiuroides* (Munro) Hack (Poaceae)	Whole Plant	The key transcriptional regulators of adipogenesis, C/EBPα and PPARƔ, were expressed. Furthermore, this plant reduces AKT and GSK3 phosphorylation levels.	3T3-L1 preadipocytes	Park *et al.*, (2012)
16	*Glycine max* (L.) Merr. (Leguminosae)	Bean	In 3T3-L1 preadipocyte cells, it suppresses adipocyte development. Triglyceride accumulation was prevented, and AMPK is stimulated.	3T3-L1 preadipocytes	Kim (2014) Singh *et al.*, (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
17	*Houttuynia cordata* Thunb. (Saururaceae)	Leaf	Reduces fatty acid synthase, sterol regulatory element-binding protein-1, and glycerol 3-phosphate acyltransferase expression. In mice, the extract prevents an increase in plasma TG levels. The extracts may inhibit NEFA and glycerol absorption by inhibiting FAT/CD 36, as well as aquaproin-7.	Human HepG2 hepatocytes	Kang and Koppula (2014) Miyata *et al.*, (2010)
18	*Ilex paraguariensis* A.St.-Hil. (Aquifoliaceae)	Leaves and unripe fruits	There is also a modulatory influence on the expression of adipogenesis-related genes such as PPAR2, leptin, TNF, and C/EBPα.	3T3-L1 preadipocytes	Arçari *et al.*, (2009) (2013) Gosmann *et al.*, (2012) Resende *et al.*, (2012)
19	*Ipomoea batatas* (L.) Lam (Convlvulaceae)	Tuber	SREBP-l, Acyl-CoA Synthase, Glycerol-3-Phosphate Acyltransferase, HMG-CoA Reductase, and Fatty Acid Synthase all have high levels of expression in liver tissue.	3T3-L1 preadipocytes	Ju *et al.*, (2011)
20	*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (Irvingiaceae)	Seed	Adipogenesis in adipocytes is inhibited. The impact appears to be mediated by decreased expression of adipogenic transcription factors (PPAR-) and adipocyte-specific proteins (leptin), as well as increased expression of adiponectin.	3T3-L1 preadipocytes	Ngondi (2009)
21	*Morus australis* Poir. (Moraceae)	Root	Increases lipolytic effects such as decreased intracellular triglyceride and the release of glycerol.	3T3-L1 preadipocytes	Kim *et al.*, (2010)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
22	*Nelumbo nucifera* Gaertn. (Nelumbnaceae)	Seed epicarp, leaves, seed, petals	Preadipocyte differentiation was inhibited by the extracts. In cultured human adipocytes, flavonoids suppress both adipocyte development and pancreatic lipase activity, accumulation, and expression of PPARγ, GLUT4, and leptin, showing that it reduces the differentiation of pre-adipocytes into adipocytes.	3T3-L1 adipocyte, NIH3T3 mouse fibroblast, L-02 normal hepatocyte cells CHO-K1, and U2OS cells,	Ahn *et al.,* (2013) Du (2010) Velusami *et al.,* (2013) You *et al.,* (2014)
23	*Nepeta tenuifolia* Benth. (Lamiaceae)	Whole plant	Triglyceride accumulation was inhibited in 3T3-L1 adipocytes, implying anti-obesity action.	3T3-L1 preadipocytes	Roh and Jung (2012)
24	*Pericarpium zanthoxyli* (Rutaceae)	Seed	Reduces the expression of the adipogenesis-related transcription factor, PPAR-Ɣ and PPAR-Ɣ target genes, such as adipocyte protein 2 (aP2), fatty acid synthase (FAS), and other adipocyte indicators, as well as CCAAT/enhancer-binding protein β (C/EBPβ) levels in a dose-dependent manner.	OP9 cells	Kim *et al.,* (2014)
25	*Petasites japonicus* (Siebold & Zucc.)	Flower buds	The extracts inhibit three adipogenic transcription factors: peroxisome PPAR-Ɣ2, CCAAT/enhancer-binding protein, and sterol regulatory element-binding protein 1c.	3T3-L1 preadipocytes	Watanabe *et al.,* (2010)
26	*Peucedanum japonicum* Thunb. (Apiaceae)	Leaves	SREBP-1c, fatty acid synthase, and acetyl-coenzyme A are all downregulated by pteryxin. A carboxylase-1 upregulates lipid catabolizing genes in treated 3T3-L1 adipocytes and HepG2 hepatocytes. Another study found that the extract inhibits SREBP1 c, a major lipogenic activator, and paternally expressed gene 1/mesoderm-specific transcript (PEG1/MEST), an adipocyte size marker gene, in vivo in adipose tissue.	Both 3T3-L1 and HepG2 cell lines, 3T3-L1 and HepG2 cells	Nugara *et al.,* (2014)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
27	*Rubus chingii* var. suavissimus (S.K.Lee) L.T.Lu (Rosaceae).	Leaves	Adipogenesis and the expression of adiponectin and leptin both are increased by the extract. Extract stimulates the mRNA production of adipogenic transcription factors CCAAT/ enhancer binding protein and PPAR-γ in the early stages of adipocyte differentiation.	3T3-L1 preadipocytes	Ezure and Amano (2011)
28	*Salicornia herbacea* L. (Amaranthaceae)	Whole plant	Isorhamnetin 3-O—D-glucopyranoside inhibits adipogenic development via inhibiting PPARγ, CCAAT/enhancer-binding proteins, SREBP1, and adipocyte-specific proteins. Activation of AMPK confirms a specific mechanism mediating the effects of isorhamnetin 3-—D- glucopyranoside.	3T3-L1 preadipocytes	Kong and Seo (2012)
29	*Siegesbeckia pubescens*L. (Amaranthaceae)	Whole plant	CCAAA/enhancer binding proteins, as well as peroxisome proliferator-activated receptor, gene, and protein expressions, influence the anti-obesity impact.	3T3-L1 preadipocytes	Park (2013)
30	*Smilax china* L. (Smilacaceae)	Leaves	Polyphenol and flavonoid with anti—glucosidase and anti-lipid buildup activities.	3T3-L1 preadipocytes	Kang (2013)
31	*Tetrapanax papyriferus* (Hook.) K.Koch (Araliaceae)	Whole plant	CCAAT/enhancer binding proteins, as well as peroxisome proliferator-activated receptor, gene, and protein expression, all influence the anti-obesity impact.	3T3-L1 preadipocytes	Park (2013).
32	*Veratrum nigrum* L. (Melanthiaceae)	Whole plant	In 3T3-L1 cells, it reduces lipid accumulation and the expression of two main adipogenesis factors, PPARɣ and C/EBPα.	3T3-L1 preadipocytes	Park *et al.,* (2013)

Continued…

Sr. No.	Name of Plant	Part Used	Action of Plant Extract	Cell Line	References
33	*Vitis labrusca* L. (Vitaceae)	Seed	Seed extract reduces lipid accumulation in C3H10T1/2 and 3T3-L1 cells in a dose-dependent manner. Inhibition is related with decreased PPAR- Ɣ expression.	C3H10T1/2 and 3T3-L1 preadipocytes	Oh (2013)
34	*Vitis vinifera* L. (Vitaceae)	Seed flours, peel, roots, fruit	By increasing the expression of hepatic genes involved in cholesterol (CYP51) and bile acid (CYP7A1) production, as well as LDL-cholesterol absorption. Mlxp1, Stat5a, Hsl, Plin1, and Vdr, which are involved in lipid metabolism, were all down-regulated. As evaluated by real-time polymerase reaction, the extract treatment reduces expression of aP2, Fas, and Tnfa, which are recognised indicators of adipogenesis. PPAR-γ expression in the liver and adipose tissue is reduced by controlling lipid metabolism and suppressing obesity.	3T3-L1 preadipocytes, high-fat diet- induced mice, murine 3T3-LI adipocytes,	Aguilar Santamaría *et al.,* (2012) Hsu *et al.,* (2014) Kim *et al.,* (2014) Jeong (2012, 2011) Kang *et al.,* (2011) Kim (2014) Zhang *et al.,* (2012)
35	*Ziziphus jujube* Mill. (Rhamnaceae)	Fruit	Decrease fat build up and glycerol-3-phosphate dehydrogenase activity. Attenuates the expression of major adipogenic transcription factors, including PPAR- γ and CCAAT enhancer binding proteins (C/EBPs), eliciting the greatest inhibitory impact.	3T3-L1 preadipocytes	Gao *et al.,* (2013) Mostafa *et al.,* (2013) Kubota *et al.,* (2009)
36	Germinated brown rice, germinated waxy brown rice, germinated black rice, and germinated waxy black rice	Seed	This seed extract reduces body weight growth and lipid build up in the liver and epididymal adipose tissue. The seed extract reduces the mRNA levels of adipogenic transcriptional factors such as CCAAT enhancer binding protein (C/EBP)-α, SREBP(SREBP)-1c, and peroxisome proliferator activated receptors (PPAR)-, as well as associated genes (aP2, FAS).	3T3-L1 preadipocytes	Ho *et al.,* (2012)

2.9 Anti-obesity effect of plant extract on the high-fat diet-induced rats:

Oh *et. al.,* (2014) studied the effect of *Achyranthes bidentata* Blume root extract on the high-fat diet-induced rat they observed that the drug shows an effect on the differentiation of adipocytes and decreases phospho- Akt expression. Halfordinol, ethyl ether aegeline and esculetin were the active chemical elements *of Aegle marmelos* leaves responsible for the reduction in adipocyte formation. Umbelliferone and esculetin are active substances that reduce hyperlipidemia by depleting lipid content in adipocytes in high-fat diet-induced obesity in male SD rats Karmase *et al.,* (2013).

In high fat-fed rats, *Allium cepa* peel extract reduces mRNA levels of activating protein (AP2) while increasing levels of carnitine palmitoyl transferase-1 (CPT-1) and fatty acid binding protein 4 (FABP4) (Kim *et. al.,* 2012). In diet-induced obese male SD rats, it is also hypothesised that *A. cepa* raises levels of PPAR-2 mRNA (mesenteric fats) and IL-6 mRNA (perirenal and mesenteric fats) (Moon *et al.,* 2013).

Galangin, the principal component of *A. galangal* Rhizome was extracted and studied for lipase inhibition activity. After that, Galangin was fed to the obese female rats. Obesity was induced in rats by feeding a cafeteria diet. The treatment with galangin decreases serum lipids, liver weight, lipid peroxidation and the accumulation of hepatic TGs. (Kumar and Alagawadi 2013). Ethanolic extracts of *Argyreia speciosa* root were studied on cafeteria diet-induced obesity rats. The extract reduces the serum contents of leptin, total cholestrol, LDL, and triglycerides. (Kumar *et al.,* 2011).

Shikov *et al.,* (2014) discovered that *Bergenia crassifolia* leaf extracts inhibited fat buildup in obese rats produced by a high-calorie diet. Galloylbergenin derivatives 3,11-Di-O-galloylbergenin and 4,11-Di-O-galloylbergenin are active chemicals found in the leaf extracts. The extract of *Boehmeria nivea* leaf decreases fat tissue weight as well as serum alkaline aminotransferase and lactate dehydrogenase activity. In animals given leaf powder, serum triglycerides, total cholesterol, LDL-cholesterol level, atherogenic index, and cardiac risk factors are reduced, while blood HDL-cholesterol levels are elevated. This investigation was conducted on male SD rats fed a high-fat/cholesterol diet (*Lee et al.,* 2011).

The hydroalcoholic extract of *Boerhaavia diffusa* root contains sitosterol, which is structurally identical to cholesterol and lowers LDL cholesterol and cholesterol levels in plasma in female SD rats on a high-fat diet (Khalid and Siddiqui 2012). Gupta *et al.,* (2013) investigated the ability of *Bombax ceiba* stem bark extract containing gemfibrozil to reverse the effects of a high-fat diet in wistar rats. This action might be attributed to the inactivation

of acetyl-coA carboxylase as a result of AMPK activation, which is responsible for thermogenesis and FAS inhibition.

C. japonica leaf extract regulates insulin, which modulates lipid synthesis via sterol regulatory element binding protein-1c (SREBP-1c). Insulin deficiency affects hepatic triglyceride production in SD rats fed a high-fat diet (Tamaru *et. al.*, 2013).

The impact of *Undaria pinnatifida* and fucoxanthin on metabolic, physiological, and inflammatory markers linked to obesity, as well as the expression of genes involved in white adipose tissue lipid metabolism, was investigated in a diet-induced overweight rat model. By up-regulating PPARα, PGC1, PPARɣ, and UCP-1, the treatments increased energy expenditure, oxidative stress, and adipogenesis. Image processing of retroperitoneal adipose tissue, which measured cell area, perimeter, and cellular density, also stated adipogenesis. Furthermore, the treatments reduced lipid synthesis and swelling by decreasing acetyl-CoA carboxylase (ACC) gene expression, boosting serum concentration and expression of adiponectin, and decreasing IL-6 expression. Both fucoxanthin and *Undaria pinnatifida* might well be explored for treating obesity and other disorders associated. (Grasa-López *et al.*, 2016)

Yazici-Tutunis *et al.*, (2016) investigated the impact of an aqueous extract of *Phillies latifolia* L. (Oleaceae), which is frequently found in the Mediterranean area of Turkey and is utilized in folk medicine as medicinal teas for weight reduction and hyperglycemia. They fed the rats a high-energy meal with *P. latifolia* leaf extract. In the HED + PLE group, PLE treatment resulted in a significant reduction in body weight. PLE increased mechanical strength and reduced leukocyte migration in the liver and small intestine tissues. The HED group had significantly higher levels of blood glucose, leptin, total cholesterol, and LDL. In the HED group, PLE medication reduced these levels. HDL levels were greater in the HED + PLE group than in the control and HED groups. For the first time, the chemical makeup of a Turkish sample of EtOAc extract of leaves was studied, and luteolin 7-O-glucoside and chlorogenic acid were determined. *Phillyrea latifolia* leaves may help with obesity-related cellular issues and might be a suitable source of anti-diabetic therapy.

Ramírez *et al.*, (2017) investigate the effects of *Mangifera indica* L. leaves because they include mangiferin, total phenolics, and antioxidants, all of which have several functional qualities. Hot leaf extracts were fed to Wister rats on a high-fat diet. They looked at biometrics and serum biochemical factors relating to metabolic control, inflammation, and oxidative stress, as well as visceral adipose tissue histomorphometry and mRNA

expression of PPAR-Ɣ co-activator 1 α, lipoprotein lipase (LPL), and fatty acid synthase (FAS). The ingestion of the extract increased total antioxidant capacity and interleukin10 serum concentrations, decreased belly fat storage, elevated PPAR-Ɣ and LPL expression, and downregulated FAS expression. Extract offers therapeutic promise in the treatment of obesity and related illnesses by modulating the expression of transcriptional factors and adipogenesis-related enzymes.

Bounihi *et al.,* (2017) assessed the effects of fruit vinegars on high-fat diet-induced rats fed a high-fat diet. Vinegar treatments effectively reduced the HFD-induced rise in body weight and visceral fat mass, as well as plasma levels of CRP, fibrinogen, leptin, TNF-a, AST, CK-MB, and LDH. Moreover, the fruit vinegar containing pomegranate, apple, and prickly pear may prevent HFD-induced obesity and obesity-related cardiac problems, and this protection may be due to the vinegar' significant anti-inflammatory and anti-adiposity capabilities.

In 2017 El Ayed *et al.,* investigated the effect of a high-fat diet (HFD)on lung lipotoxicity, oxidative stress, fatty acid composition and proportions in lung and implications in asthma development to lower the effect of lipotoxicity on the body they used grape seed extract. The trial revealed that theta HFD caused a lipid profile imbalance, raising cholesterol and VLDL-C. HFD also caused oxidative stress, as seen by increased MDA levels and a decrease in antioxidant activity such as SOD, CAT, and POD. Overall, GSSE demonstrated a strong preventative impact against HFD-induced obesity, and so may be employed as an anti-obesity drug as well as a beneficial agent with prospective uses against lung tissue damage.

CitroliveTM is a trademark for the combination of citrus fruit and olive leaves. CitroliveTM may regulate metabolic disorders associated with obesity and their side effects because citrus fruit and olive leaves contain bioactive compounds such as biophenols, which were shown to improve adiposity conditions through anti-hyperlipidaemic and anti-inflammatory effects, as well as by regulating lipoproteins and saturated fat body levels. CitroliveTM was discovered to inhibit pancreatic lipase after an animal study revealed that the CitroliveTM treatment substantially lowered the liver-to-body-weight ratio, as assisted by lower plasma transaminases, but non - significantly depleted plasma low-density lipoprotein (LDL) and postprandial TAG plasma levels. Finally, therapy alleviates symptoms of hepatotoxicity that are increased by the consequences of a high-fat diet. (Merola *et al.,* 2017).

Ekeleme-Egedigwe *et al.*, (2017) investigated the impact of *Vernonia amygdalina* leaves on obese wistar rats on a high-fat diet. Serum lipid profile, blood glucose concentrations, body weight, adiposity index, feed intake, faecal loss, and relative organ mass were all measured after twelve weeks of feeding. The researchers discovered that obese rats fed *Vernonia amygdalina* extract prevented HFD-induced weight gain and adiposity in rats while simultaneously increasing serum TG and TC levels as compared to obese rats fed a regular diet. The results show that feeding obese rats *Vernonia amygdalina* extract reversed fatty infiltration, resulting in lower weight gain and visceral fat mass in the rats.

2.10 *Dioscorea* species and anti-obesity:

Some species from the genus *Dioscorea* were screened for anti-obesity activity. *Dioscorea* species shows the different modes of action like lipase inhibition, which inhibits adipocytes differentiation.

Table No. 2.5: *Dioscorea* species reported for anti-obesity activity

Name of the species	Mode action	Compound	References
Dioscore nipponica	Lipase inhibitory	Dioscin	Kwon *et. al.,* (2003)
Dioscorea steriscus	Lipase and α-amylase inhibitors	flavonoids	Dzomba and Musekiwa (2014)
Dioscorea oppositifolia	Liapse inhibition Inhibit adipogensis	batasin	Jeong *et. al.,* (2016) Yang (2014)
Dioscorea batatas	Liapse inhibition	Dioscin	Jeon *et. al.,* (2006)
Marketed	Inhibits adipogenesis through the AMPK/MAPK pathway	Dioscin	Poudel (2014)
Marketed	Reduce weight in high-fat diet rat	Diosgenin	Son *et. al.,* (2007)
Dioscorea tokoronis	Suppresses the expression of SREBP-1 as well as that of fatty acid synthase in adipose and liver tissues.	-----	Song *et. al.,* (2009)

Herbal extracts have been traditionally used as an anti-obesity treatment due to their low or no negative effects. The current study attempted to investigate the anti-obesity effects of selected *Dioscorea* species. The study's purpose was to prevent the development of obesity and obesity-related complications by suppressing adipogenesis and lipogenesis.

3. MATERIAL AND METHODS:

3.1 Germplasm collection of different *Dioscorea* species:

3.1.1 Survey and collection of *Dioscorea* species:

Dioscorea species were collected through extensive field tours during June to November from 2018 to 2020 to different localities (Fig 3.1). The germplasm of collected *Dioscorea* species was maintained in Botanical Garden, Shivaji University, Kolhapur. The photographs were taken in field and after cultivation in garden by using Cannon Eos-1500D and mobile camera. The plant material was authenticated by consulting protologues and relevant literature. The herbarium and experts were also consulted for the purpose of identification of the species.

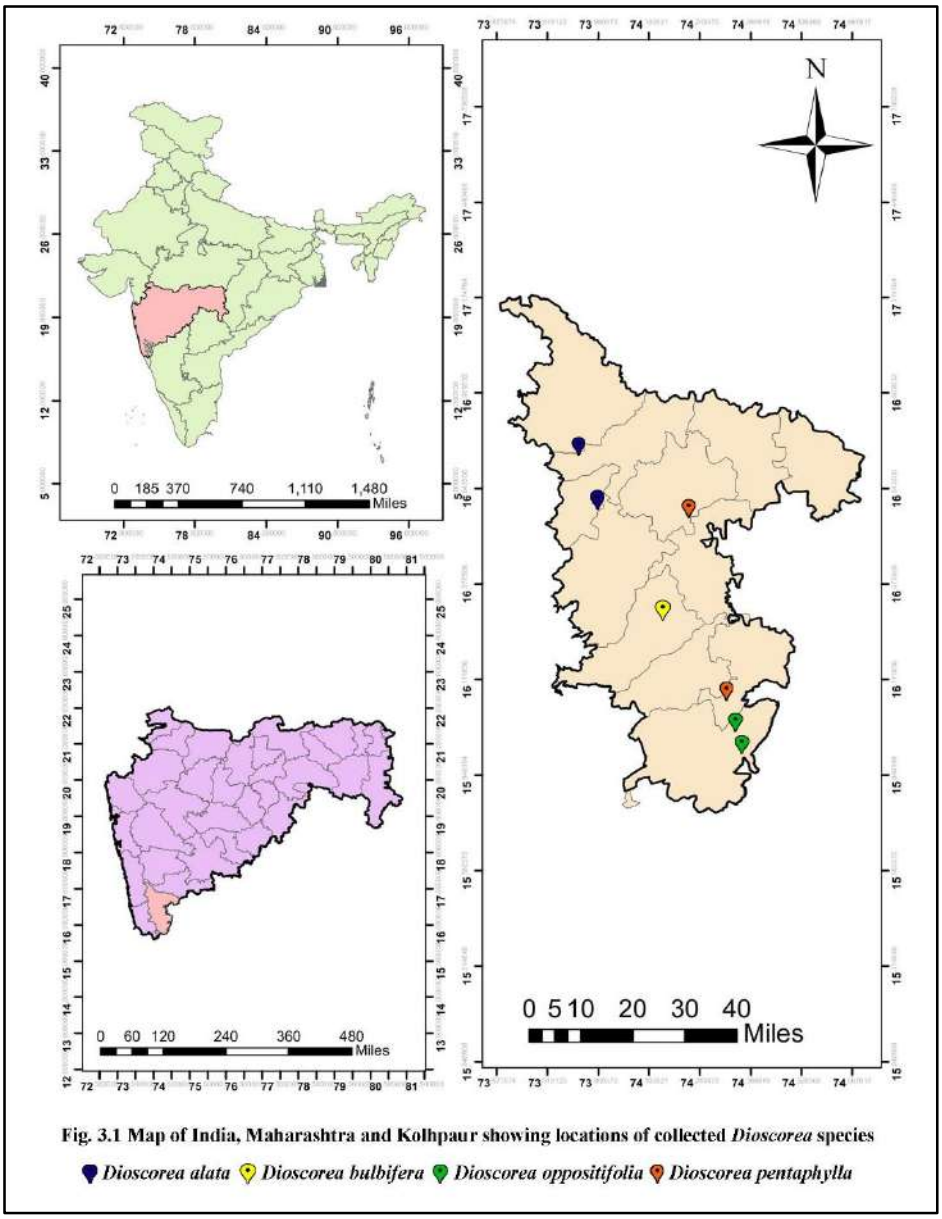


Fig. 3.1 Map of India, Maharashtra and Kolhpaur showing locations of collected *Dioscorea* species

3.1.2 Preparation of herbarium specimens:

Some of the specimens from collected plant material with proper phenological stage were used to prepare herbarium specimens. Specimens were dipped in 40% formalin for two days and pressed in blotting paper under wooden press. These specimens were dried using paper sheets with frequent changing. The dried specimens were poisoned, mounted and labelled on the herbarium sheet by standard method (Forman and Bridson, 1989). The processed herbarium specimens of *Dioscorea* species were placed in a well-labelled folder for each species containing photographs of habit and phenological stages**.** These specimens were deposited in the herbarium of Department of Botany, Shivaji University, Kolhapur.

3.1.3 Preparation of different extracts:

Extraction was carried out by a modified method by Sarker and Nahar, (2012). For extraction equal amount (10 gm) of dry plant powder and diatomaceous earth swas mixed and filled into a 66 ml stainless steel extraction cell. The cell was loaded in an accelerated solvent extractor (Dionex™ ASE™ 350 Thermo Scientific). The extraction was carried out using the following conditions, heating at 45˚C and pressurized for 5 min with static extraction under pressure 1300-1500 PSI. Then, flushed with fresh solvent for 0.5 min and purged with nitrogen for 1.0-2.0 min. Extracts [methanolic (350 mbar) and aqueous (72 mbar)] were collected and evaporated by rotary evaporator under vacuum at 64˚C and 100˚C, respectively. After evaporation, the dry residues were removed and dissolved in the respective solvents to obtain concentrations of 10 mg/ ml for each assay. And stored at -20˚C until used.

3.2. Chemo profiling of *Dioscorea species* for specific antiobesity molecules:

3.2.1 Estimation of Total Phenolic Content:

The total phenolic content was estimated as follows; 50µl of distilled water was distributed in all the wells; 12.5µl of plant extract and 12.5µl of Folin–Ciocalteu reagent were added, then incubated at room temperature for 10 min. after incubation 7% sodium carbonate was added and the reaction volume was made up with 100µl of distilled water and incubated in the dark at 37°C for 90 min. After incubation, optical density was measured at 760 nm on a 96-well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Phenolic content was determined by the calibration curve of standard gallic acid (20 to 100µg/ml). (Wolfe and Lui 2003)

3.2.2 Estimation of Total Flavonoid Content:

Total flavonoid content was estimated by the Luximon-Ramma *et al.,* (2002) method, with 100 µl of plant extract and 100µl of 2% aluminium chloride mixed together and incubated at room temperature for 10 min. Then optical density was measured at 368 nm on a 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Flavonoid content was determined by the calibration curve of standard quercetin (20 to 100µg/ml).

3.2.3 Estimation of Total Alkaloid Content:

The total alkaloid content was determined by using the method described by Singh *et al.,* (2004) with minor modification, 100 µl of plant extract was mixed with 100 µl of 0.05M Phenanthroline solution and 100 µl of 0.025M $FeCl_3$. The final volume was made to 1 ml using distilled water. The reaction mixture was incubated at 70^oC for 30 minutes in the water bath. Absorbance was measured at a 510 nm wavelength. Colchicine was used as a standard at varying concentrations (20 µg to 100 µg). The total alkaloid content was calculated from the calibration curve and the results were expressed as mg of Colchicine equivalent per gm fresh or dry weight.

3.2.4 Estimation of Total Terpenoid Content:

Total terpenoid content was determined by colorimetry using the following procedure (Chang *et al.,* 2012) 50µl of plant extract was mixed with the 75µl of 5% vanillin-glacial acetic acid solution and 250µl of perchloric acid, and the samples were heated at 45^oC for 60 min. After incubation cooled on an ice bath, 1.125 ml of glacial acetic acid was added and then optical density was recorded at 548 nm on a 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific).

3.2.5 Estimation of Total Saponin Content:

Saponin content in *Dioscorea* spices was determined according to the method described by Hiai *et al.,* (1976) with slight modification. In a 1.5 ml vial, 50µl of standard and plant extract were dispensed in triplicate. After that, 50µl of 8% vanillin was added to each tube. Sulphuric acid in the amount of 500µl was added to each tube. The reaction tubes were incubated in a water bath for 10 minutes at 60^oC. After cooling, optical density was recorded at 544 nm was measured using a 96well plate reader (Multiskan sky 96well spectrophotometer, Thermo scientific).

3.2.6 Anti-oxidant assays:

A. Determination of Free radical scavenging activity by DPPH:

The free radical scavenging activity of the plant extract was analysed with the method of Blois (1958), Rosidah *et al*,.(2008). For this, 10µl of plant extract was mixed with 290µl of DPPH solution (200µM in methanol). After addition, the plate was incubated at 37ºC for 30 min in the dark, and optical density was measured at 517nm on a 96-well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Ascorbic acid (20 to 100 µg/ml) was used as a standard. The formula was used to calculate the percentage of DPPH radical scavenging activity.

% Inhibition = [(Control OD – Sample OD)/Control OD] x100

B. Determination of ABTS scavenging activity:

The protocol of Patil *et al.,* (2020) was followed with some modifications to determine the ABTS scavenging poetical of plant extracts. To generate ABTS radicals, ABTS (7mM) and potassium persulfate (2.4mM) were mixed in equal parts and allowed to stand at room temperature for 16 hours in the dark. Then absorbance was measured at 734nm, to obtain O.D. of 0.700. After getting OD 0.700 the 10µl plant extracts were treated with 290µl of ABTS in 96 well plate. Before taking the readings at 734nm. Ascorbic acid (20 to 100µg/ml) was used as standard. % ABTS radical scavenging activity was estimated by using formula.

% Inhibition = [(Control OD – Sample OD)/Control OD] x100

C. Determination of FRAP (Ferric Reducing Antioxidant Power) assay:

The ferric reducing antioxidant power of plant extract was determined by the Benzie and Strain (1996) method. In a 10:1:1 ratio, 0.3M acetate buffer (pH 3.6), 10mM TPTZ (31.23 mg added to 10 ml of 40mM HCl), and 20 mM $FeCl_3$ (54.06 mg dissolved in 10 ml of D/W) were combined to make FRAP reagent. The test was performed in a 96-well plate.10 µl of plant extract was mixed with 290µl and incubated for 15 min at 37ºC. Then optical density was measured at 595nm on a 96well plate reader (Multiskan sky 96well spectrophotometer, thermo scientific). Ascorbic acid (20 to 100µg/ml) was used as a standard.

3.3 To evaluate species from genus *Dioscorea* for antiobesity activity:

3.3.1 Testing of crude extracts for lipase inhibition activity:

The activity of Porcine Pancreatic Lipase (PPL, type II) was evaluated using the substrate p-nitropheny l butyrate (p-NPB). Earlier published technique for assessing pancreatic lipase activity was adapted for this study (Kim *et al.*, 2010 and Zheng *et al.*, 2010). PPL stock solutions (0.6 mg/ml) were prepared in a 0.5 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl and kept at 20˚C. The lipase inhibitory action was evaluated using extracts or Orlistat (concentrations ranging from 1 mg/mL to 5 mg/mL) For the lipase inhibition reaction, 100 µl of buffer, 50 µl of lipase enzyme, 25 µl of plant extract or Orlistat, and 25 µl of p-nitrophenyl butyrate substrate were added, and the optical density (OD) at 400 nm was measured using a spectrophotometer (Multiskan sky 96 well spectrophotometer, Thermo scientific). For control reaction, 125 µl buffer, 50 µl of lipase enzyme, and 25 µl of p-nitrophenyl butyrate substrate were added. The inhibitory activity was calculated according to the following formula:

$$\%\text{Inhibition} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

3.3.2 Testing of crude extracts on cell line 3T3-L1 pre adipocytes:

A. 3T3-L1 Cell Culture:

3T3-L1 preadipocytes were obtained from the National Centre for Cellular Sciences (NCCS) Pune, India. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with high glucose (Himedia laboratories India) containing 10% Fetal Bovine Serum (FBS) (Invitrogen, Waltham, Massachusetts, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin, in a humidified environment of 5% CO_2 at 37˚C. Cells were used for additional experiments once they reached 80% confluency.

B. Cell viability assay:

The effect of *Dioscorea* extract on the viability of 3T3-L1 preadipocytes cells was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Himedia laboratories India) assay. 3×10^4 cells were seeded on a 96 well plate and incubated for 24 hrs before treating with various *Dioscorea* species extracts at varying concentrations (6.25, 12.5, 25, 50, and 100 µg/ml). Extracts were diluted with DMEM media, then incubated for 24 hrs at 37˚C in 5% CO_2. Following incubation, cells were

treated with MTT for 4 hrs to form crystals. The mitochondrial reduction process of the live cell reduces MTT into formazan crystals that were dissolved in Dimethylsulfoxide (DMSO), and the O.D. was measured at 570 nm using a 96-well plate reader (Multiskan Sky Thermo fisher scientific).

C. 3T3-L1 Cells Differentiation:

For differentiation of 3T3-L1 preadipocytes into adipocytes, 3×10^4 cells were seeded in a 24 well plate and incubated for 48 hrs. After incubation, the cells were stimulated with a hormone cocktail comprising 0.5 mM 3-isobutyl-1-methylxanthine (sigma Aldrich, USA), 1 M Dexamethasone (Himedia laboratories India), and 1µg insulin (Sigma Aldrich, USA) coupled with various extracts of different *Dioscorea species* at varying concentrations (25 and 75 µg/ml) for 48 hrs. Then medium was changed with DMEM supplemented with 10% FBS and 1µg insulin, along with the plant extract, and kept at 37˚C with 5% CO_2 until day 8. Differentiated adipocytes were observed under microscope (Yang *et al.*, 2014).

D. Oil Red O Staining of Differentiated adipocytes:

For Oil Red O staining of differentiated adipocytes, on day 8 after induction of differentiation, the medium was withdrawn from the 24 well plates, and the cells were washed twice with 1X PBS. For 30 mins, the cells were fixed in 70% ethanol. The fixed cells were rinsed twice with 1X PBS to remove the ethanol. After that, Oil Red O stain [0.6% Oil Red O, Sigma Aldrich, USA in isopropanol: water (3:2)] was added 300µl to each well and incubated for 1 hr at room temperature and then washed twice with distilled water. The staining of differentiated adipocytes was examined under an inverted Microscope (INVI Inverted Microscope, Magnus Opto Systems India).

E. Quantification of lipid accumulation:

Accumulated lipids were quantified by the eluted Oil O Red stain that remained in the cells after rinsing with isopropanol and quantified by measuring the absorbance at 500 nm. The percentage of inhibition of lipid accumulation was calculated by the following formula:

% Inhibition of lipid accumulation = (OD of control – OD of the sample)/OD of control × 100

F. Gene expression studies:

Total mRNA was isolated by using the trizol method with some modification. The harvested cells were suspended in 500µl of trizol solution then 250µl of the chloroform was added and mixed vigorously and incubated at room temperature for 5 min. Then centrifuged at 10000 rpm for 10 min. After centrifugation upper aqueous layer was collected in to the fresh tube. The precipitation was carried out by adding 550µl of isopropanol in the aqueous layer and incubated at -20ºC for 15 min. Afterward centrifuged at 14000 rpm for 30 min. and supernatant was discarded. The pellet was washed twice with 75% ethanol. Later pellet was air dry at room temperature for 15 min. Dry pellet was then suspended in 50µl of the DEPC treated water and stored at -20ºC or used for c DNA synthesis. c DNA synthesized by using the High-Capacity cDNA Reverse Transcription Kits (4368814 thermofisher scientific) the 2X master mix was prepared according to instructions given in kit. **(Table no 3.1)** and 10µl of master mix was mixed with 10µl RNA. Then tube was kept in thermal cycler with condition given in table 3.2.

Table No. 3.1: Preparation of Master Mix for c DNA Reaction

Sr. No.	Content	Volume in µl for 1 reaction
1	10X RT Buffer	2.0
2	25X dNTP Mix (100 mM)	0.8
3	10X RT Random Primers	2.0
4	MultiScribe™ Reverse Transcriptase	1.0
5	Nuclease-free H_2O	4.2
	Total volume	**10µl**

Table No. 3.2: Thermal cycling conditions for cDNA Synthesis

parameter	Step 1	Step 2	Step 3	Step 4
Temp.	25ºC	37ºC	85ºC	4ºC
Time	10	120	5	∞

G. Real time PCR:

For gene expression study Applied Biosystems' Quant Studio™ 3D Digital qPCR instrument was used. Master mix was prepared as per mentioned in to the **Table 3.3** and mixed with 1.3µl c DNA and gene specific primers (kicqstart primer Sigma Aldrich USA) generated from sequences obtained from the NCBI nucleotide sequence database for

amplification**.** The polymerase chain reaction was carried out at the following temperatures (95ºC for 45 sec, gene specific **(Table 3.4)** annealing temp for 45 sec, 72ºC for 45 sec for 40 cycles) and fluorescence measurement during annealing and extension stage. All of the responses were carried out in triplicate. Glyceraldehyde 3 phosphate dehydrogenase was used as a reference gene to normalize the gene expressions.

Table No. 3.3: PCR reaction master mix (Invitrogen, Waltham, Massachusetts, USA).

Sr. No.	content	Volume in µl for 1 reaction
1	SYBR Green Master Mix	5.0
2	gene specific forward primers	1.1
3	gene specific reversed primers	1.1
4	Nuclease free water	1.5
5	cDNA	1.3
	Total volume	10

Table No. 3.4 Primer sequences used for qPCR reaction

Genes	Forward primer	Reverse primer	Temp. ^{O}C
PPARɣ	AAAGACAACGGACAAATCAC	GGGATATTTTTGGCACTCTG	60.0
SREBP 1	AATAAATCTGCTGTCTTGCG	CCTTCAGTGATTTGCTTTTG	59.7
FAS	GATTCAGGGAGTGGATATTG	CATTCAGAATCGTGGCATAG	59.55
CPT1	ACTAACTATGTGAGTGACTGG	TGGCATAATAGTTGCTGTTC	55.5
C/EBP α	AAGGGTGTATGTAGTAGTGG	AAAAAGAAGAGAAGGAAGCG	56.15
GAPDH	TGAACGGGAAGCTCACTGG	TCCACCACCCTGTTGCTGTA	60.2

(PPARγ): Peroxisome proliferator-activated receptor-gamma; **(SREBP 1):** Sterol regulatory element binding protein 1) ; (**FAS):** Fatty acid synthase; **(CPT1):** Carnitine palmitoyltransferase1; (**C/EBPα):** CCAAT/Enhancer binding protein**; (GAPDH):** Glyceraldehyde-3-phosphate dehydrogenase.

H. LCMS-MS analysis:

To analyse the metabolites the 6200 series TOF/6500 series Q-TOF B.09.00 (B9044.0) UHD accurate mass QTOF LC/MS was used. The extracted aliquot was filtered and diluted with mobile phase before injection for LC separation and subsequently to the hybrid mass analyser. For LCMS/MS analysis, 5 µl of extract was injected. The gradient mode was used to separate the samples. Mobile phase gradient is given in the **Table No. 3.5.**

Table No.: 3.5 Flow profile of mobile phase

Time (min)	Flow (ml/min)	Solvent A [0.1% formic acid in H_2O (v/v)]	Solvent B Acetonitrile
0- 18 min	300	95%	5%
18.10-25 min	300	5%	95%
25-25.10 min	300	95%	5%
25.10-30min	300	5%	95%

The column runoff was then continued for ionization under positive polarity and mass spectrometer m/z analysis. The Mass spectrometry was run in high-resolution mode with the following commands: The timer for the analytical run was set to 30 minutes, and the flow profile of the mobile phase, Gas Temp 325°C, Gas Flow (l/min) 8, Nebulizer (psig), Sheath Gas Temp 300°C, Sheath Gas Flow 10, etc., with a scan rate of 2 spectra/min and an Acquisition Mode of 60 -1700 (m/z). Mass Hunter Workstation Qualitative software was used to process the data (version B.06.00, Agilent Technologies, Santa Clara, CA, USA). For screened potential metabolites, the analysis of accurate mass and prediction of molecular formula in databases such as CAS, HMP, LMSD, KEGG, METLIN, and Mass Bank. METLIN's Personal Compound Database includes a mass MS/MS library with correct mass data (PCDL). All metabolites with extra accurate masses (Q-TOF MS/MS library reference spectra) are included in the METLIN PCDL.(Patil *et al.,* 2020)

3.4 Evaluation of anti-obesity of extracted compounds using cell line (3T3-L1 pro adipocytes):

3.4.1 Fractionation of the *D. oppositifolia* methanolic extract:

Methanolic extract was obtained through an accelerated solvent extractor. The methanol was evaporated by using a rotary evaporator to get a dry residue. Then, dry

residue powder was resuspended in to 30 ml distilled water to make a suspension. Then an equal volume of hexane was added, mixed well, and allowed to stand until two clear layers were formed. The hexane layer (upper one) was separated. Again, washed with hexane twice, and hexane fraction was collected. The process repeated with chloroform, ethyl acetate and n-butanol solvent. The remaining aqueous fraction contains water soluble compounds. All solvents were evaporated from each fraction, and the collected residue was suspended in DMSO at 1 mg/ml concentration. (Abubakar and Haque 2020)

3.4.2 Extraction of diosgenin:

Diosgenin was extracted using a modified of Shaha and Lee (2012) by acid hydrolysis process. 5 gm root powder was mixed with 100ml of isopropanol with 20% H_2 SO_4 and refluxed at 65^{o}C for 8 hours. Whatman filter paper was used to filter the refluxed material. The obtained filtrate was washed three times with hexane. The collected hexane extract was combined and washed three times with 5% NaOH. Again, rinsed with distilled water after the alkali wash. Finally, the solvent was evaporated in a rotary evaporator, and the dry powder produced was redissolved in ethanol.

3.4.3 All the fractions and extracted diosgenin were tested on the 3T3-L1 preadipocytes by following methods given in section 3.3.2 B, C, D, E, F, and G.

3.4.4 Identification of anti-obesity metabolites in chloroform fraction by LCMS-QTOF by following methods given in section **3.3.2 H** with modification in mobile phase gradient given in **Table No. 3.6.**

Table no 3.6: Flow profile of mobile phase

Time (min)	**Flow** (ml/min)	**Solvent A** [0.1% formic acid in H2O (v/v)]	**Solvent B** Acetonitrile
0-2 min	300	95%	5%
2-5 min	300	75%	25%
5-10 min	300	60%	40%
10-15 min	300	40%	60%
15-20 min	300	20%	80%
20-25 min	300	5%	95%
25-28 min	300	95%	5%
28-30 min	300	95%	5%

3.4.5 Detection of extracted diosgenin by HPLC

The JASCO Auto-sampler with UV-Visible detector was used for high performance liquid chromatography. Separations were performed on a C-18 column (250mmX 5mmX 5 microns) at a flow rate of 1 ml/min, at a temperature of 35°C, and peaks were detected at

absorption spectra 200 nm. With isocratic elution of Acetonitrile: water 90:10 (V/V) yielded well resolved chromatograms. The standard diosgenin retention time is 3.19 minutes. The extracted diosgenin was detected by matching the retention time with standard diosgenin.

3.5 *In vivo* studies

The animal study was carried out by using the Munshi *et al.,* (2014) method with some modification

3.5.1 Experimental design:

Obese model was developed by using the high fat diet containing 3ml/kg fat emulsion for 6 weeks along with normal diet and water. Animals were weighed, Labelled and randomly divided into 5 groups, each group containing 6 animals. All the animals were taken care with ethical consideration and protocol was dually approved by ethical committee **(1825/PO/EReBi/15/CPCSEA/2/2021, dated on 19 May 2021)**

Group I - Normal control (NC) - animal feed with Standard pellet diet and water for 24hr over a period of 6 weeks.

Group II – Diseased Control (DC) - animals feed with Standard pellet diet and water with 3ml/kg/day high fat diet.

Group III- Standard control (SC) - animal feed with Standard pellet diet and water with 3ml/kg/day high fat diet and 10 mg/kg/day of Orlistat.

Group IV- Test 1 group - animal feed with Standard pellet diet, water with 3ml/kg/day high fat diet and *D. oppositifolia* extract 200mg/kg/day.

Group V- Test 2 group - animal feed with Standard pellet diet, water with 3ml/kg/day high fat diet and *D. oppositifolia* extract 400mg/kg/day.

During the experiment daily weight of each animal was measured, and at the end of the experiment, Animals were taken by group wise and blood was collected using the retroorbital method.

3.5.2 Preparation of orlistat:

Orlistat was used as standard. Orlistat (Resheep) capsule content was dissolved in to the water to make 12mg/ml stock.

3.5.3 Experimental animals:

Wistar rats of 130 -150 gm were used for the study. Animals were housed in group of 6 animal each for 1 week in 12:12 h light and dark cycle in controlled temperature and humidity. After adaptation period the animals were used for the study.

3.5.4 High fat diet:

The high-fat diet was prepared by mixing Indian vanaspati ghee and coconut oil in a 3:1(V/V) ratio. It was fed to the rat every day at the dose of 1ml/kg of body weight.

3.5.5 Weight:

During the experiment the weight of each animal was recorded and noted for the determination of the dose of the drug every day.

3.5.6 Blood biochemical analysis:

At the end of the experiment the blood was collected group wise by retro orbital puncture and subjected to the centrifugation to separate serum. Total cholesterol, triglycerides (TG), LDL cholesterol, VLDL cholesterol and HDL cholesterol were analysed to check the lipids intake in the rat body.

3.6. Data analysis:

The data were shown as mean ± standard deviation and analysed using one-way ANOVA test with Dunnett's Multiple Comparison Test, and two-way ANOVA, $P < 0.05$ was considered as level of significance. The data were analysed using GraphPad Prism 5 Software, San Diego, California, USA.

4. RESULTS AND DISCUSSION

4.1 Germplasm collection

4.1.1 Germplasm collection of different *Dioscorea* species:

Dioscorea species were collected from various locations in the Kolhapur district during June to October 2018 to 2020. Frequent collection visits were conducted at various times of the year to obtain material in both vegetative and reproductive phases **(Plate No. 1-4). Table No. 4.1** shows the geographical coordinates with latitude, longitude, and voucher specimen numbers (**Plate No. 5**).

Table No. 4.1: Survey and collection sites of *Dioscorea* species

Sr. No.	Plant name	GPC location	Village name	Voucher specimen numbers.
1	*D. oppositifolia*	N $15^0$57'52.9, E$74^0$21'42.7 N $16^0$02'05.2, E$74^0$21'31.0	Kini Hadalge	RSP 001
2	*D. bulbifera*	N $16^0$20'21.3, E$74^0$08'47.1 N $16^0$20'11.9, E$74^0$08'46.4	Kalnakwadi Kalnakwadi	RSP 002
3	*D. alata*	N $16^0$38'36.5, E$73^0$57'02.4 N $16^0$38'37.33, E$73^0$57'01.51	Mandukali Karanjfen	RSP 003
4	*D. pentaphylla*	N $16^0$37'15.2, E$74^0$12'29.3 N $16^0$7'6.27", E$74^0$20'19.67	Katyayani hill Lakudwadi ghat	RSP 004

4.1.2 Preparation of plant extract and crude yield:

For the preparation of the crude extracts 10 gm the dry plant powder was used. From the 10 gm of dry plant powder in case of the methanolic extraction the yield of the crude residue is less than the aqueous extraction. The amount of the crude extract powder is mentioned in the **Table No. 4.2.** The highest yield was obtained in *D. alata* aqueous extract *i.e.,* 1700mg/10 gm of the dry powder and lowest yield was obtained in the *D. pentaphylla* methanolic extract *i.e.,* 250 mg/10 gm of the dry powder. While comparison between solvent the aqueous all the aqueous extract had the highest yield than the methanolic extract. For the further study extracts were diluted to the 1mg/ml.

Table No. 4.2: Amount of the crude extract powder after extraction

Sr. No.	Plant Name	Solvent	mg/10gm
1	*D. alata*	Methanol	720
		Aqueous	1700
2	*D. bulbifera*	Methanol	600
		Aqueous	1200
3	*D. pentaphylla*	Methanol	250
		Aqueous	740
4	*D. oppositifolia*	Methanol	700
		Aqueous	1500

4.2 Chemo profiling of *Dioscorea* species for specific anti-obesity molecules:

4.2.1 Estimation of Total phenolic content:

The total phenolic content of various parts of four yam species is shown in the **Table No. 4.3** and **Figure 4.1.** The phenolic content of four yam species differed significantly. The *D. oppositifolia* dry whole plant methanolic extract has the highest phenolic content, i.e. 128.14±1.70 mg/gm GAE of dry weight, while the lowest content was found in *D. pentaphylla* methanolic extract i.e. 34.23±0.70 mg/gm GAE of dry weight. When comparing the extraction solvents, the methanolic extract had a higher phenolic content than the aqueous extract of the plants. Phenolics are widely distributed throughout plants. Antioxidant and other biological activity features have been linked to Phenolics. Bhandari *et al.,* (2004) investigated the four *Dioscorea* species found in Nepal: *D. bulbifera, D. versicolor, D. deltoidea, and D. triphylla,* and found that *D. bulbifera* has the greatest phenolic content, while *D. triphylla* has the lowest. Sakthidevi and Mohan (2013) worked on the *D. alata* and reported that *D. alata* have 0.68mg/100gm of phenolic content in the methanolic extracts. The phenolic content of the different vaterites of *D. alata* and *D. esculenta* from the Philippine were studied by the Cornago *et al.,* (2010) reported the 69.9 to 421.8 mg gallic acid equivalent (GAE)/100 gm dry weight. Ghosh *et al.,* (2013) prepared *D. bulbifera* bulbs extract in different solvents and examined the phenolic content and reported that methanolic extract contains the highest phenolics *i.e.,* 145.446± 3.29 µg/ml than the other solvents used.

Table No. 4.3: Comparison of the phenolic, flavonoids, alkaloid, terpenoids and saponins content in different *Dioscorea* extract,

Extract	DAM	DBM	DPM	DOM	DAA	DBA	DPA	DOA
Total Phenolic Content	110.09 ± 1.80	37.68 ±2.04	34.23 ±0.70	128.14 ±1.70	68.94 ±1.33	39.63 ±2.07	55.03 ±2.08	65.38 ±7.83
Total Flavonoid Content	95.56 ± 0.645	39.50 ±0.308	34.11 ±0.867	94.45 ±0.308	82.15 ±0.760	24.11 ±0.760	47.10 ±0.534	42.83 ±0.226
Total Alkaloid Content	385.28 ±1.359	147.10 ±1.125	137.81 ±0.614	407.20 ±5.290	284.78 ±0.505	139.63 ±0.863	102.25 ±0.789	219.22 ±0.440
Total Terpenoid Content	288.90 ±5.94	307.77 ±18.26	282.70 ±5.12	349.70 ±2.54	14.00 ±4.04	6.93 ±12.60	-42.27 ±4.51	6.43 ±2.66
Total Saponins Content	543.87 ±4.06	350.53 ±3.53	458.87 ±2.33	547.53 ±2.19	424.87 ±5.84	482.87 ±2.40	420.53 ±3.76	579.53 ±5.33

Values presented in ± SE (n=3). DAM- *D. alata* methanolic extract, DBM- *D. bulbifera* methanolic extract, DPM- *D. pentaphylla* methanolic extract, DOM- *D. oppositifolia* methanolic extract, DAA- *D. alata* aqueous extract, DBA- *D. bulbifera* aqueous extract, DPA- *D. pentaphylla* aqueous extract, DOA- *D. oppositifolia* aqueous extract.

4.2.2 Estimation of Total flavonoid content:

Flavonoids are a class of naturally occurring polyphenol that are distinguished by their flavone nucleus. These are currently regarded an essential component in a wide range of nutraceutical, pharmaceutical, medical, cosmetic, and other products. Flavonoids promote good health and prevent illness (Karak 2019). While comparing the flavonoid content in different *Dioscorea* extract The maximum flavonoid content was found in *D. alata* methanolic extract *i.e.,* 94.45±0.308 mg/gm quercetin equivalent of dry weight and the lowest content found in *D. bulbifera* aqueous extract *i.e.,* 24.11±0.760 mg/gm quercetin equivalent of dry weight **(Table No. 4.3 and Figure No. 4.2)**. Both methanolic and aqueous the extracts of *D. bulbifera* and *D. pentaphylla* showed the lower flavonoid content than all other extracts. Sakthidevi and Mohan (2013) reported *D. alata* have 1.12gm/100gm of flavonoid content in the methanolic extracts. Ghosh *et al.,* (2013) have reported 27.866±0.18 µg/ml of the flavonoid content in ethyl acetate extracts of *D. bulbifera* bulbs, other three solvents show the less flavonoid content. Sonibare and Abegunde (2012) studied the Nigerian *D. dumetorum* (Kunth) Pax and *D. hirtiflora* for their biological activity. They have reported the flavonoid content 13.33±0.33 and 25.60±0.20 mg/gm of the dry weight respectively. Dzomba and Musekiwa

(2014) isolated the flavonoids from the *D. steriscus* and by using TLC and tested for the lipase inhibition activity.

4.2.3 Estimation of Total alkaloid Content:

Alkaloids are among the most significant and well-known secondary metabolites, alkaloids are the end products of nitrogen metabolism in plants, Because of toxicity in yam tuber, alkaloid content is crucial. These toxic metabolites can be found in variable amounts in yam tubers (Poornima and Rai 2009). While comparing the alkaloid content in the different *Dioscorea* extracts **(Table No. 4.3 and Figure No. 4.3)** the maximum alkaloid were found in the methanolic extract of the *D. oppositifolia i.e.,* 407.20±5.290 mg/gm of Colchicine equivalent and the minimum was found in the aqueous extract of the *D. pentaphylla i.e.,* 102.25±0.789 mg/ml Colchicine equivalent. Also, both the extracts of the *D. bulbifera* and *D. pentaphylla* showed less alkaloid content. Poornima and Ravishankar (2009) reported the alkaloids content 0.68 mg 100-1g in *D. belophylla.* Ogbuagu (2008) studied effect of cooking on the alkaloid content in *D. bulbifera and D. dumentorum*. After cooking in both the tubers the alkaloid content was reduced. Ezeabara and Anona (2018) investigated the alkaloid content in four different yams from Nigeria namely *D. alata, D. bulbifera, D. cayenensis and D. rotundata* where they found that highest alkaloid is present in the *D. bulbifera i.e.,* 0.64 ± 0.01 mg/100g.

4.2.4 Estimation of Total Terpenoid content:

Terpenoids, also defined as isoprenoids, are a wide and diversified group of naturally occurring organic compounds formed from isoprene, a 5-carbon molecule, and isoprene polymers called as terpenes. Terpenoids are the most common type of plant secondary metabolite and they have significant pharmacological bioactivity. The terpenoid content in the different extracts of the *Dioscorea* species shows in the **(Table No. 4.3 and Figure 4.4).** The highest terpenoid content was observed in the methanolic extracts of the *D. oppositifolia i.e.,* 349.70±2.54 mg/gm of ursolic acid equivalent. Adeosun *et al.,* (2016) studied the phytochemicals properties of the *D. bulbifera* and reported the 20.40 mg/gm of the terpenoids. Odimegwu *et al.,* (2013) extracted Essential oil from *D. floribunda* and *D. composite* and carried out the GCMS analysis they found 76 compounds in *D. floribunda* and 37 from *D. composita* essential oil, major compounds were terpenoids. The comparison in the methanolic

and aqueous extracts of *Dioscorea,* most of the terpenoid extracted in the methanol than the water.

4.2.5 Estimation of Total Saponins Content:

Saponins are major compounds found in the *Dioscorea* species, these are water soluble compound commonly found in the toxic plants. The *Dioscorea* tuber contains high amount of saponins. Saponins were estimated by using method described by Hiai *et al.,* (1976). The highest saponins content was found in the in the aqueous extract of the *D. oppositifolia i.e.,* 579.53±5.33mg/gm of Diosgenin equivalent and the lowest content of saponins observed in the methanolic extract of *D. bulbifera*. i.e. 350.53 ±3.5 mg/gm of Diosgenin equivalent **(Table No. 4.3 and Figure 4.5).** *D. belophylla* has the saponin content of 18.46 mg 100-1g (Poornima and Ravishankar 2009) in *D. bulbifera* and *D. diementorum* saponin content was reported as 79.48mg/100gm and 84.62mg/100gm respectively (Ogbuagu 2008).

Through multiple approaches, phytochemicals have a role in the treatment of obesity. Polyphenols have anti-obesity potential because they block fat metabolizing enzymes such as pancreatic lipase, lipoprotein lipase, and glycerol phosphate dehydrogenase. Polyphenols have the ability to lower blood glucose levels and lipid profiles, as well as boost energy expenditure and fat burning. Flavonoids are plant secondary metabolites that have previously been shown to have a key function in inhibiting pancreatic lipase activity. (Slanc *et al.*, 2009).

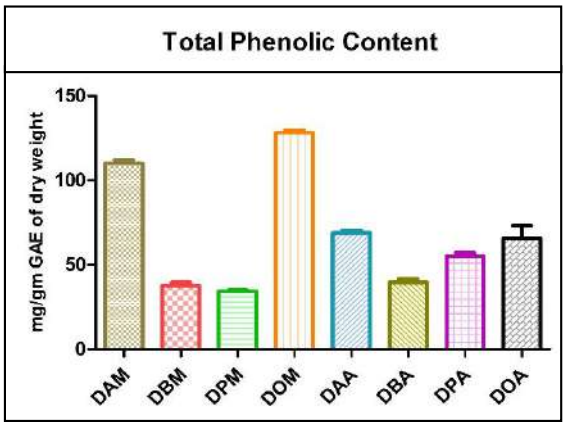


Figure No. 4.1: Total phenolic content in Different *Dioscorea* species.

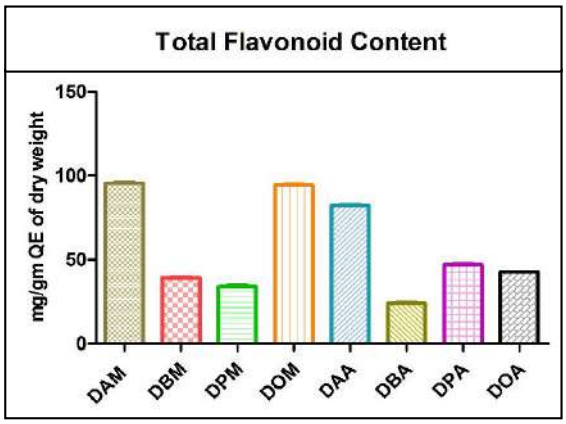


Figure No. 4.2: Total flavonoid content in Different *Dioscorea* species.

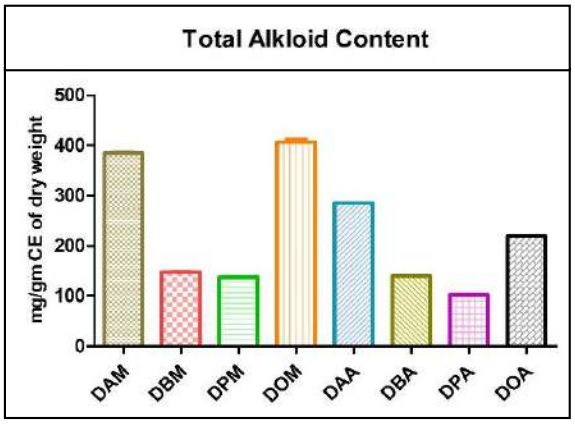


Figure No. 4.3: Total alkaloid content in Different *Dioscorea* species.

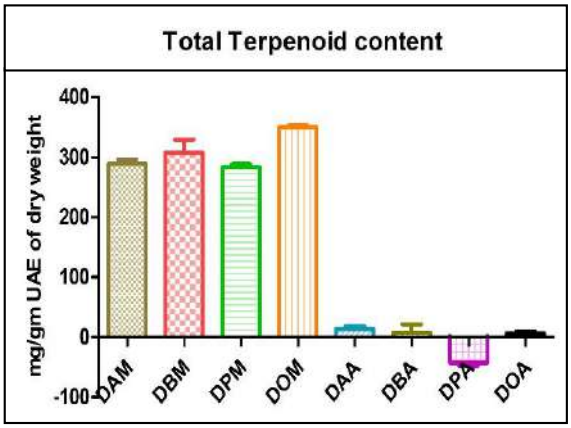


Figure No. 4.4: Total terpenoid content in Different *Dioscorea* species.

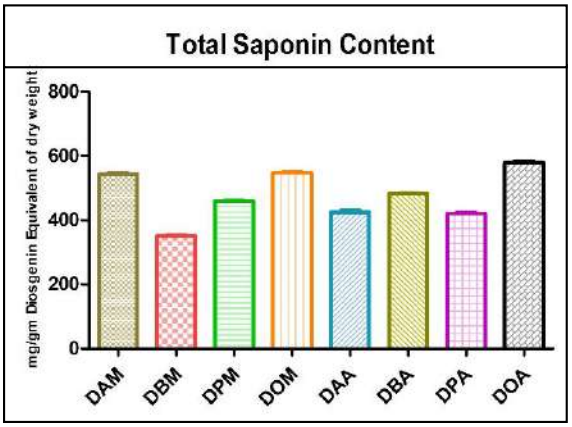


Figure No. 4.5: Total saponin content in Different *Dioscorea* species.

Results showed significantly difference in One-way analysis of variance (< *P=0.05*). (DAM - *D. alata* methanol, DBM - *D. bulbifera* methanol, DPM - *D. pentaphylla* methanol, DOM - *D. oppositifolia* methanol, DAA - *D. alata* aqueous, DBA - *D. bulbifera* aqueous, DPA - *D. pentaphylla* aqueous, DOA - *D. oppositifolia* aqueous)

4.2.6 Anti-oxidant activity:

During the obesity development inflammation and oxidative stress occur. Plant extracts have the ability to scavenge free radicals and reduce oxidative stress will be useful in the treatment of obesity. To evaluate the ability of the plant extracts to scavenge a free radicals developed during the obesity, antioxidant activity was analysed by an array of *in vitro* antioxidant assays **(Table No. 4.4)**.

The comparison of DPPH free radical scavenging activity of the Different *Dioscorea* extracts were presented in **Figure No.4.6**. The antioxidant activity is expressed as % inhibition. The highest DPPH radical scavenging activity was observed in methanolic extract of the *D. oppositifolia i.e.,* 80.30±4.919 % and the lowest was observed in *D. bulbifera* aqueous extracts *i.e.,* 5.65±1.326 %. In case of the ABTS radical scavenging activity the *D. oppositifolia* methanolic extract showed the highest % inhibition *i.e.,* 92.26± 0.484%. Also, both extracts of *D. alata* showed the above 90% inhibition of the ABST radical. **(Figure No 4.7)**. The lowest radical scavenging was shown by *D. bulbifera* methanolic extracts *i.e.,* 34.12± 1.345 %. While in the ferrous reducing antioxidant power assay the *D. alata* methanolic extract showed the highest ability i.e. 124.26±3.383 mM/gm of Ascorbic acid equivalent. Although minimum was observed in *D. bulbifera* aqueous extract 31.19± 1.136. **(Figure No 4.8)**

Table No.4.4: Comparison of the antioxidant activities Different *Dioscorea* extract.

Extract	DAM	DBM	DPM	DOM	DAA	DBA	DPA	DOA
DPPH % Inhibition	69.90± 7.388	16.09± 4.588	10.33± 0.393	80.30± 4.919	23.49± 2.690	5.65± 1.326	12.71± 1.117	25.16± 1.152
ABTS % Inhibition	92.11± 0.558	34.12± 1.345	50.48± 2.607	92.26± 0.484	91.82± 0.848	47.97± 2.014	91.24± 0.713	70.86± 1.302
FRAP mM/gm of AAE	124.26± 3.383	33.56± 4.608	31.43± 1.794	114.95± 6.461	80.26± 1.881	31.19± 1.136	51.58± 0.839	59.57± 1.639

Values presented in ± SE (n=3). DAM- *D. alata* methanolic extract, DBM- *D. bulbifera* methanolic extract, DPM- *D. pentaphylla* methanolic extract, DOM- *D. oppositifolia* methanolic extract, DAA- *D. alata* aqueous extract, DBA- *D. bulbifera* aqueous extract, DPA- *D. pentaphylla* aqueous extract, DOA- *D. oppositifolia* aqueous extract.

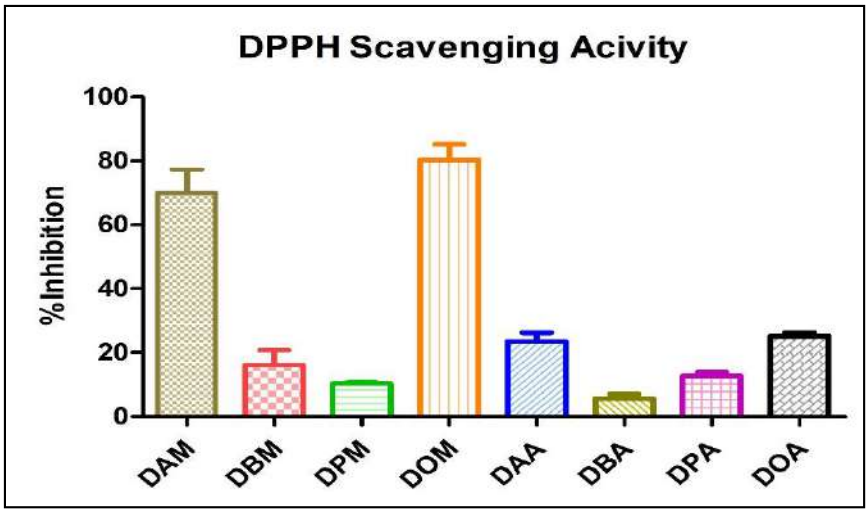


Figure No. 4.6: DPPH Free radical scavenging activity of different *Dioscorea* extracts

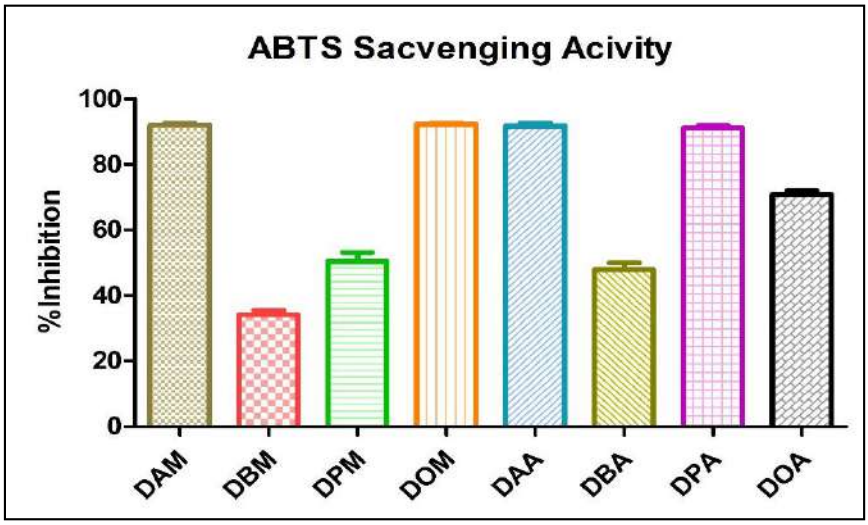


Figure No. 4.7: ABTS Free radical scavenging activity of different *Dioscorea* extracts

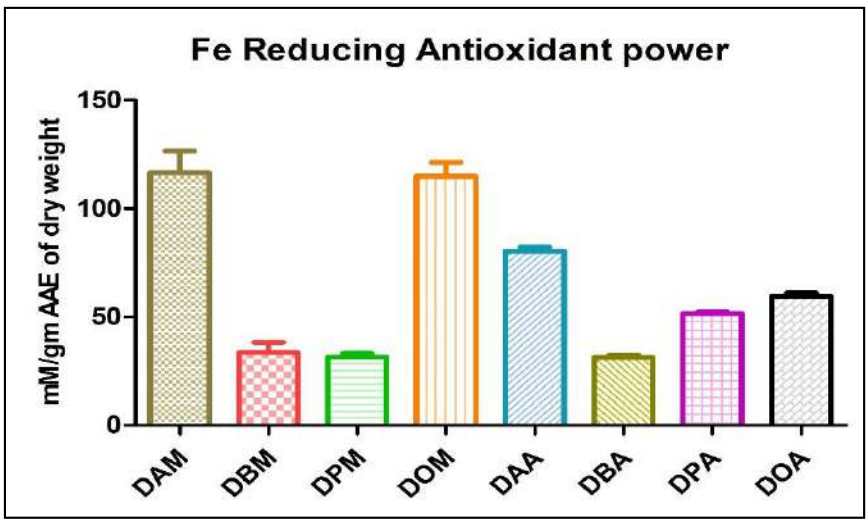


Figure No. 4.8: FRAP activity of different *Dioscorea* extracts

(DAM - *D. alata* methanol, DBM - *D. bulbifera* methanol, DPM - *D. pentaphylla* methanol, DOM - *D. oppositifolia* methanol, DAA - *D. alata* aqueous, DBA - *D. bulbifera* aqueous, DPA - *D. pentaphylla* aqueous, DOA - *D. oppositifolia* aqueous)

Obesity is characterised by low-grade inflammation caused by an increase in adipose tissue mass, which causes hypoxia and the release of pro-inflammatory cytokines. This results in cardiovascular disorders. Herbal extracts' ability to scavenge free radicals limits oxidation of macromolecules, hence avoiding a variety of obesity-related illnesses (Yang *et al.,* 2009; Mukherjee 2003).

It has been observed that phenolic chemicals have a variety of biological effects, including antioxidant activity. The existence of several antioxidant components in plant tissues, particularly fruits and vegetables, makes measuring each antioxidant component independently problematic. Bhandari *et al.,* (2004) evaluated the antioxidant activity of the four *Dioscorea* species from Nepal. They have reported *D. bulbifera* showing the strong DPPH scavenging activity. While the *D. deltoidea* showing the highest Fe reducing power than the other species. Also, the polyphenol content showing the correlation with the antioxidant activity. Sakthidevi and Mohan (2013) studied the antioxidant potential of the *D. alata.* They have reported leaf methanol extracts with strong antioxidant activity this activity may be because of the high phenolic and flavonoid content. Ghosh *et al.,* (2013) studied the antioxidant properties of the *D. bulbifera* by different *in vitro* assays and in all the studied assays the methanolic extracts exhibited the highest activity.

Yam (*Dioscorea* sp.) extracts have been shown to exhibit more than 70% antioxidant activity when tested using a model system including b-carotene and linoleic acid, and are classified as a vegetable with high antioxidant activity (Kaur & Kapoor, 2002). Farombi *et al.,* (2000) discovered a significant degree of antioxidant activity in several farmed yam species from Nigeria using the ABTS technique. Hsu *et al.,* (2003) discovered exceptional antioxidant properties in various cultivated yam species from Taiwan, as measured by DPPH radical scavenging, ferrous ion chelating, reducing power, and ABTS assays. Reports on the antioxidant activity of wild yam species, on the other hand, are quite rare in the literature. As a consequence, comparing our findings to those of earlier research is extremely challenging. Sonibare and Abegunde (2012) studied the antioxidant activity by DPPH activity of *D. hirtiflora, D. dumetorum* (edible) *D. dumetorum* (non-edible) and reported that *D. hirtiflora* exhibit the highest % of inhibition than *D. dumetorum*. Also, correlation between the total phenolic content and DPPH activity reported with R^2=0.892. Dzomba and Musekiwa (2014) isolate the different flavonoids from the *D. steriscus* and antioxidant potential was studied by

the DPPH assay. They have correlated the antioxidant and with the biological activity (lipase inhibition and amylase inhibition) also they suggested the flavonoids from the *D. steriscus* have the anti-obesity potential. Liu (2016) have reported the activity of the antioxidant activity of the fresh peel and flesh of *D. opposita* and got the highest free radical scavenging activity in the aqueous extract of peal, also studied the anticancerus properties of the extracts and reported the bioactive compound from the *D. opposita*.

4.2.7 Correlation of total phenolic, flavonoid, alkaloid, terpenoid, and saponin content of *Dioscorea* Species:

The Pearson correlation coefficients between antioxidant activities and TPC, TFC were represented in **Table No. 4.5** TPC strongly correlate with DPPH, and FRAP assays with r= 0.972, 0.968 respectively but with ABTS showing significant correlation with r=0.752, whereas TFC exhibited a strong correlation with FRAP activity with r = 0.962 and significant correlation with DPPH and ABTS with r=0.876, 0.790 respectively. Overall, the result revealed the existence of a positive and correlation between tested phytochemicals and antioxidant assays. TAC showing the stronger correlation with DPPH with r=0.940 and FRAP with r= 0.952. While with ABTS showing less correlation. Total terpenoid content and total saponin content were not showing significant correlation with any of the antioxidant assay.

Table No. 4.5: Correlation between antioxidant activity and phenolic, flavonoid, alkaloid, terpenoid, and saponins content of *Dioscorea* Species

Parameters	DPPH	ABTS	FRAP
TPC	0.972**	0.752*	0.968**
TFC	0.876**	0.790*	0.962**
TAC	0.940**	0.633	0.952**
TTC	0.583	0.426	0.558
TSC	0.572	-0.169	0.340

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed). TPC: Total phenolics content, TFC: Total flavonoids content, TAC: total alkaloid content, TTC: total terpenoid content, TSC: total saponin content. DPPH: 2, 2-diphenyl-1-picrylhydrazyl, ABTS: 2, 2'-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant property assay.

4.3. Evaluation of species from genus *Dioscorea* for anti-obesity activity:

4.3.1 Lipase inhibition activity:

Pancreatic lipase is primarily responsible for the hydrolysis of triglycerides into free fatty acids and monoglycerides. It's a lipolytic enzyme that catalysis the hydrolysis of the triacylglycerol's' ester bonds. The enzyme operates by removing the fatty acids from sites 1 and 3 of the triglyceride, leaving a 2-monoglyceride and two free fatty acids in their place. (Lowe, 1997)

The inhibitory activities of extracts towards pancreatic lipase are represented in **Table No. 4.6.** Among the 8 extracts both methanolic and aqueous extracts of *D. pentaphylla* crude extracts showed lipase inhibition activity better than other extracts and *D. alata* and *D. bulbifera* extracts showing the moderate lipase inhibition at concentration 5 mg/ml **(Table No. 4.6).** The significant inhibition of Pancreatic lipase was observed in *D. pentaphylla* aqueous extract 75.83±1.661% with IC_{50} 0.986 mg/ml, although in *D. pentaphylla* methanolic extract with 71.73±1.729 % with IC_{50} 3.088 mg/ml. the lowest inhibition was observed in *D. oppositifolia* aqueous extract *i.e.,* 44.73±0.272% with high IC_{50} value 5.59mg/ml. The orlistat which was used as positive control showed the 86.33±0.832% inhibition at 5mg/ml concentration with IC_{50} value 0.957 mg/ml **(Figure No. 4.9).** Orlistat, a Hydrogenated derivative of lipstatin, is the pancreatic lipase inhibitor currently approved for a long-term treatment of obesity. Similarly inhibitory effect Dioscin, diosgenin, and saponins from *D. nipponica* were isolated and tested for lipase inhibitory efficacy in rats, finding a substantial inhibitory effect by Kwon *et al.,* (2003). Bioactive flavonoids were extracted from *D. steriscus* tubers that can inhibit lipase and α-amylase, making them useful for the development of anti-obesity therapeutics (Dzomba and Musekiwa, 2014). Jeong *et al.,* (2016) has reported n-butanolic extracts of *D. oppositifolia* has bioactive compound which inhibit lipase and reduces fat absorption in high fat induced mice.

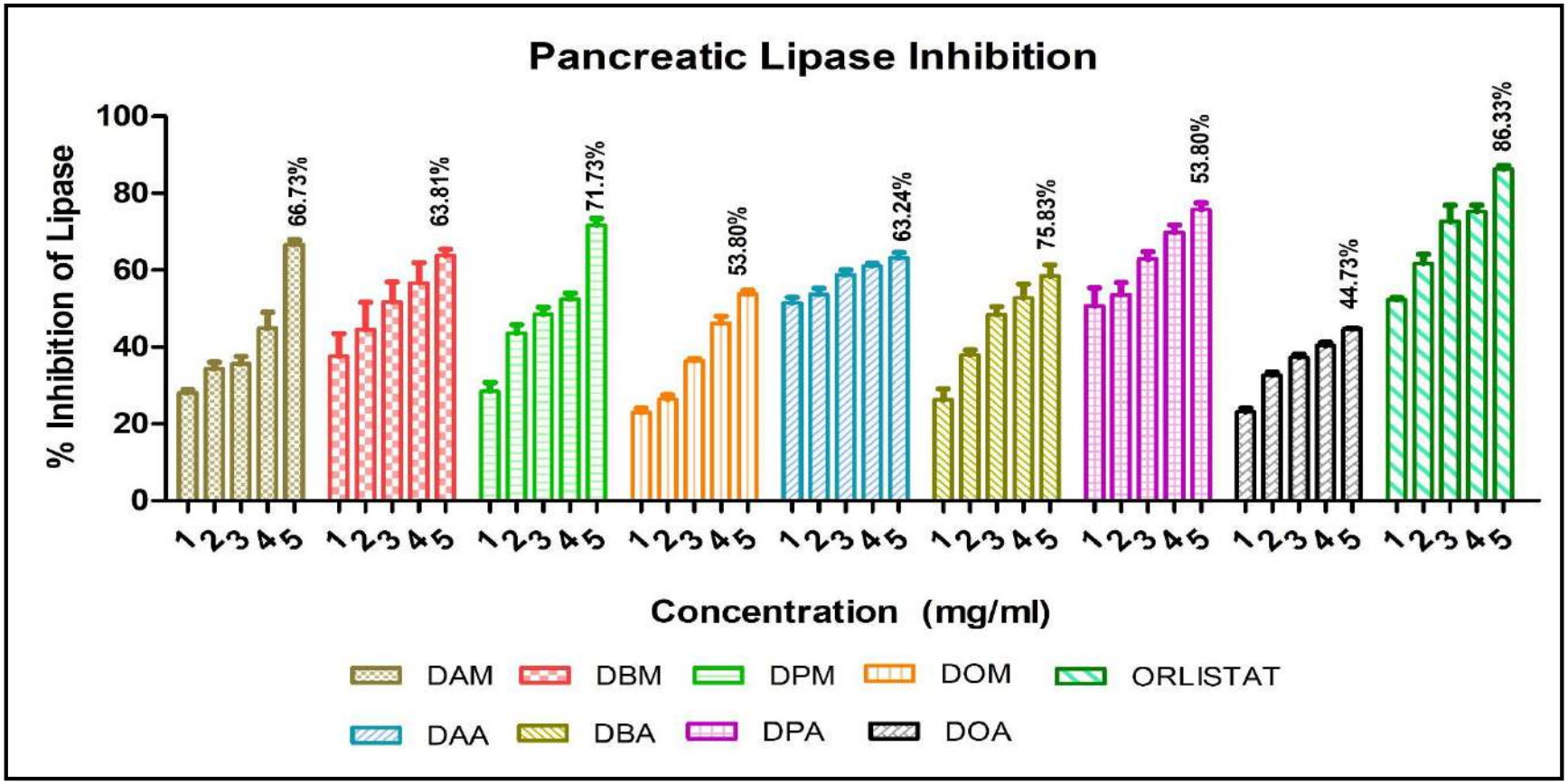


Figure No. 4.9: Inhibitory effect of orlistat and plant extract pancreatic lipase activity. Two-way ANOVA was calculated *p- value* is statistically significant (*P=< 0.0001*) (DAM= *D. alata* methanol, DBM= *D. bulbifera* methanol, DPM *D. pentaphylla* methanol, DOM= *D. oppositifolia* methanol, DAA =*D. alata* aqueous, DBA =*D. bulbifera* aqueous, DPA= *D. pentaphylla* aqueous, DOA =*D. oppositifolia* aqueous)

Table No. 4.6: Lipase inhibition activity of *Dioscorea* extracts values expressed in % inhibition with ± SE

Sr. No.	Plant Name	Solvent	%Inhibition at 5mg/ml	IC_{50} mg/ml
1	***D. alata***	Methanol	66.73 ± 1.109	4.461
		Aqueous	63.24 ± 0.889	0.971
2	***D. bulbifera***	Methanol	63.81 ± 1.658	2.903
		Aqueous	58.47 ± 2.925	3.097
3	***D. pentaphylla***	Methanol	71.73 ± 1.729	3.088
		Aqueous	75.83 ± 1.661	0.986
4	***D. oppositifolia***	Methanol	53.80 ± 0.889	4.332
		Aqueous	44.73 ± 0.272	5.59
5	**Orlistat**	DMSO	86.33 ± 0.832	0.957

4.3.2 Testing of crude extracts on cell line 3T3-L1 pre adipocytes

A. Cell viability assay:

The cell viability assay was carried out of the all the extracts was carried out for the determination of the IC_{50} value. From the IC_{50} value treatment concentration was decided for the differentiation assay. The toxicity of the extract was investigated using the MTT test at various doses (6.25-100 µg/ml). Methanolic extracts of all four plant extracts were shown to be toxic to cells at higher concentrations *i.e.,* 100µg/ml whilst aqueous extracts were found to be slightly toxic to cells at the tested doses. The IC_{50} values are mentioned in **Table No. 4.7,** and **Figure No. 4.10** and **4.11** are presenting the % viability of the 3T3-L1 cells at different tested concentrations.

Table No. 4.7: IC_{50} values of the methanolic and aqueous extracts.

Sr. No.	Plant Name	IC_{50} of methanol extract	IC_{50} of Aqueous extract
1	*D. alata*	81.31 µg/ml	97.50 µg/ml
2	*D. bulbifera*	83.90 µg/ml	93.17 µg/ml
3	*D. pentaphylla*	77.89 µg/ml	95.57 µg/ml
4	*D. oppositifolia*	78.59 µg/ml	69.04 µg/ml

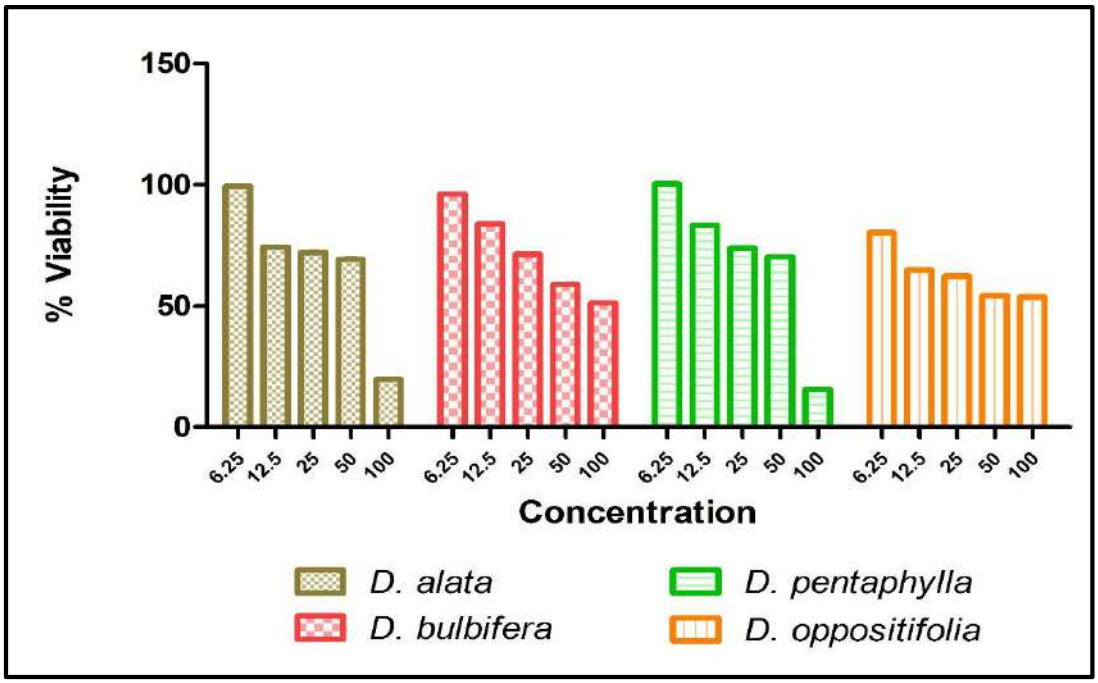


Figure No. 4.10: Cell viabilty test by MTT assay cells treated with methanolic extract (6.25 to 100µg/ml concentration)

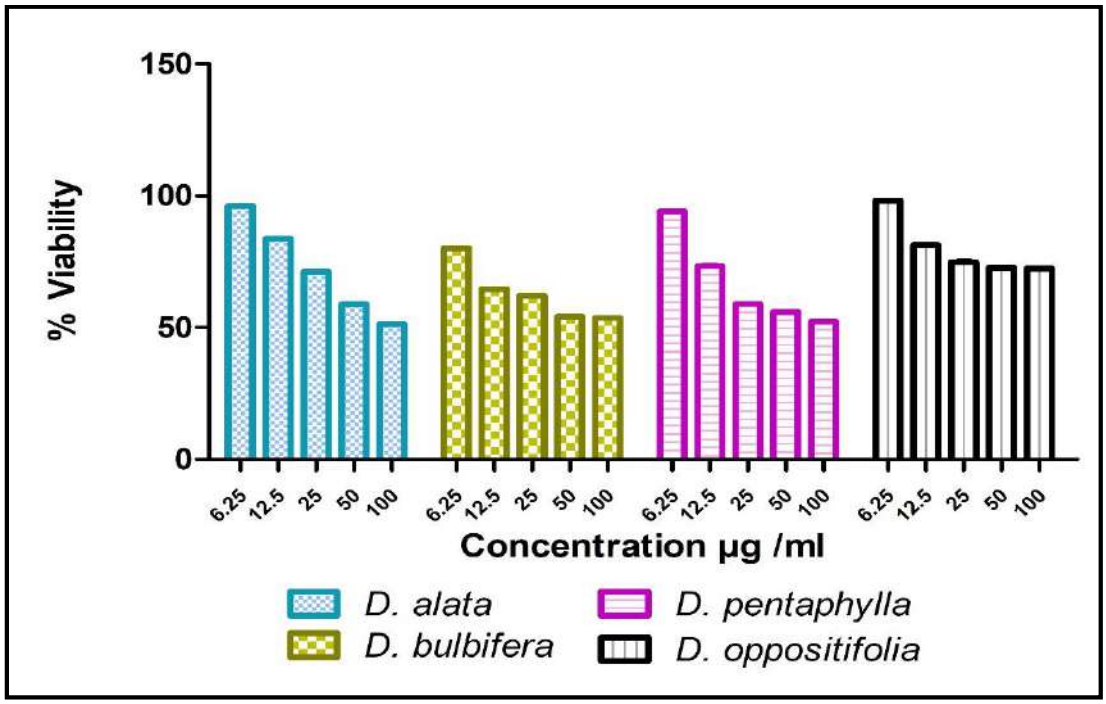


Figure No. 4.11: Cell viabilty test by MTT assay cells treated with aqueous extracts (6.25 to 100µg/ml concentration)

B. 3T3-L1 Cells Differentiation:-

The effects of the plant extracts were studied at different time intervals on 3T3-L1 cells. The treatments given on day 4 to day 6 and day 6 to day 8 did not exhibit inhibitory activity on the differentiation of the adipocytes. Whereas, the cells treated with plant extracts from day 2 up to day 8 with a hormone cocktail and insulin show a more effective inhibition of the adipocytes. The decrease in lipid deposition was measured by comparing the cells that had been stimulated with a hormone cocktail.

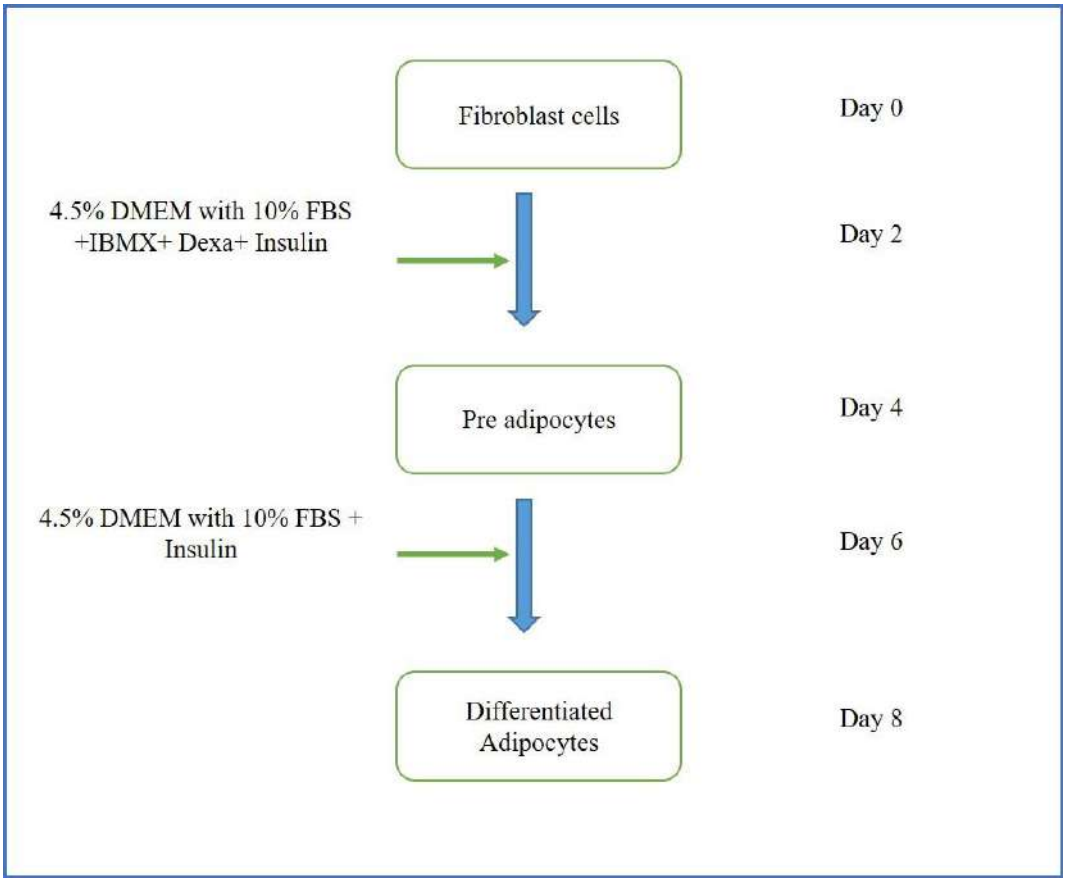


Figure No. 4.12: Flow chart of adipogenesis

C. Effect of plant extracts on inhibition of lipid droplet accumulation in differentiation of 3T3-L1 cell line:

The Lipid quantification was carried out on the eighth day of the experiment. Cells treated with a hormone cocktail had 2.19 times increase in lipid content as compared to non-induced cells. In comparison with control group the lipid accumulation was highly significant

(*P<0.001*) for induced group whereas in other tested groups the lipid accumulation was significantly decreased (*P<0.001*) in comparison with induced group, among the tested four *Dioscorea* species. The lipid deposition in cells treated with *D. oppositifolia* methanolic and aqueous extracts was found to reduce by 1.8 and 2.2 times, respectively. Furthermore, *D. pentaphylla* methanolic and aqueous extracts inhibit lipid formation in cells by 1.7 and 1.6 times, respectively **(Figure No. 3.13).** *D. pentaphylla* and *D. oppositifolia* were chosen based on the decrease in lipid accumulation among the four species for gene expression study **(Plate No. 6 and 7).**

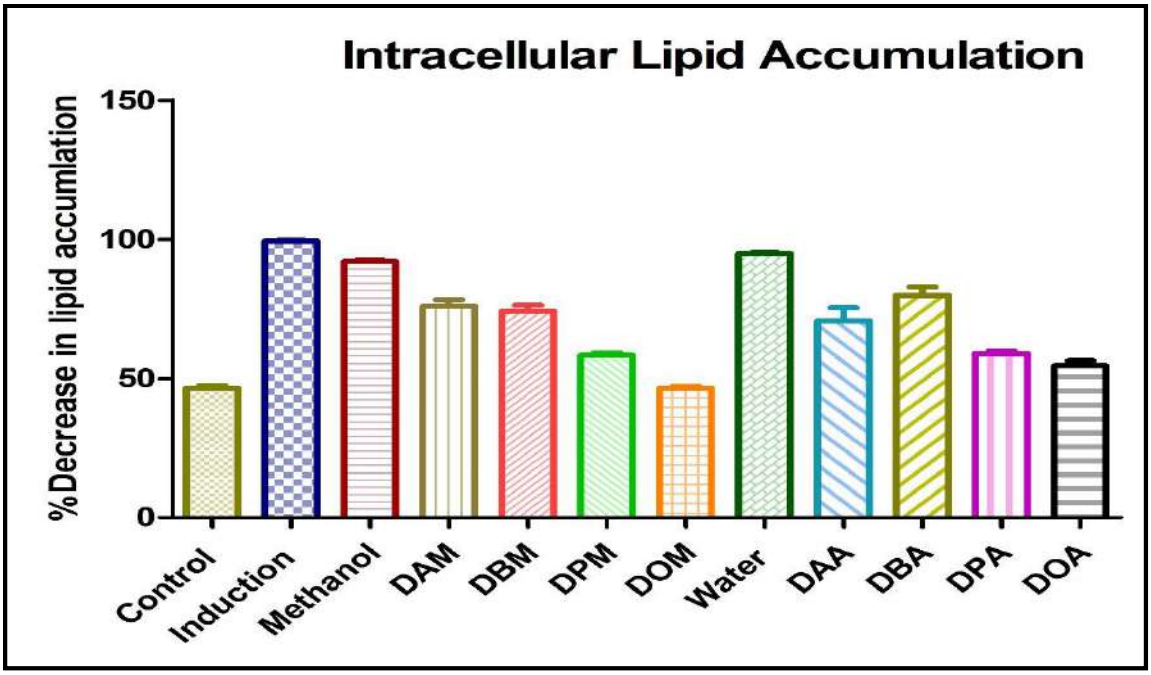


Figure No. 4.13: Intracellular lipid accumulation in 3T3-L1 cells by isopropanol extraction method ******P< 0.001* **as compare to induction and control using one way ANOVA followed by tukyes test. (DAM= *D. alata* methanol, DBM= *D. bulbifera* methanol, DPM *D. pentaphylla* methanol, DOM= *D. oppositifolia* methanol, DAA =*D. alata* aqueous, DBA =*D. bulbifera* aqueous, DPA= *D. pentaphylla* aqueous, DOA =*D. oppositifolia* aqueous)

D. Effect of *D. oppositifolia* and *D. pentaphylla* on gene expression in 3T3-L1 cells:

While considering the results obtained from the lipase inhibition activity and lipid droplet accumulation in differentiated of 3T3-L1 cell. The whole plant extracts of *D. pentaphylla* and *D. oppositifolia* were selected for further gene expression study.

Methanolic and aqueous whole plant extracts of *D. oppositifolia* and *D. pentaphylla* were tested on 3T3-L1 preadipocytes cells. Comparison of all four extracts of *D. oppositifolia* and *D. pentaphylla*, both methanolic and aqueous extracts of *D. oppositifolia* exhibited superior anti-adipogenesis action on 3T3-L1 cells than both the extracts *D. pentaphylla* extract.

D. oppositifolia methanolic and aqueous extracts can down regulates adipogenic gene expression. The expression of PPARγ **(Figure No. 4.14 A, 4.15 A)**, C/EBPα **(Figure No. 4.14 B, 4.15 D)**, and SREBP-1 **(Figure No. 4.14 C, 4.15 C)** genes were assessed in comparison to the control. At greater concentrations of the extracts, cells treated with methanolic and aqueous extracts down-regulated the adipogenic genes PPARγ, C/EBPα, and SREBP-1 by up to 99 %, 97 %, and 98 %, respectively. Methanolic extracts also regulated the fatty acid synthase (FASN) gene expression. The FASN was down-regulated up to 93 and 99 % of the extracts at 25 µg/mL and 75 µg /mL, respectively **(Figure No. 4.14 D)**. In the case of the treatment of the aqueous extract, the expression of FASN increased with the increasing concentration of the *D. oppositifolia* aqueous extract. The FASN up-regulation was observed, up-regulated up to 26% and 124% at 25 µg /mL and 75 µg /mL concentration respectively. **(Figure No. 4.15 D)** The methanolic and aqueous extracts of *D. pentaphylla* were found to up regulate the expression of PPARγ **(Figure No. 4.16 A and 4.17 A),** SREBP-F1 **(Figure No. 4.16 C and 4.17 C),** and C/EBPα **(Figure No. 4.16 B and 4.17 B).** Whereas the methanolic extract of *D. pentaphylla* inhibited FAS expression, the aqueous extracts stimulated it **(Figure No. 4.16 D and 4. 17 D).**

D. oppositifolia showed promising results in the down-regulation of the adipogenic genes. PPARγ was first identified as a factor that is activated during adipocyte differentiation (Ferré, 2004 and Matsusue *et al.,* 2004) and is well recognized for its function in regulating adipogenic and lipogenic pathways. Similarly, it is important for modulating gene networks involved in glucose homeostasis, especially boosting the expression of glucose transporter type 4 (Glut4). Expression of the C/EBPα is stimulated by the PPARγ (Ahmadian *et al.,* 2013). C/EBPα helps in the differentiation and development of the adipocytes. It is a key transcription factor that helps to regulate adipogenic genes. It is highly expressed in the liver and adipocytes in humans and rodents (Louise *et al.,* 2008). Previously, Yang *et al.,* (2014) had extracted batatasin I from the butanolic fraction of *D. oppositifolia* root extract and tested it against 3T3-L1 cells, obtaining a 90% down regulation of the PPARγ gene and a 20.9% decrease in C/EBPα

expression. *D. oppositifolia* methanolic and aqueous extract showed the 99% down-regulation the PPARγ gene. In the case of C/EBPα, a drop of up to 98% was observed. This significant down-regulation of PPARγ gene may be due to the cumulative action of phytochemicals from the extracts. This reduction of adipogenic transcription factors may aid in regulating adipocyte differentiation and lowering adipocyte lipid accumulation. There is a correlation between SREBP-F1 and FASN gene. If the level of SREBP-F1 increases, it ultimately elevates the level of the FASN gene and thereby increases the fat accumulation. During differentiation, FASN helps in the *de novo* synthesis of the new lipid molecules in the cells (Laliotis *et al.*, 2010). Thus, the target in the present study was to examine the effectiveness of the screened extract towards the gene expression. In the present investigation, methanolic extract of the *D. oppositifolia* reduced 99% expression of the FASN in 3T3-L1 cells, while in the case of the aqueous extract of *D. oppositifolia*, up-regulation of the FASN was observed at higher concentration of the extracts. But at a lower concentration, it down regulated the expression of the FASN up to 73%. **(Figure 4.15 D)**

There is another way to control obesity by triggering lipolysis. Up regulation of the CPT-1 helps increase the transport of the cytosolic long-chain fatty acids in the mitochondria for the β oxidation of the lipids (Ju *et al.*, 2011). If CPT-1 gene expression is inhibited, there is an increase in the risk of obesity (Yang *et al.*, 2014). The *D. oppositifolia* methanolic extract at 25 µg/mL and 75 µg/mL increased the CPT-1 expression by ≅ 2 and 6.9 times, respectively **(Figure No. 4.14 E).** In the case of the aqueous extract, the CPT-1 was up-regulated in dose dependent manner; 7.8 folds, and 5.3 folds increased as compared to the control at 25 µg /mL and 75 µg /mL, respectively **(Figure No. 4.15 E).** Both methanolic and aqueous extracts of *D. oppositifolia* increased CPT-1 gene expression by ≅ 7 times the control that will help to reduce fat accumulation in the cells by activating the assisting the lipolysis pathway.

Both *D. pentaphylla* extracts suppressed the expression of CPT-1 **(Figure No. 4.16 E and 4.17 E).** *D. pentaphylla* extracts stimulated adipogenesis by activating adipogenic transcription factors inhibiting the expression of CPT-1, which is a crucial enzyme that assists in lipolysis. When CPT-1 is inhibited, the rate of fatty acid oxidation is decreased, and fatty acid accumulation is increased. As a result, *D. pentaphylla* exhibited the anti-lipase activity but did not show the down-regulation of the adipogenic genes.

This study's goal was to assess potential *Dioscorea* species for anti-obesity effects. The lipase inhibition ability of all four *Dioscorea* species extracts were investigated, both methanolic and aqueous extracts of *D. pentaphylla* inhibited lipase more effectively than *D. alata, D. bulbifera*, and *D. oppositifolia* extracts. All extracts were also tested for antiadipogenic activity in 3T3-L1 preadipocytes. The uptake of the oil red O stain in cells provides information on lipid build-up in the cells. All extracts of *D. alata, D. bulbifera*, and *D. pentaphylla* revealed less antiadipogenic efficacy than *D. oppositifolia* methanolic and aqueous extracts. On the basis of the oil red o staining assay, both methanolic and aqueous whole plant extracts of *D. oppositifolia* and *D. pentaphylla* were carried forward for the gene expression study.

In a gene expression analysis, *D. oppositifolia* methanolic extracts suppressed adipogenic genes more than *D. oppositifolia* aqueous extracts and *D. pentaphylla* methanolic and aqueous extracts. From the obtained results, the *D. oppositifolia* whole plant extract was used for the solvent fraction extraction study and the animal study.

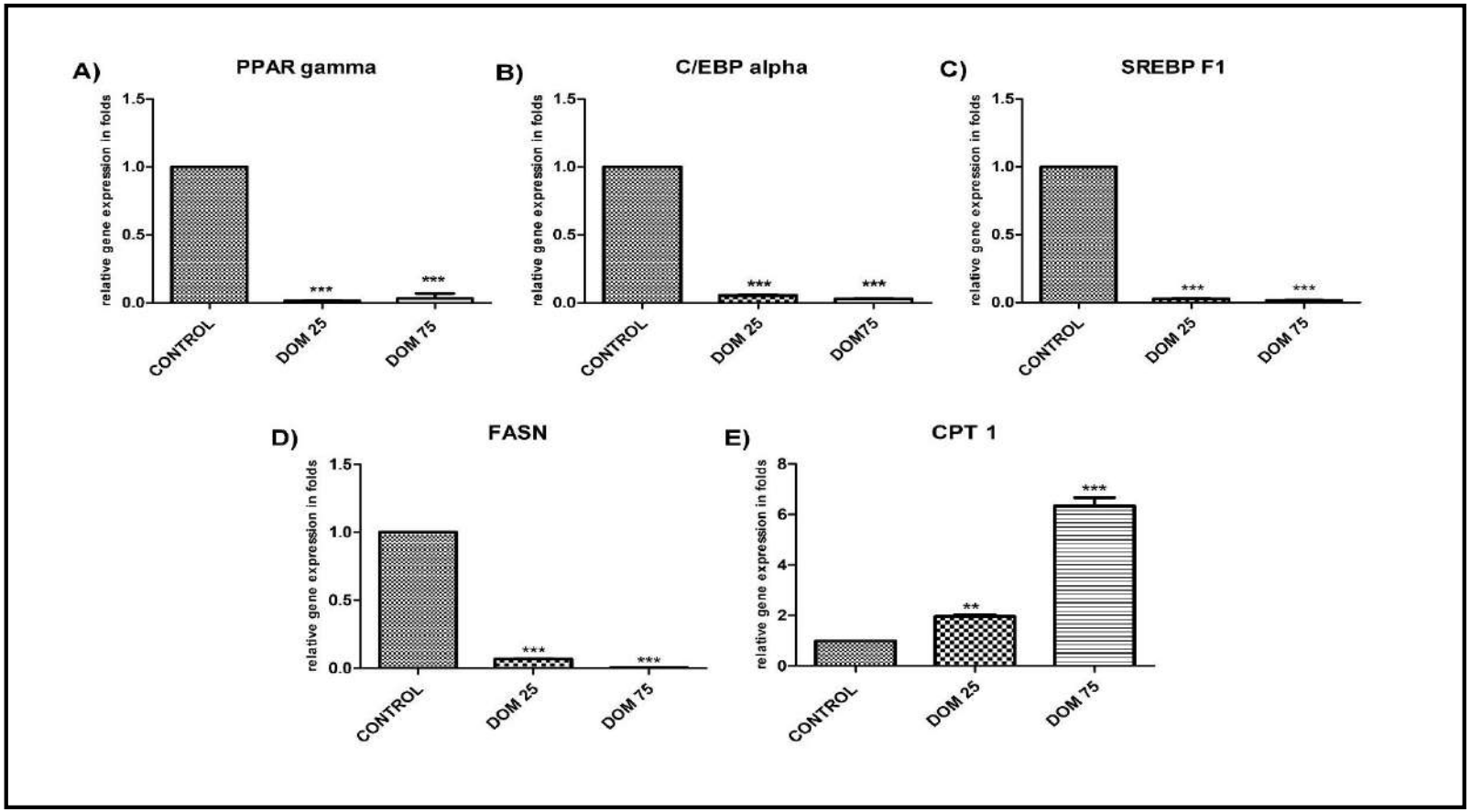


Figure No. 4.14: Effect of *D. oppositifolia* methanolic (DOM) extract on Relative mRNA expression of (a) peroxisome proliferator-activated receptor-gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one-way ANOVA followed by Dunnett's Multiple Comparison Test.**

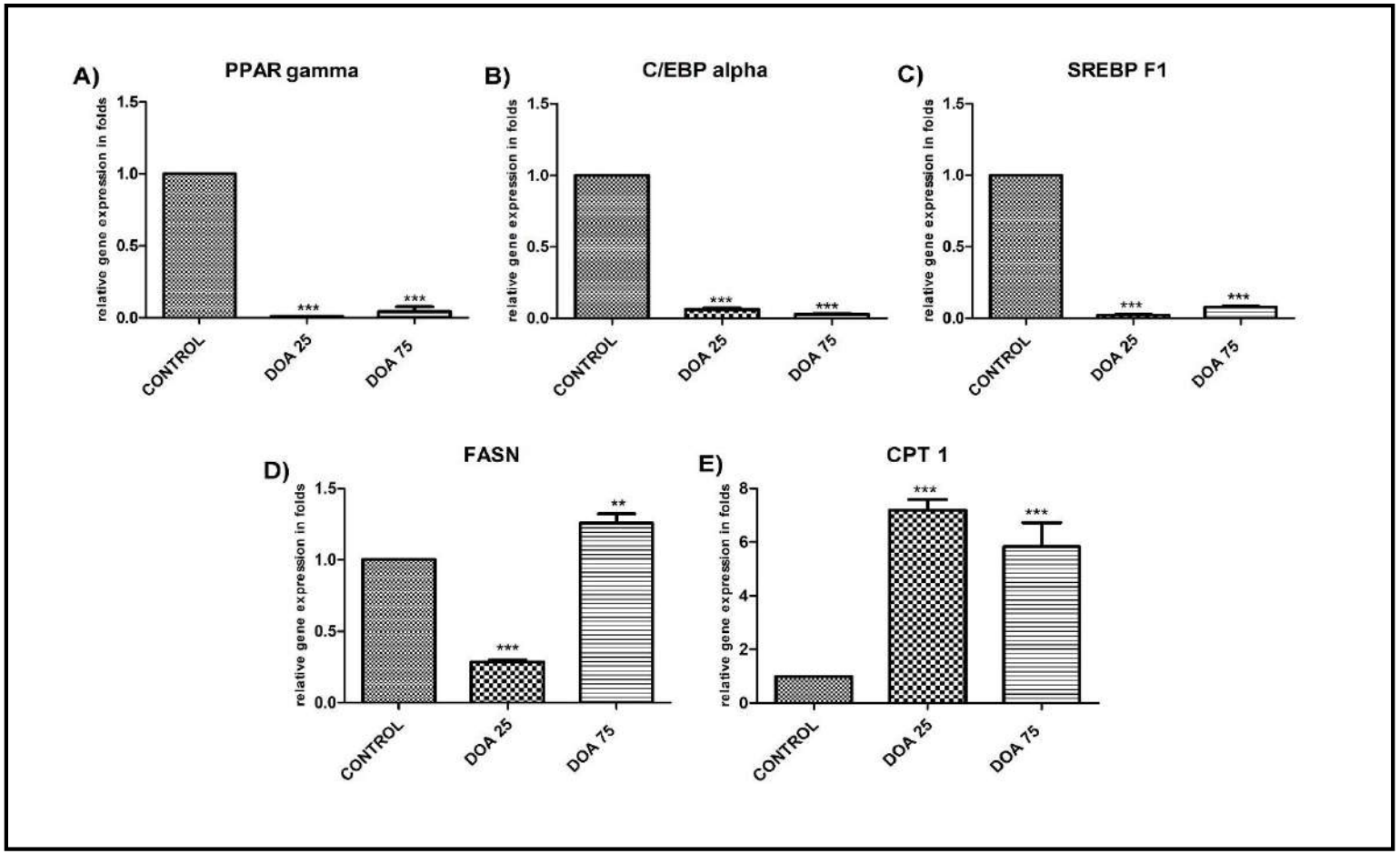


Figure No. 4.15: Effect of *D. oppositifolia* aqueous (DOA) extract on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

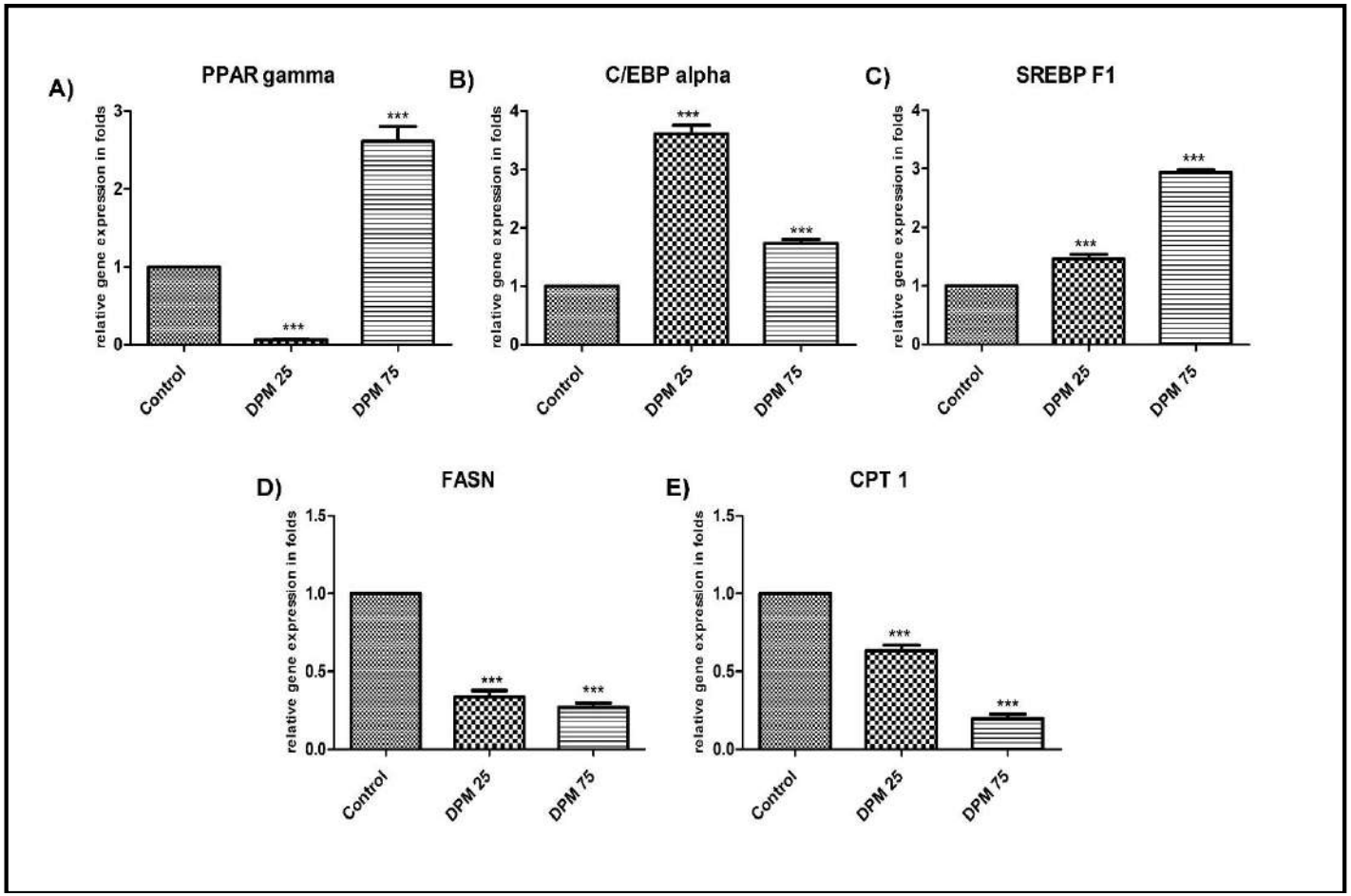


Figure No. 4.16: Effect of *D. pentaphylla* methanolic (DPM) extract on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

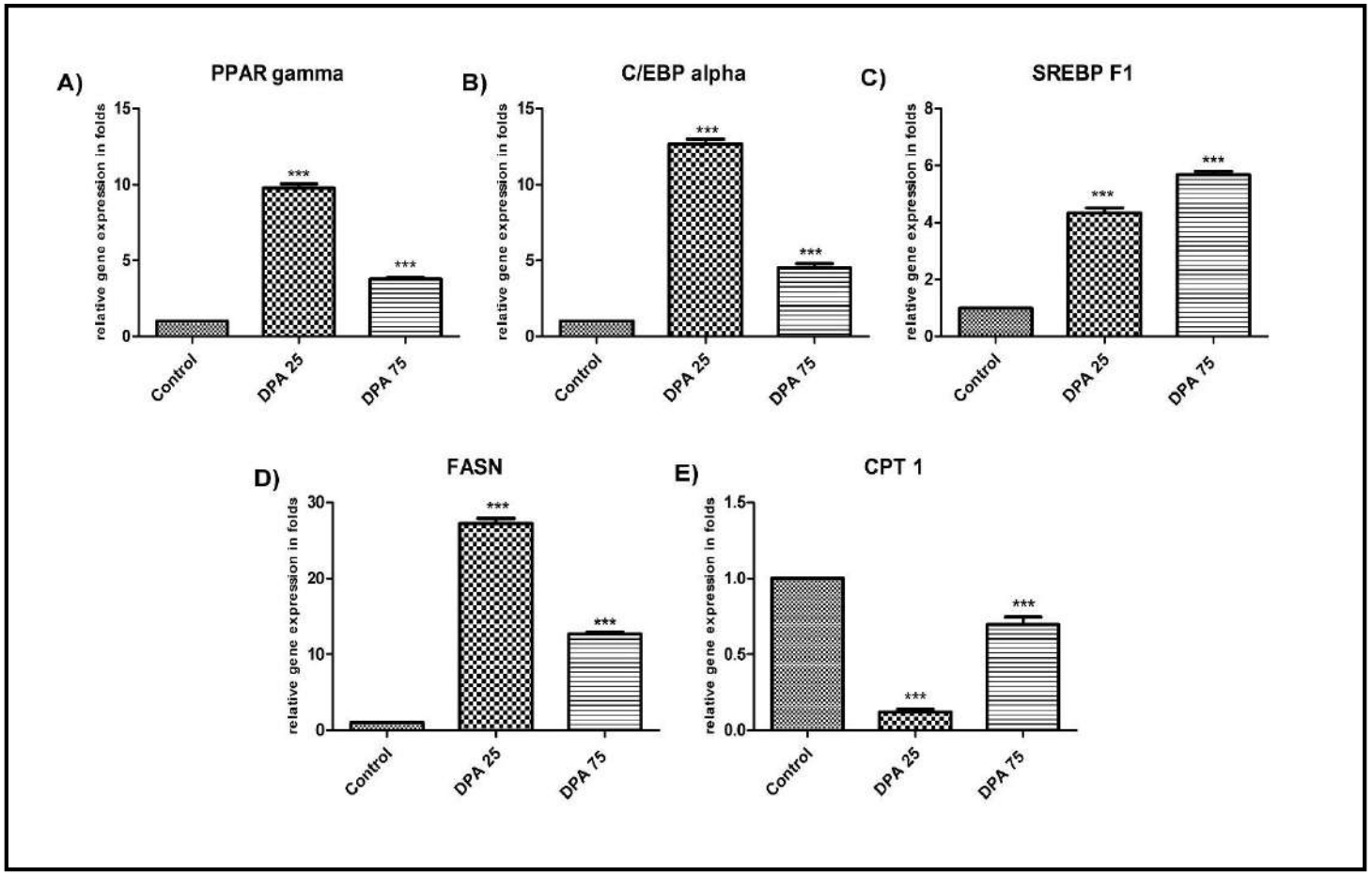


Figure No. 4.17: Effect of *D. pentaphylla* aqueous (DPA) extract on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

In context with results obtained from the lipase inhibition, lipid accumulation in 3T3-L1 cells, phytochemical analysis, and antioxidant assay the *D. oppositifolia* methanolic extracts has a good anti-oxidant potential and it also inhibiting the lipid accumulation in 3T3-L1 cells and *D. pentaphylla* has a good lipase inhibition potential so these two extracts were carried forward for the metabolite profiling by the LCMS method to identify the antiobesity compounds present in the plant extracts.

E. LCMS analysis of the *D. oppositifolia* and *D. pentaphylla* methanolic extract:

The LCMS-MS analysis of methanolic extracts of *D. oppositifolia* and *D. pentaphylla* was carried out. The collected data showed many classes of substances including fats/lipids, alkaloids, sugars, peptides, phenolic, steroids, glycosides, terpenoid, flavonoids, and cyanidins. Identified compounds are listed in **Table No. 4.8** and **4.9. Figure No. 4.18** and **4.19** shows the LCMS chromatogram of *D. oppositifolia* and *D. pentaphylla* respectively.

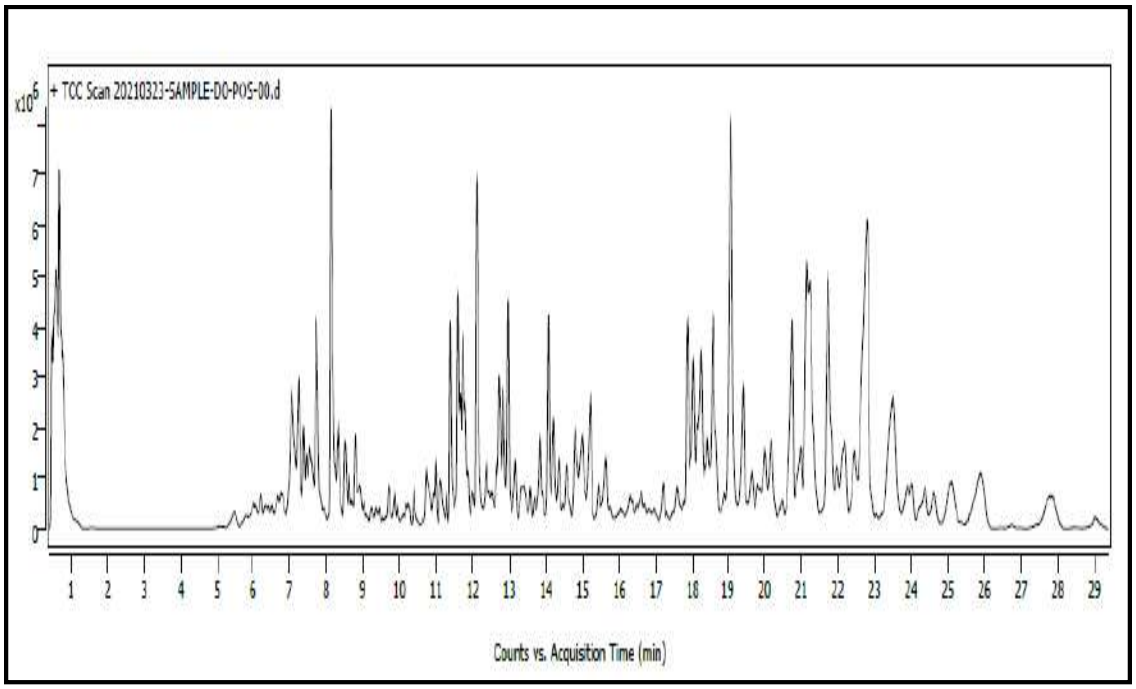


Figure No. 4.18: LCMS chromatogram of *D. oppositifolia* whole plant methanolic extract

Table No. 4.8: List of identified compound from *D. oppositifolia* using LSMS

	Name	Formula	RT	Mass
Phenolics	Crosatoside B	$C_{20}H_{30}O_{11}$	5.818	446.1785
	2,6-Dimethoxy-4- propylphenol	$C_{11}H_{16}O_3$	7.109	196.1099
	Octyl gallate/ gallic acid	$C_{15}H_{22}O_5$	8.557	282.1464
	3"-Hydroxygeranylhydroquinone	$C_{16}H_{22}O_3$	10.624	262.1567
	Nogalonic acid	$C_{20}H_{14}O_8$	12.524	382.0688
	R1128A	$C_{17}H_{14}O_5$	12.670	298.0833
	5-Heptadecyl-1,3- benzenediol	$C_{23}H_{40}O_2$	13.804	348.3021
	Ethyl vanillin isobutyrate	$C_{13}H_{16}O_4$	13.834	236.1043
	Oryzarol	$C_{26}H_{42}O_3$	14.797	402.3130
Flavonoid	Cinnamtannin A1	$C_{45}H_{38}O_{18}$	6.355	866.2065
	Apigenin 6-C-glucoside	$C_{26}H_{28}O_{14}$	6.595	564.1487
	"4'-O-Methyldelphinidin 3- O-beta-D-glucoside"	$C_{22}H_{23}O_{12}$	6.743	479.1194
	Moracin I	$C_{20}H_{20}O_4$	7.009	324.1363
	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	7.156	464.0952
	Cyanidin 3-rhamnoside 5-glucoside	$C_{27}H_{31}O_{15}$	7.454	595.1665
	7,8,3',4'-Tetrahydroxyisoflavone	$C_{15}H_{10}O_6$	7.457	286.0476
Terpenoid	"3-Hydroxy-6,8-dimethoxy-7(11)-eremophilen-12,8-olide	$C_{17}H_{26}O_5$	6.491	310.1770
	Gibberellin A38 glucosyl ester	$C_{26}H_{36}O_{11}$	7.083	524.2260
	(-)-trans-Carveol	$C_{10}H_{16}O$	7.375	152.1200
	Unshuoside A	$C_{16}H_{28}O_7$	7.518	332.1838
	Ganoderenic acid A	$C_{30}H_{42}O_7$	8.590	514.2936
	Acuminoside	$C_{21}H_{36}O_{10}$	8.785	448.2304
	Eremopetasidione	$C_{14}H_{20}O_3$	9.017	236.1414
	Valerosidatum	$C_{21}H_{34}O_{11}$	9.087	462.2100
	Neryl glucoside	$C1_6H_{28}O_6$	9.200	316.1883
	Zerumbone	$C_{15}H_{22}O$	9.354	218.1668
	"2,2,4,4,-Tetramethyl-6-(1-oxopropyl)-1,3,5-cyclohexanetrione	$C_{13}H_{18}O_4$	10.104	238.1201
	Ethyl menthane carboxamid	$C_{13}H_{25}NO$	12.038	211.1936
	2-oxophytanic acid	$C_{20}H_{38}O_3$	12.523	326.2821
	Celastrol	$C_{29}H_{38}O_4$	12.545	450.2770
	Armillaripin	$C_{24}H_{30}O_6$	12.756	414.2044
	Celastrol	$C_{29}H_{38}O_4$	12.759	450.2768
	Isomytiloxanthin	$C_{40}H_{54}O_4$	12.779	598.4029
	Pristanic acid	$C_{19}H_{38}O_2$	13.465	298.2867
	Isomytiloxanthin	$C_{40}H_{54}O_4$	13.731	598.4025
	Lactapiperanol D	$C_{18}H_{28}O_5$	13.833	324.1935
	all-trans-heptaprenyl	$C_{35}H_{60}O_7P_2$	13.907	654.3817

	Name	Formula	RT	Mass
Terpenoid	3L,7D,11D-phytanic acid	$C_{20}H_{40}O_2$	14.102	312.3025
	"all-trans-heptaprenyl diphosphate	$C_{35}H_{60}O_7P_2$	14.216	654.3821
	6-O-Acetylaustroinulin	$C_{22}H_{36}O_4$	17.674	364.2613
	22-Angeloyltheasapogenol A	$C_{35}H_{56}O7$	20.681	588.4030
	3-cis-Hydroxy-b,e-Caroten-3'-one	$C_{40}H_{54}O$	21.138	550.4177
Lipids	Cucurbic acid	$C_{12}H_{20}O_3$	8.125	212.1405
	9,10-Dihydroxy-12,13-epoxyoctadecanoate	$C_{18}H_{34}O_5$	10.169	330.2407
	2-Tetradecanone	$C_{14}H_{28}O$	10.790	212.2137
	Octadecanedioic acid	$C_{18}H_{34}O_4$	10.793	314.2455
	9,10,13-Trihydroxystearic acid	$C_{18}H_{36}O_5$	10.822	332.2560
	Phenethyl decanoate	$C_{18}H_{28}O_2$	11.769	276.2087
	(R)-2-Hydroxysterculic acid	$C_{19}H_{34}O_3$	11.779	310.2503
	(Z)-15-Oxo-11-eicosenoic acid	$C_{20}H_{36}O_3$	11.850	324.2666
	Phytosphingosine	$C_{18}H_{39}NO_3$	12.115	317.2930
	DGlucosyldihydrosphingosine	$C_{24}H_{49}NO_7$	12.385	463.3505
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	12.718	352.2608
	Octadecanedioic acid	$C_{18}H_{34}O_4$	12.761	314.2456
	Stearamide	$C_{18}H_{37}NO$	12.832	283.2873
	MG(0:0/18:1(11Z)/0:0)	$C_{21}H_{40}O_4$	12.922	356.2926
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	12.965	352.2614
	LysoPC(18:3(6Z,9Z,12Z))	$C_{26}H_{49}NO_7P$	13.130	518.3245
	MG(0:0/20:1(11Z)/0:0)	$C_{23}H_{44}O_4$	13.521	384.3236
	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	13.678	678.3814
	MG(0:0/18:3(6Z,9Z,12Z)/0:0)	$C_{21}H_{36}O_4$	13.837	352.2611
	22-Oxo-docosanoate	$C_{22}H_{42}O_3$	14.265	354.3126
	LysoPE(0:0/16:0)	$C_{21}H_{44}NO_7P$	14.460	453.2845
	alpha,alpha'-Trehalose 6-	$C_{28}H_{52}O_{12}$	14.988	580.3465
	Phenethyl decanoate	$C_{18}H_{28}O_2$	15.216	276.2089
	9-HOTE	$C_{18}H_{30}O_3$	15.217	294.2199
	"8, 11, 14, 17-icosatetraenoic acid;C20:4n-3,6,9,12	$C_{20}H_{32}O_2$	15.246	304.2400
Glycosides	Pelargonidin 3-(6-pcoumaroyl)glucoside	$C_{30}H_{27}O_{12}$	5.453	579.1508
	Cyanidin 3-rhamnoside	$C_{27}H_{31}O_{15}$	6.033	595.1669
	Lucuminic acid	$C_{19}H_{26}O_{12}$	6.188	446.1423
	Cyanidin 3-rhamnoside	$C_{27}H_{31}O_{15}$	6.203	595.1666
	Peonidin-3-galactoside	$C_{22}H_{22}O_{11}$	6.215	462.1162
	cis-3-Hexenyl b-primeveroside	$C_{17}H_{30}O_{10}$	6.652	394.1840
	Cyanidin 3-glucogalactoside	$C_{27}H_{31}O_{16}$	7.053	611.1625
	Linalool oxide D 3-[apiosyl-(1->6)-glucoside]	$C_{21}H_{36}O_{11}$	7.375	464.2253
	Petunidin 3-rhamnoside	$C_{28}H_{33}O_{16}$	7.543	625.1778
	Peonidin	$C_{16}H_{13}O_6$	7.549	301.0711

	Name	Formula	RT	Mass
Glycosides	Linalyl propionate	$C_{13}H_{22}O_2$	7.596	210.1623
	Isolariciresinol 9-O-beta-D-glucoside	$C_{26}H_{34}O_{11}$	7.667	522.2102
	Malvidin	$C_{17}H_{15}O_7$	7.723	331.0813
Alkaloids	N-Methylcalystegine C1	$C_8H_{15}NO_5$	0.605	205.0946
	Maculosine	$C_{17}H_{17}NO_6$	0.906	331.1055
Lignan	Isolariciresinol 9-O-beta-D-glucoside	$C_{26}H_{34}O_{11}$	6.992	522.2099
	Austrobailignan 7	$C_{20}H_{22}O_5$	15.043	342.1464
Coumarins	Coriandrone C	$C_{13}H_{10}O_5$	10.282	246.0529
	4-Hydroxy-8-methoxy-2H-furo[2,3-h]-1-benzopyran-2-one	$C_{12}H_8O_5$	13.425	232.0372
	Avocadenofuran	$C_{17}H_{28}O$	15.217	248.2142
Oligosaccharides.	((S)-Nerolidol 3-O-[a-LRhamnopyranosyl-(1->4)-a-Lrhamnopyranosyl-(1->2)-b-Dglucopyranoside]	$C_{33}H_{56}O_{14}$	12.719	676.3666
Amino Acid	Arginyl-Proline	$C_{11}H_{21}N_5O_3$	0.689	271.1647
	L-Leucine	$C_6H_{13}NO_2$	0.691	131.0942
	N(alpha)-t-Butoxycarbonyl-L-leucine	$C_{11}H_{21}NO_4$	0.729	231.1468
	N-(1-Deoxy-1-fructosyl)phenylalanine	$C_{15}H_{21}NO_7$	0.780	327.1320
	2-Carboxy-1-[5-(2-carboxy-1-yrrolidinyl)-2-hydroxy-2,4-pentadienylidene]pyrrolidinium	$C_{15}H_{21}N_2O_5$	7.826	309.1442
	NPhenylacetylphenylalanine	$C_{17}H_{17}NO_3$	7.933	283.1207
	Mucronine B	$C_{28}H_{36}N_4O_4$	13.389	492.2737
	L-Leucine	$C_6H_{13}NO_2$	0.691	131.0942
Steroids	Diosgenin 3-[glucosyl-(1- >4)-rhamnosyl-(1->4)-[rhamnosyl-(1->2)]-glucoside]	$C_{51}H_{82}O_{21}$	8.855	1030.5371
	4,4-Difluoropregn-5-ene-3,20-dione	$C_{21}H28F_2O_2$	10.383	350.2061

In case of LCMS profile of the methanolic whole plant extract of *D. oppositifolia* total 224 compounds were acquired from the LCMS data base. From that data 97compunds were identified by using pubchem, human metabolome database and KEGG database. The data revealed a wide range of metabolite, including 25 molecules from fats/lipids, 26 molecules from terpenoid, 13 molecules from glycosides, 9 molecules from phenolic, 7 molecules from flavonoids, 8 molecules from amino acids, 3 molecules from coumarins, and 2 molecules from each group of alkaloids, steroids, and lignin. (**Table No. 4.8)**

In LCMS data of the *D. pentaphylla* 316 compounds were found. From the data 132 compounds were identified including by using pubchem, human metabolome database and KEGG database. The results indicated a diverse set of metabolite including 55 molecules from fats/lipids, 19 molecules from terpenoid, 21 molecules from glycosides, 11 molecules from phenolic, 15 molecules from flavonoids, 8 molecules from steroids and 3 molecules from alkaloids. (**Table No. 4.9)** among these compounds 11 have been reported for the anti-obesity properties **Table No. 4.10**

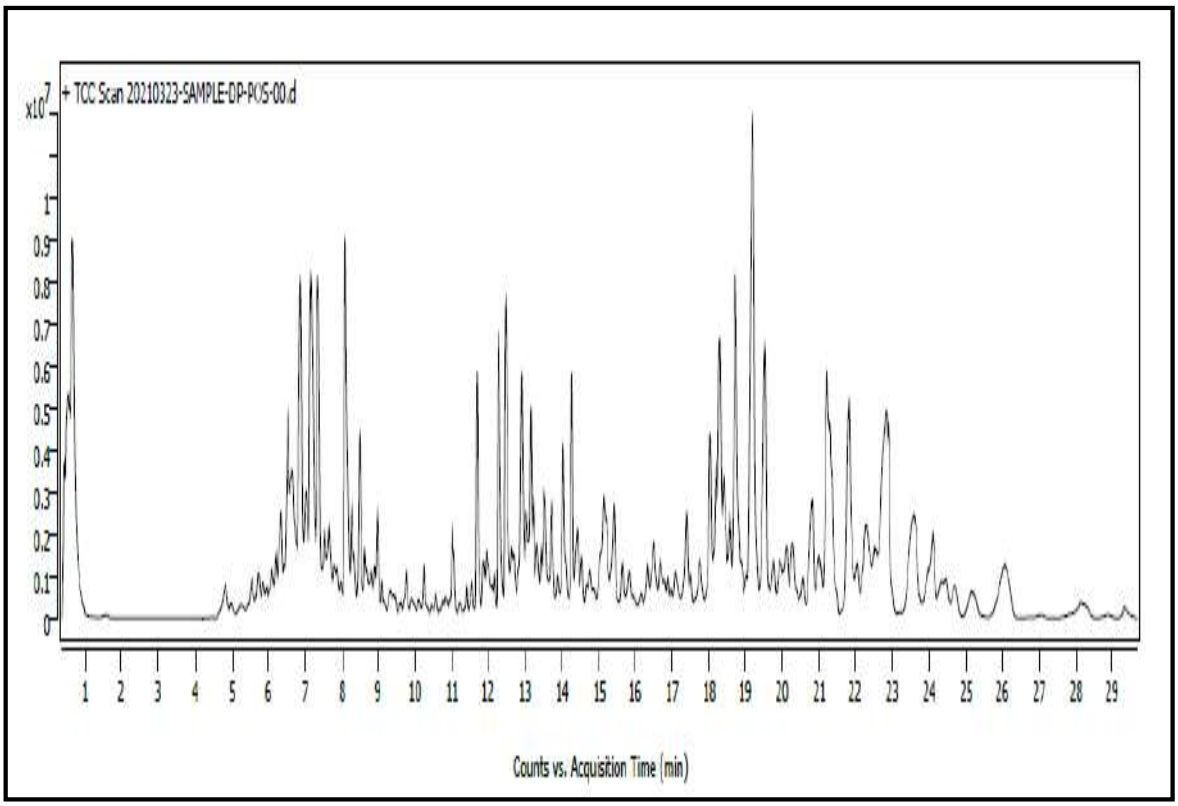


Figure no 4.19: LCMS-MS chromatogram of *D. pentaphylla* whole plant methanolic extract

Table No. 4.9: List of identified compound from *D. pentaphylla* using LSMS

	Name	Formula	RT	Mass
Phenolic	Reboxetine	$C_{19}H_{23}NO_3$	0.506	313.1679
	N-Methyltyramine	$C_9H_{13}NO$	0.685	151.0996
	Oxprenolol	$C_{15}H_{23}NO_3$	6.154	265.1675
	2,6-Dimethoxy-4-propylphenol	$C_{11}H_{16}O_3$	7.057	196.1097
	Alteichin	$C_{20}H_{14}O_6$	7.159	350.0783
	Linusitamarin	$C_{17}H_{22}O_9$	7.525	370.1266
	"3-Hydroxychavicol 1- glucoside	$C_{21}H_{30}O_{11}$	7.702	458.1788
	"1-Methoxy-3-(4- propenal 4'-glucoside"	$C_{16}H_{22}O_7$	7.95	326.1356
	Dihydrocapsaicin	$C_{18}H_{29}NO_3$	8.689	307.2142
	Ethyl vanillin isobutyrate	$C_{13}H_{16}O_4$	14.004	236.1045
	Oryzarol	$C_{26}H_{42}O_3$	14.996	402.3139
Flavonoid	Coumestrin	$C_{21}H_{18}O_{10}$	6.528	430.0895
	Quercitrin	$C_{21}H_{20}O_{11}$	6.528	448.1001
	Rothindin	$C_{22}H_{20}O_{10}$	6.651	444.1062
	Prunitrin	$C_{22}H_{22}O_{10}$	7.119	446.1214
	6-beta-DGlucopyranosyl- 4',5- dihydroxy-3',7-dimethoxyflavone	$C_{23}H_{24}O_{11}$	7.319	476.1310
	6"-O-Acetyldaidzin	$C_{23}H_{22}O_{10}$	7.348	458.1212
	Barpisoflavone A	$C_{16}H_{12}O_6$	7.486	300.0635
	Pelargonidin 3-rhamnoside 5-glucoside	$C_{27}H_{31}O_{14}$	7.503	579.1717
	7,8,3',4'-Tetrahydroxyisoflavone	$C_{15}H_{10}O_6$	7.555	286.0478
	Molludistin 2"-rhamnoside	$C_{27}H_{30}O_{13}$	7.622	562.1698
	Coumestrin	$C_{21}H_{18}O_{10}$	7.816	430.0906
	Apimaysin	$C_{27}H_{28}O_{13}$	8.181	560.1533
	Maysin 3'-methyl ether	$C_{28}H_{30}O_{14}$	9.102	590.1646
	Grossamide	$C_{36}H_{36}N_{28}$	9.292	624.2472
	5-Hydroxyflavone	$C_{21}H_{20}O_8$	9.797	400.1159
Terpenoid	Leonuridine	$C_{15}H_{24}O_9$	7.654	348.1422
	6Z-8-Hydroxygeraniol 8- O-glucoside	$C_{16}H_{28}O_7$	7.456	332.1835
	(2S,4R,6S)-2-[2-(4- hydroxy-3-methoxyphenyl)ethyl]tetrahydro-6-(4-hydroxy-3,5-dimethoxyphenyl)- 2H-pyran-4-ol	$C_{22}H_{28}O_7$	7.641	404.1840
	Leonuridine	$C_{15}H_{24}O_9$	7.654	348.1422
	Eremopetasidione	$C_{14}H_{20}O_3$	8.990	236.1415
	2,2,4,4,-Tetramethyl-6-(1-oxopropyl)-1,3,5-cyclohexanetrione	$C_{13}H_{18}O_4$	10.117	238.1201
	(5alpha,10alpha)- 3,7(11)-Eudesmadien-2-one	$C_{15}H_{22}O$	9.362	218.1670
	Austroinulin	$C_{20}H_{34}O_3$	11.406	322.2499
	Celastrol	$C_{29}H_{38}O_4$	12.706	450.2768
	Armillaripin	$C_{24}H_{30}O_6$	12.918	414.2039
	Isomytiloxanthin	$C_{40}H_{54}O_4$	12.966	598.4028
	2-oxophytanic acid	$C_{20}H_{38}O_3$	13.349	326.2825
	Lactapiperanol D	$C_{18}H_{28}O_5$	14.002	324.1935

	Name	Formula	RT	Mass
Terpenoid	Tricycloekasantal	$C_{12}H_{18}O$	15.200	178.1356
	Erythrodiol	$C_{30}H_{50}O_2$	17.186	442.3811
	Triptohypol F	$C_{31}H_{52}O_2$	17.340	456.3967
	(ent-2b,4S,9a)-2,4,9-Trihydroxy-10(14)-oplopen-3-one 2-(2 methylbutanoate) 9-(3-methyl-2E-pentenoate)	$C_{26}H_{40}O_6$	17.519	448.2825
	6-O-Acetylaustroinulin	$C_{22}H_{36}O_4$	17.846	364.2611
	(2S,4R,6S)-2-[2-(4-hydroxy-3-methoxyphenyl) ethyl]tetrahydro-6-(4-hydroxy-3,5-dimethoxyphenyl)-2H-pyran-4-ol	$C_{22}H_{28}O_7$	7.641	404.1840
Glycosides	Mandelonitrile rutinoside	$C_{20}H_{27}N_{10}$	6.073	441.1635
	Cyanidin 3-rhamnoside	$C_{27}H_{31}O_{15}$	6.553	595.1664
	Cyanidin 3-sambubioside	$C_{26}H_{29}O_{15}$	6.240	581.1513
	Isopeonidin 3-galactoside	$C_{22}H_{23}O_{11}$	6.651	463.1241
	Netilmicin	$C_{21}H_{41}N_{57}$	6.664	475.2996
	Pelargonidin 3-rhamnoside 5-glucoside	$C_{27}H_{31}O_{14}$	6.958	579.1712
	Cyanidin 3-Cyanidin 3-glucogalactoside	$C_{27}H_{31}O_{16}$	6.978	611.1621
	Luteolinidin 3-Oglucoside	$C_{21}H_{21}O_{10}$	7.004	433.1137
	Prunitrin	$C_{22}H_{22}O_{10}$	7.119	446.1214
	Peonidin 3-rhamnoside	$C_{22}H_{23}O_{10}$	7.158	447.1280
	Linalool oxide D 3-glucoside]	$C_{21}H_{36}O_{11}$	7.307	464.2255
	6-beta-DGlucopyranosyl-4',5-dihydroxy-3',7-dimethoxyflavone	$C_{23}H_{24}O_{11}$	7.319	476.1310
	6''-O-Acetyldaidzin	$C_{23}H_{22}O_{10}$	7.348	458.1212
	6Z-8-Hydroxygeraniol 8-O-glucoside	$C_{16}H_{28}O_7$	7.456	332.1835
	Linusitamarin	$C_{17}H_{22}O_9$	7.525	370.1266
	Eriojaposide A	$C_{24}H_{38}O_{11}$	7.574	502.2419
	Malvidin	$C_{17}H_{15}O_7$	7.665	331.0817
	3-Hydroxychavicol 1-glucoside]	$C_{21}H_{30}O_{11}$	7.702	458.1788
	1-Methoxy-3-(4-propenal 4'-glucoside	$C_{16}H_{22}O_7$	7.950	326.1356
	Piceatannol 4'-galloylglucoside	$C_{27}H_{26}O_{13}$	8.906	558.1384
	(3b,16b,20R)-Pregn-5-ene-3,16,20-triol 3-glucoside	$C_{27}H_{44}O_8$	14.036	496.3031
Steroid	Polypodoside A	$C_{45}H_{72}O_{17}$	15.087	884.4781
	6-Azaequilenin+AN3:AS9	$C_{17}H_{17}NO_2$	9.968	267.1258
	3-Deoxyestradiol	$C_{18}H_{24}O$	12.111	256.1826
	3beta-(1-Pyrrolidinyl)-5alpha-pregnane-11,20-dione	$C_{25}H_{39}NO_2$	12.550	385.2988
	Terminaline	$C_{23}H_{41}NO_2$	13.296	363.3139
	Ophiopogonin C'	$C_{39}H_{62}O_{12}$	16.662	722.4252
	Lycoperoside D	$C_{39}H_{65}N_{12}$	16.662	739.4513
	26-Hydroxybrassinolide	$C_{28}H_{48}O_7$	16.720	496.3401
Alkaloid	Muricinine	$C_{18}H_{19}NO_4$	4.975	313.1318
	Hydrocodone	$C_{18}H_{21}NO_3$	5.556	299.1520
	Desacetylcolchicine	$C_{20}H_{23}NO_5$	8.916	357.1579

	Name	Formula	RT	Mass
	Malonylcarnitine	$C_{10}H_{18}NO_6$	0.567	248.1135
	Aminocaproic acid	$C_6H_{13}NO_2$	0.681	131.0947
	Ginkgolide C	$C_{20}H_{24}O_{11}$	7.221	440.1327
	Corchorifatty acid F	$C_{18}H_{32}O_5$	9.753	328.2254
	"9,10-Dihydroxy-12,13-epoxyoctadecanoate"	$C_{18}H_{34}O_5$	10.237	330.2409
	C16 Sphinganine	$C_{16}H_{35}NO_2$	10.832	273.2668
	"9,10,13-Trihydroxystearic acid"	$C_{18}H_{36}O_5$	10.936	332.2561
	MG(0:0/15:0/0:0)	$C_{18}H_{36}O_4$	11.530	316.2613
	"3-Methyl-5-pentyl-2-furanundecanoic acid"	$C_{21}H_{36}O_3$	11.557	336.2667
	"2-methoxy-hexadecanoic acid"	$C_{17}H_{34}O_3$	11.683	286.2509
	"10,20-Dihydroxyeicosanoic acid"	$C_{20}H_{40}O_4$	11.852	344.2926
	Phytosphingosine	$C_{18}H_{39}NO_3$	11.858	317.2928
	"(Z)-15-Oxo-11-eicosenoic acid"	$C_{20}H_{36}O_3$	12.000	324.2665
	10Z-nonadecenoic acid	$C_{19}H_{36}O_2$	12.466	296.2713
	"D Glucosyldihydrosphingosine"	$C_{24}H_{49}NO_7$	12.580	463.3509
	"Polyoxyethylene 40 monostearate"	$C_{20}H_{40}O_3$	12.634	328.2972
	Methyl linoleate	$C_{19}H_{34}O_2$	12.806	294.2556
	"Polyoxyethylene (600) mono- ricinoleate"	$C_{21}H_{40}O_3$	12.872	340.2973
	MG(18:0/0:0/0:0)	$C_{21}H_{42}O_4$	12.900	358.3079
Lipids	"MG(0:0/18:3(6Z,9Z,12Z)/0:0)"	$C_{21}H_{36}O_4$	12.901	352.2609
	Stearamide	$C_{18}H37NO$	13.015	283.2874
	Docosatetraenoyl	$C_{24}H_{41}NO_2$	13.047	375.3140
	"MG(0:0/18:3(6Z,9Z,12Z)/0:0)"	$C_{21}H_{36}O_4$	13.152	352.2610
	"(Z)-13-Oxo-9-octadecenoic acid"	$C_{18}H_{32}O_3$	13.155	296.2350
	LysoPC(18:3(6Z,9Z,12Z))	$C_{26}H_{49}NO_7$	13.315	518.3250
	Stearoylethanolamide	$C_{20}H_{41}NO_2$	13.367	327.3139
	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	13.609	678.3838
	LysoPC(16:0)	$C_{24}H_{51}NO_7$	15.056	496.3412
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	14.037	352.2610
	LysoPE(0:0/18:2(9Z,12Z)	$C_{23}H_{44}NO_7$	14.082	477.2861
	LysoPC(18:2(9Z,12Z))	$C_{26}H_{51}NO_7$	14.124	520.3404
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	14.269	352.2606
	LysoPC(18:2(9Z,12Z))	$C_{26}H_{51}NO_7$	14.383	520.3406
	22-Oxo-docosanoate	$C_{22}H_{42}O_3$	14.462	354.3137
	LysoPE(0:0/16:0)	$C_{21}H_{44}NO_7$	14.666	453.2860
	omega-hydroxy behenic	$C_{22}H_{44}O_3$	14.771	356.3291
	7-Oxostigmasterol	$C_{29}H_{46}O_2$	14.934	426.3501
	Phenethyl decanoate	$C_{18}H_{28}O_2$	15.418	276.2088
	9-HOTE	$C_{18}H_{30}O_3$	15.419	294.2197
	8, 11, 14, 17-icosatetraenoic acid;C20:4n3,6,9,12	$C_{20}H_{32}O_2$	15.447	304.2404

	Name	Formula	RT	Mass
	LysoPC(18:1(11Z))	$C_{26}H_{53}NO_7$	15.462	522.3564
	(22Alpha)-hydroxycampest-4-en-3-one	$C_{28}H_{46}O_2$	15.950	414.3496
	Tridecyl phloretate	$C_{22}H_{36}O_3$	16.434	348.2665
	MG(0:0/18:3(6Z,9Z,12Z)	$C_{21}H_{36}O_4$	16.556	352.2608
	10-Hydroxy-2,8-decadiene-4,6-diynoicacid	$C_{10}H_8O_3$	17.088	176.0474
	Oleoyl Ethanolamide	$C_{20}H_{39}NO_2$	17.767	325.2975
	Docosatrienoic Acid	$C_{22}H_{38}O_2$	17.787	334.2867
Lipids	10-Hydroxy-2,8-decadiene-4,6-diynoicacid	$C_{10}H_8O_3$	18.000	176.0473
	2-Hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinol	$C_{38}H_{58}O_3$	18.098	562.4386
	MG(0:0/22:1(13Z)/0:0)	$C_{25}H_{48}O_4$	18.447	412.3549
	MG(0:0/16:0/0:0)	$C_{19}H_{38}O_4$	18.572	330.2770
	13Z,16Z-docosadienoic	$C_{22}H_{40}O_2$	18.656	336.3028
	Soyacerebroside I	$C_{40}H_{75}NO_9$	22.766	713.5432
	10-hydroperoxy-8E,12Zoctadecadienoicacid	$C_{18}H_{32}O_4$	9.332	312.2297
	10-Hydroxy-2,8-decadiene-4,6-diynoicacid	$C_{10}H_8O_3$	8.491	176.0474

The LCMS data provided insight into the phytochemical distribution in the extracts. When compared to previously reported data, the 11 compounds identified in the data exhibit anti-obesity action **Table No. 4.10.**

Rahim *et al.,* (2015) studied the lipase inhibition effect of the Apigenin 6-C-glucoside (IC_{50} was 5.28E+11 µM) and suggested this compound can be used to treat obesity. Song *et al.,* (2021) studied the anti-obesity effect of the *Vaccinium bracteatum* Thunb. fruit extract on high fat diet obese mice and found that extract reduces the body weight of mice. The phytochemicals analysis of the plant extracts found that Apigenin 6-C-glucoside one of the compounds that inhibit the pancreatic lipase.

The effect of delphinidin 3-O-beta-D-glucoside (D3G) was studied on the 3T3-L1 cells. D3G inhibit the lipid accumulation in the cells in doses dependent manner. D3G down regulate the expression of the PPARϒ, C/EBPα, SREBP-1, and FASN genes and up regulate the expression of the CTP-1 gene in the 3T3-L1 cells. D3G inhibits adipogenesis and enhances lipid metabolism through activating AMPK-mediated signaling, and hence has the potential to play a therapeutic role in the management and treatment of obesity. (Park *et al.,* 2019)

Yamamoto and Oue (2006) reported the effect of quercetin in rats fed with a high-fat high-sucrose diet they observed that quercetin reduce fat accumulation in 3T3-L1 preadipocytes by down regulating the PPARϒ.

Table No. 4.10: List of anti-obesity compounds from studied *Dioscorea* species.

Sr. No.	Compound name	Molecular Formula	RT	Mass	References
1	Apigenin 6-C-glucoside	$C_{26}H_{28}O_{14}$	6.595	564.1487	Rahim *et al.*, (2015) Song *et al.*, (2021)
2	Delphinidin 3-O-beta-D-glucoside	$C_{22}H_{23}O_{12}$	6.743	465.4121	Park *et al.*, (2019)
3	Quercetin 3-galactoside	$C_{21}H_{20}O_{12}$	7.156	464.0952	Yamamoto and Oue (2006) Abdul *et al.*, (2017)
4	(-)-trans-Carveol	$C_{10}H_{16}O$	7.375	152.1200	Kazemipoor *et al.*, (2013) Ali-Shtayeh *et al.*, (2019)
5	Peonidin	$C_{16}H_{13}O_6$	7.549	301.0711	Sari *et al.*, (2020)
6	Octyl gallate/ gallic acid	$C_{15}H_{22}O_5$	8.557	282.1464	Totani *et al.*, (2011) Song *et al.*, (2020)
7	Ganoderic acid A	$C_{30}H_{42}O_7$	8.590	514.2936	Zhu *et al.*, (2018) Sharma *et al.*, (2019)
8	Diosgenin	$C_{51}H_{82}O_{21}$	8.855	1030.5371	Son *et al.*, (2007) Uemura *et al.*, (2010) Wang *et al.*, (2015) Hua *et al.*, (2016)
9	Zerumbone	$C_{15}H_{22}O$	9.354	218.1668	Tzeng *et al.*, (2014) Ahn (2017)
10	Gingerglycolipid B	$C_{33}H_{58}O_{14}$	13.678	678.3814	Ilavenil *et al.*, (2016)
11	Oleoyl Ethanolamide	$C_{20}H_{39}NO_2$	17.596	325.2975	Romano *et al.*, (2014)

Chemical structures of all compounds represented in **(Plate 8-9).**

A study conducted by Kazemipoor *et al.*, (2013) reported that the effect of *Carum carvi* L. on overweight and obese women as clinical trial they treat the obese women with the aqueous 10% seed extract for the 90 days with their diet the observed that the significant weight loss. In the GCMS analysis of the extracts found the Trans Carveol as one compound. Trans-Carveol from the mentha has lipase inhibition activity Ali-Shtayeh *et al.,* (2019).

Sari *et al.,* (2020) did the virtual screening of anthocyanins from black rice for anti-obesity activity by targeting the TLR4 and JNK pathways. According to the findings of this study, cyanidin, peonidin, cyanidin-3-O-glucoside, and peonidin-3-O-glucoside directly inhibited TLR4 and JNK proteins at their critical regions. All black rice anthocyanins may have anti-obesity activity.

In 2011 Totani *et al.,* reported the effect of gallic acid ester and octyl gallate on the obese rats which are feed with 7% frying oil for the 12 weeks. In observation the octyl gallate and ester from the gallic acid were effectively reduces the weight in the rat.

The *Diospyros kaki* extract inhibits the development of 3T3-L1 pre-adipocyte cells into mature adipocytes. Gallic acid is a significant bioactive component of extract that Inhibit Fatty Acid Synthesis in 3T3-L1 Cells by activating AMPK. The observed that the fermented *Diospyros kaki* extract effect on metabolic parameters of mice feed with high-fat diet (HFD). Fermented extracts supplementation resulted in a 15% reduction in body weight also decreased abdominal and liver fat, and decreased blood levels of triglycerides, total cholesterol, and glucose Song *et al.,* (2020).

Zhu *et al.,* (2018) studied the effect of ganoderic acid A on the high fat diet induce mice and 3T3-L1 adipocytes. ganoderic acid A (GAA) inhibits SREBP expression and lowers cellular levels of cholesterol and fatty acids. Also reduces body weight gain and fat accumulation in the liver or adipose tissues, as well as improving serum lipid levels and insulin sensitivity in obese mice. GAA has the potential to be a leading compound in the development of drugs for the prevention of obesity and insulin resistance.

Fatty acid synthase is the key enzyme involved in the DE novo synthesis of the fatty acids in adipocytes and liver tissue. Kaushal *et al.,* (2019) did the bioinformatics study on the

interaction of the ganoderic acid and fatty acid synthase gene. The inhibition of the fatty acid synthase helps in the controlling the obesity.

Wang *et al.,* (2015) studied the effect of diosgenin on the 3T3-L1 cells and high fat diet induced mice. When treat the 3T3-L1 cells and mice with the diosgenin the PPARϒ gene was suppressed in both cases. They concluded that in response to diosgenin stimulation, ERβ interacted with RXR α and detached RXRα from PPARϒ resulting in a decrease in transcriptional activity. The study shows that ER-β mediated control of PPAR expression and activity is essential for diosgenin-inhibited adipocyte differentiation.

Son *et.al.* (2007) examined the effect of the diosgenin on the high fat diet induced rat which is feed with 0.1% to 0.5% diosgenin for 6 weeks. The treatment with diosgenin found reduction in the total cholesterol level in plasma and liver. From this study they conclude that diosgenin can be used for the treatment of the hypercholesterolemia.

In pregnant diabetic mice, Diosgenin reduced the expression of sterol regulatory element-binding transcription factor-1 (SREBP-1) and its target genes, including fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1) and acetyl coenzyme A carboxylase (ACC). Furthermore, overexpression of SREBP-1 by LV-SREBP-1 injection might significantly reduce diosgenin protective impact against disorders of glucose and lipid metabolism as well as oxidative stress in diabetic mice. The findings suggest that SREBP-1 is a significant target of diosgenin that mediates its anti-diabetic effects in diabetic mice. Hua *et.al,* (2016).

Uemura *et al.,* (2010) studied the effect of the *Trigonella foenum-graecum* (fenugreek) extract on the high fat induced the rat the examined the hepatic tissue for the lipid accumulation the fond reduction of the lipid accumulation in the liver tissue. Further they did the fractionation of the extracts and again did the same experiment. In this study they found fraction containing diosgenin inhibiting the lipid accumulation in the hepatic tissue.

Lipid- reducing effects of zerumbone was investigated by the Tzeng *et al.,* (2014) on high-fat diet-induced hyperlipidemic hamsters. Zerumbone is a natural cyclic sesquiterpene of *Zingiber zerumbet* Smith, in the experiment hamster feed with the high fat diet for two weeks. Then apply a dose of the zerumbone in different concentrations for 8 weeks. They

observed that the zerumbone decreases in plasma levels of TC, TG, and LDL-C, as well as hepatic lipid concentrations, were seen, with an increase in faucal lipids occurring simultaneously. Zerumbone down regulate the gene expression of fatty acid synthase, malic enzyme, sterol-regulatory element binding protein, and 3-hydroxy-3-methyl-glutaryl-CoA reductase in the liver.

The effect of the zerumbone on the high fat diet induced mice and gene regulatory effect on 3T3-L1 cells. Zerumbone dysregulated lipid metabolism in the white adipose tissues of mice. In 3T3-L1 induced AMPK activation and phosphorylation of acetyl-CoA carboxylase, and effectively decreased the adipogenesis. The administration of zerumbone was shown to efficiently reverse the significant increase of microRNA-146b. In zerumbone-treated differentiated adipocytes, the levels of SIRT1, a direct target of microRNA-146b, was increased Ahn *et al.*, (2017)

Abdul *et al.,* (2017) studied the antilipase activity of the eight herbs. From the studied plant *Cosmos caudatus* was used for the farther study. Ethanolic extracts of *Cosmos caudatus* was used for metabolite profiling by UHPLC-MS/MS. The presence of quercetin-3-rhamnoside, catechin, kaempherol, kaempherol glucoside, quercetin, quercetin-3-glucoside, quercetin-O-pentoside, quercetinrhamnosyl galactoside, quinic acid, 1-caffeyolquinic acid, monogalloyl glucose, and procyanidin B1 was found in the extracts. *Cosmos caudatus* was discovered to be a promising therapeutic plant for the creation of novel functional foods with enormous applications in obesity. lavenil *et al.*, (2016) examined the anti adipogenic effect of *Chlorella vulgaris.* Ethanolic extract inhibit the adipogenic expression. They did the LCMS analysis of the extracts in that Cecropiacic acid, briarellin-A, Platycodigenin, Martiriol, Ergost-7-ene-2,3,5,6,9,11,19- heptol, Gingerglycolipid-A, gingerglycolipid-B, gingerglycolipid-C are the major compound.

According to the behavioral satiety sequence paradigm, mice given identical dosages (5 or 10 mg/kg, i.p.) of Oleoyl Ethanolamide or rimonabant were studied for the gradual expression of spontaneous behaviors (feeding, grooming, rearing, locomotion, and resting) throughout the onset of satiety (BSS). Both medications lowered food intake (wet mash) to a similar level. The OEA therapy reduced eating activity within the first 30 minutes and induced a brief increase in resting time, which was not followed by a decrease in horizontal, vertical,

or total motor activity. Rimonabant, in addition to reduced eating activity, increased grooming time and decreased horizontal motor activity, changes that might be suggestive of adverse no motivational effects on feeding. These finding suggest that OEA reduces appetite by increasing fullness, and that its profile of action may indicate safer effects in humans as a potential anti-obesity medication. (Romano *et al.*, 2014)

These 11 chemicals discovered in the LCMS profiles of *D. pentaphylla* and *D. oppositifolia* provide evidence that this plant contains anti-obesity metabolites that work at the levels of lipase inhibition, adipogenic gene regulation, and appetite suppression.

4.4 Evaluation of anti-obesity activity of extracted compounds using cell line (3T3-L1 pro adipocytes):

4.4.1 Fractionation of the *D. oppositifolia* methanolic extract

In the gene expression study *D. oppositifolia* methanolic extract down regulated adipogenic genes and up regulated the expression of the CPT-1 gene. From these results the *D. oppositifolia* carry forward for the fractionation. Fractions were prepared by using the different solvent like Hexane, ethyl acetate, chloroform, n- Butanol and water. After fractionation the yield of each fraction was calculated. **(Table No. 4.11)** residue was diluted in DMSO for the required concentration.

4.4.2 Extraction of diosgenin from *D. oppositifolia* root

78 mg of crude diosgenin was recovered from 5 gm of *D. oppositifolia* dry root powder (Table no 4.11). After diosgenin extraction, the dry residue was weighed and diluted in methanol to make a 10 mg/ml stock.

Table No. 4.11: Amount of the crude extract powder after fractionation

Sr. No.	Plant Name	Residue in mg
1	Hexane fraction	300
2	Chloroform fraction	90
3	Ethyl acetate fraction	200
4	n- Butanol fraction	148
5	Remaining water fraction	247
6	Extracted Diosgenin	78

4.4.3 Cell viability assay:

The cell viability of all the fractions was carried out for the determination of the IC_{50} value. Form the IC_{50} value treatment concentration was decided for the differentiation assay. The toxicity of the fraction was investigated using the MTT test at various doses (6.25-100 µg/ml). All fractions were shown to be moderately toxic to cells at higher concentrations *i.e.*, 100µg/ml at the tested doses. The IC_{50} values mentioned in **Table No. 4.12, Figure No. 4.15** presenting the % viability of the 3T3-L1 cells at different tested concentrations.

Table No. 4.12: IC_{50} values of the fractions

Sr. No	Fraction Name	IC_{50}
1	Hexane fraction	58.97 µg/ml
2	Chloroform fraction	60.60 µg/ml
3	Ethyl acetate fraction	70.31 µg/ml
4	n- Butanol fraction	65.89 µg/ml
5	Remaining water fraction	66.88 µg/ml
6	Extracted Diosgenin	62.89 µg/ml
7	Std. Diosgenin	75.51 µg/ml

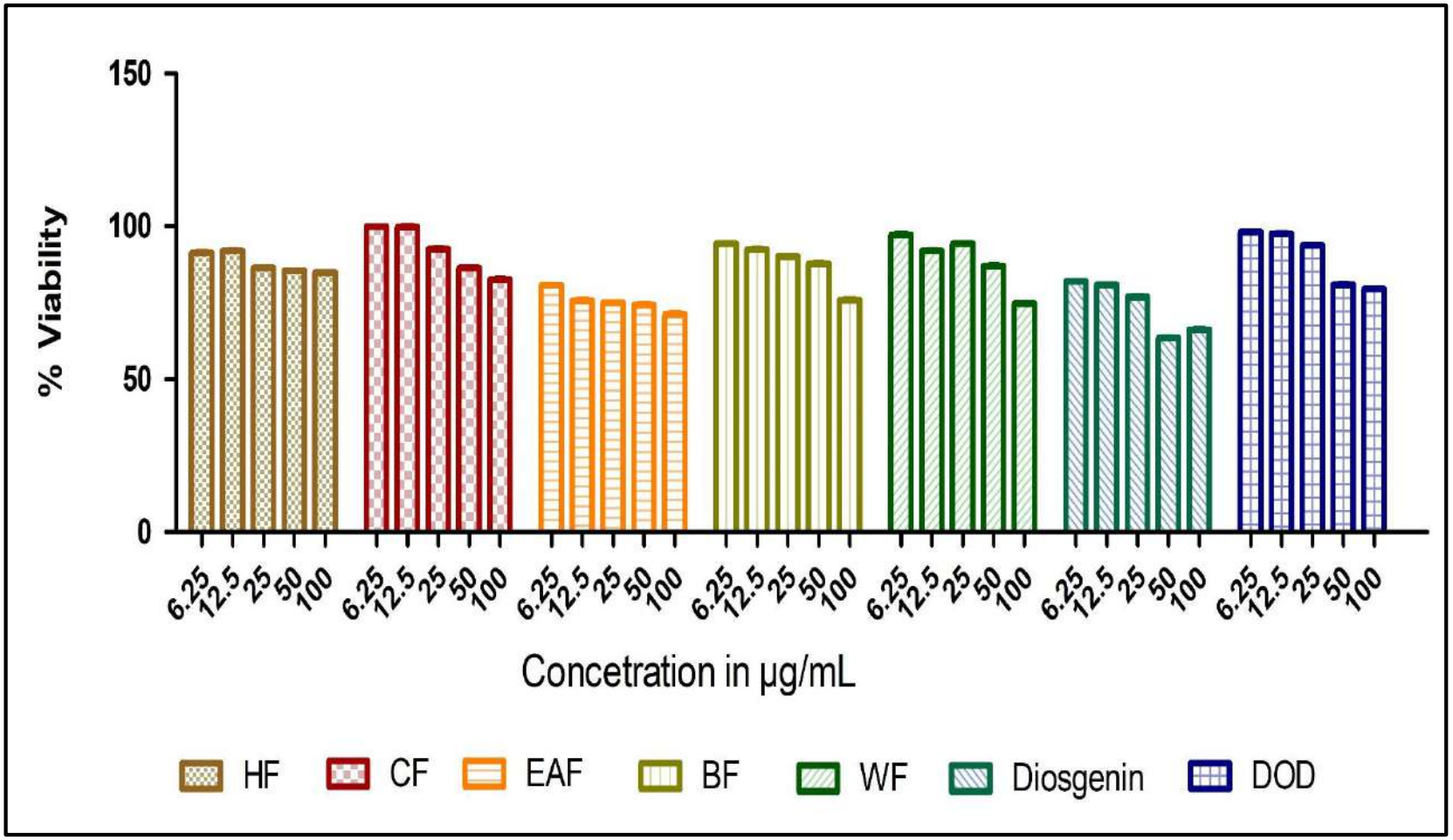


Figure No. 4.20 : Cell viabilty test by MTT assay cells treated with different fractions and extracted Diosgenin and Standerd Diosgenin (6.25 to 100 µg/ml concentrations) (HF: hexane fraction, CF: Chloroform fraction, EAF: ethyl acetate fraction, BF: n-butanolic fraction and WF: water fraction, SD: Standard Diosgenin, DOD: *D. oppositifolia* Diosgenin)

4.4.4 Effect of *D. oppositifolia* methanolic extract fractions on inhibition of lipid droplet accumulation in 3T3-L1 cell line:

The Lipid quantification was carried out, on the eighth day of the experiment. The decrease in lipid deposition was measured by comparing the cells that had been stimulated with a hormone cocktail. Cells treated with a hormone cocktail had 2.65 times increase in lipid content as compared to non-induced cells. In comparison with control group the lipid accumulation was highly significant (*P<0.001*) for induced group whereas in other tested groups the lipid accumulation was significantly decreased (*P<0.001*) in comparison with induced group. Among the tested 5 fractions and extracted diosgenin from the *D. oppositifolia,* the lipid deposition in cells treated with extracted Diosgenin from the *D. oppositifolia* and Standard diosgenin were found to reduce by 2.4 and 2.2 times, respectively in comparison with induced group. Furthermore, the cells treated with different fractions (hexane fraction, Chloroform fraction, ethyl acetate fraction, n-butanolic fraction and water fraction) of *D. oppositifolia* have not shown significant decrease in the lipid accumulation in the differentiated 3T3- L1 cells when comparing with induced group. In this chloroform fraction inhibit lipid accumulation in the cells by 1.1 times as compere to the induced cells. . The cells treated with all the fractions did not show significant difference in lipid accumulation, so all the fraction were selected for the gene expression study. (**Plate No. 10)**

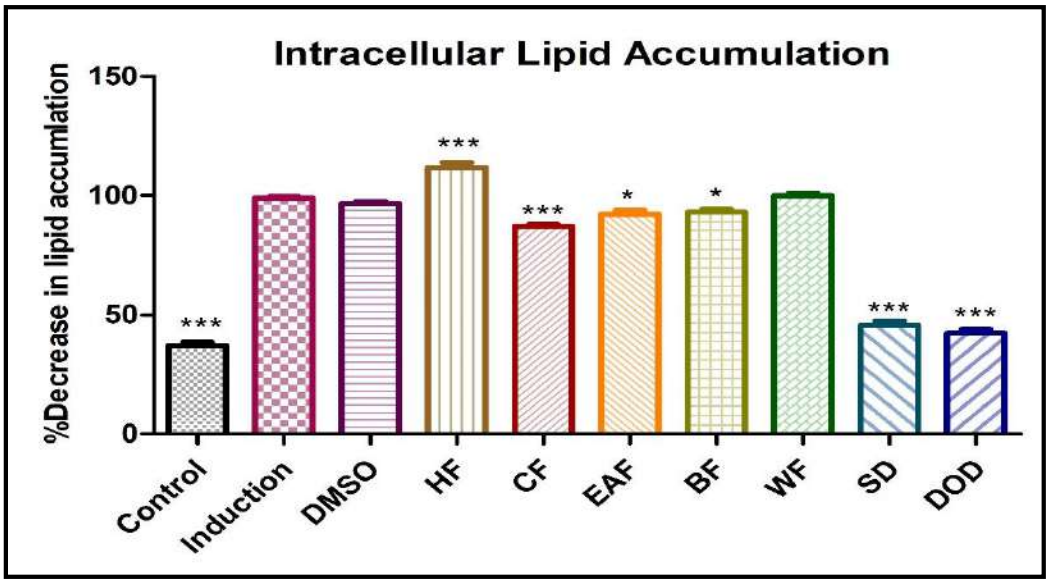


Figure No. 4.21: Intracellular lipid accumulation in 3T3-L1 cells by isopropanol extraction method ****P*< 0.001 and **P*< 0.001 as compare to induction and control using one way ANOVA followed by tukyes test. (HF: hexane fraction, CF: Chloroform fraction, EAF: ethyl acetate fraction, BF: n-butanolic fraction and WF: water fraction, SD: Standard Diosgenin, DOD: *D. oppositifolia* Diosgenin)

4.4.5. Effect of different fractions on mRNA expression of adipogenic genes:

According to the results of the oil red staining, no significant alterations were seen in cells after treatment with all fractions. For that, the influence of all the fractions explored by gene expression studies.

A. Effect of hexane fraction:

The effect of hexane fraction on adipogenic gene regulation is shown in **Figure No. 4.22,** where the expression of the PPARɣ, C/EBPα, SREBP- F1, and FASN genes is compared to the control. Up regulation of the PPARɣ, C/EBPα, SREBP-F1, and FASN genes were seen in cells treated with the hexane fraction at both doses. At 50 µg/ml concentration, PPARɣ and C/EBPα were up regulated ≈35 times and ≈8.7 times, respectively, compared to the control **(Figure No. 4.22 A, B).** SREBP F1 and FASN expression were also up to ≈4.4 and ≈5.6 times higher than in control, respectively **(Figure No. 4.22 C, D).** In the case of CPT-1 gene expression, the hexane fraction inhibits the gene at both doses; at 25 µg/ml, 98% down regulation was seen, and at 50 µg/ml, 53% down regulation was detected as compared to control cells **(Figure No. 4.22 E).**

B. Effect of chloroform fraction:

The adipogenic genes down regulated during the differentiation of 3T3-L1 cells when cells were treated with chloroform fraction. The gene expression of PPARɣ, C/EBPα, and SREBP-F1 were suppressed at both treated concentrations. The chloroform fraction reduced PPAR expression by up to 42% and 72%, respectively **(Figure No. 4.23 A).** The C/EBPα gene was suppressed by up to 74% and 62% at 25µg/ml and 50 µg/ml as compared to the control **(Figure No. 4.23 B).** In the case of 50 µg/ml the expression was slightly increased as compared to the cells treated with the 25 µg/ml concentration. The lower concentration gave better suppression of the C/EBPα gene. The expression of SREBP-F1 was reduced as compared to the control. An increase in the concentration of the chloroform fraction decreases the expression of the gene. Down regulation of the SREBP-F1 gene was observed at 25 µg/ml (7%), and 50 µg/ml (46%) **(Figure No. 4.23 C)**. The fatty acid synthase gene was up regulated at the lower concentration of the chloroform fraction almost 3.1 times at 25µg/ml and the higher 50 µg/ml concentration of the chloroform fraction slightly reduces the FASN gene expression up to 23% **(Figure No. 4.23 D)**. In the case of the CPT-1 gene, up regulation was

observed. At 25 µg /ml and 50 µg /ml, chloroform fraction increases CPT-1 expression by 1.8 and 1.9 times, respectively **(Figure No. 4.23 E).** Over all, the chloroform fraction contains some compounds that can down regulate the adipogenic genes PPARɣ, C/EBPα, and SREBP-F1. While it also contains compounds that stimulate the genes, like CPT-1, which helps to increase the lipolysis activity.

C. Effect of ethyl acetate fraction:

The effect of the ethyl acetate fraction on adipogenesis was examined, and diverse outcomes on adipogenic gene expression were found. The PPARɣ gene was up regulated by the ethyl acetate fraction, which enhanced PPARɣ gene expression about 40 times over the control at 25 µg/ml, and 7 folds over the control at 50 µg/ml **(Figure No. 4.24 A)**. While the expression of the C/EBPα gene was increased by 3 folds at 25 µg/ml compared to the control. However, treatment with a greater dose of 50µg/ml reduces up to 96 % when compared to the control **(Figure No. 4.24 B).** SREBP-F1 was down regulated at both ethyl acetate fraction dosages. Cells treated with 25 µg/ml and 50 µg/ml concentrations reveal 81 and 85 % down regulation of SREBP, respectively **(Figure No. 4.24 C).** The 37% down regulation of fatty acid synthase was shown with a lower dosage of ethyl acetate fraction. The higher dose up-regulates expression 8.6 times more than the control **(Figure No. 4.24 D).** The CPT-1 gene is up regulated 43 times at 25 µg/ml, whereas only 3.5 percent is down regulated at 50 µg/ml **(Figure No. 4.24 E).**

D. Effect of n-butanol fraction:

The expression of adipogenic genes is increased in cells treated with the n-butanolic fraction. The PPARɣ was up-regulated by 3.1 times at 25 µg/ml and 19.9 times at 50 µg/ml when the concentration was raised. The PPARɣ was shown to be up regulated **(Figure No. 4.25 A).** The 25 µg/ml butanolic fraction increases C/EBPα expression 7-fold, but high dosages reduce C/EBPα expression by up to 85 % **(Figure No. 4.25 B).** The butanolic fraction at 25 µg/ml increased SREBP-F1 expression. A tenfold increase was seen, but at 50 µg/ml, a fivefold increase in gene expression was recorded when compared to the control. In this case, a rise in concentration leads to a reduction in SREBP-1 expression **(Figure No. 4.25 C).** At 25 µg/ml and 50 µg/ml, respectively, the butanolic fraction elevated FASN about 199 times and 134 times higher than the control **(Figure No. 4.25 D).** The expression of the CPT-1 gene

increased in a concentration-dependent manner. CPT-1 gene expression was enhanced by 4.2 and 7.8 times at 25 and 50 µg/ml, respectively, as compared to the control **(Figure No. 4.25 E).**

E. Effect of water fraction:

The residual water fraction was also examined for gene expression after separation to determine the influence of the water-soluble chemical on gene expression. The PPARɣ was up regulated in a concentration dependent manner, with a 17-fold and 52-fold increase in gene expression seen at 25 and 50 µg/ml, respectively **(Figure No. 4.26 A).** C/EBPα exhibits a considerable up regulation of expression at both tested doses of 25 µg/ml 2.4 times and 50 µg/ml 1.6 times increase **(Figure No.4.26 B).** However, at 50 µg/ml, the expression of SREBP -F1 was decreased compared to the lower dosage of the water fraction, with only 6.5 times increase in gene expression detected **(Figure No. 4.26 C).** The treatment with the water fraction lowers FASN gene expression. As the concentration rises, so does the expression of the FASN gene 8.8 percent down regulation was observed at 25 µg/ml. The water fraction down regulated up to 70% of the FASN gene at 50 µg/ml **(Figure No. 4.26 D).** At 25 µg/ml, water fraction up regulated the CPT-1 gene 200 times more than the control **(Figure No. 4.26 E).** This increased expression of the CPT-1 gene assists in increasing the rate of the lipolysis process, which supports in the reduction of stored fat in the cells.

While comparing the effects of each fraction, the chloroform fraction inhibits the expression of all examined adipogenic genes more than the other four fractions. It also stimulated the activity of the CPT-1 gene. The PPARɣ, C/EBPα, SREBP-F1, and FASN genes are up regulated by hexane fraction, and down regulates the CPT-1 gene. Treatment with the Ethyl acetate fraction raises PPARɣ, C/EBPα, and FASN levels. SREBP-F1 gene expression was lower than in the control group. The CPT-1 is down regulated at greater concentrations of ethyl acetate and up regulated at lower concentrations. At the studied concentrations, cells treated with n-butanol and water fraction up regulated all adipogenic genes. At greater concentrations of the n-butanol fraction, C/EBPα was down regulated.

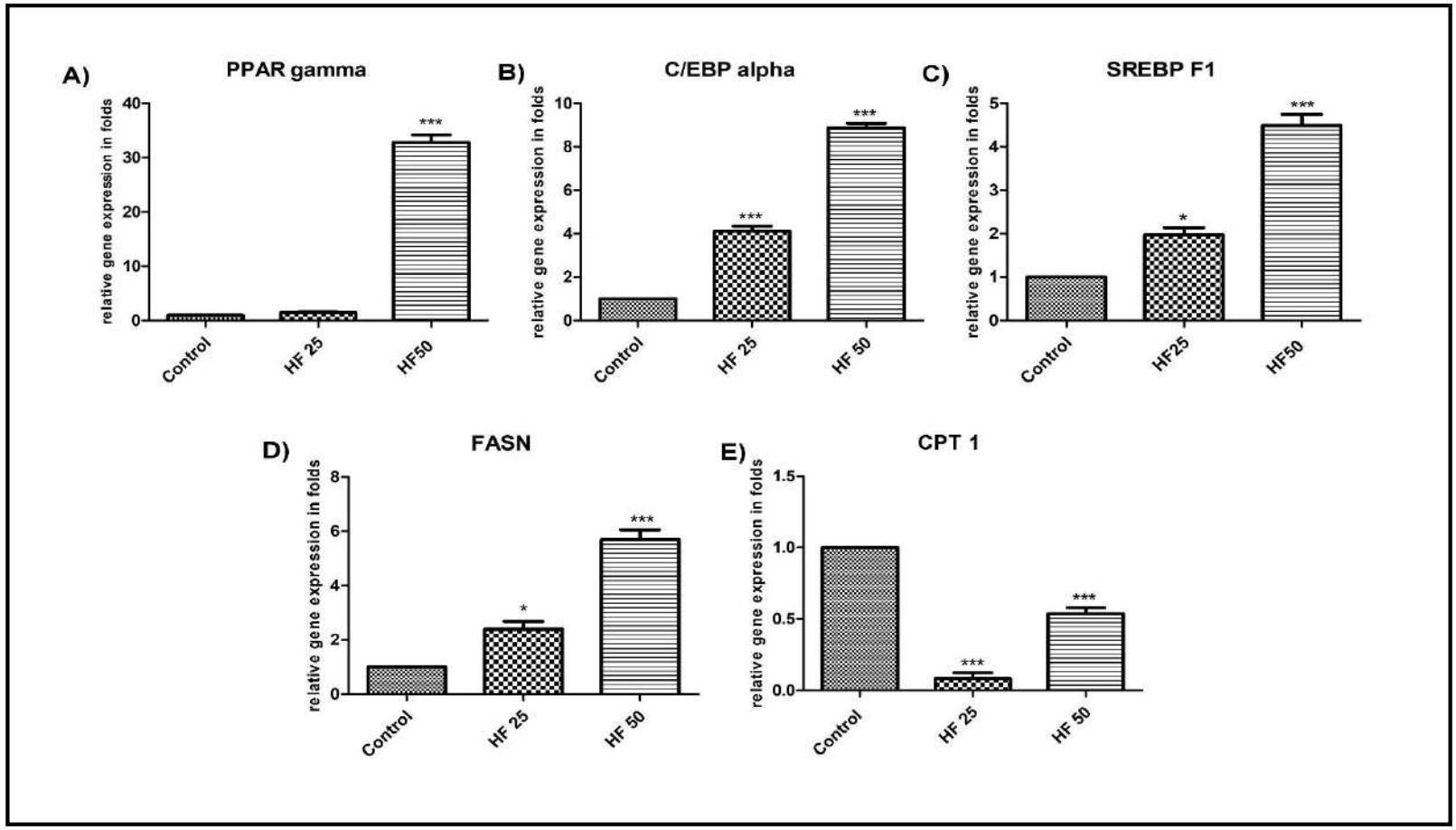


Figure No. 4.22: Effect of hexane fraction (HF) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

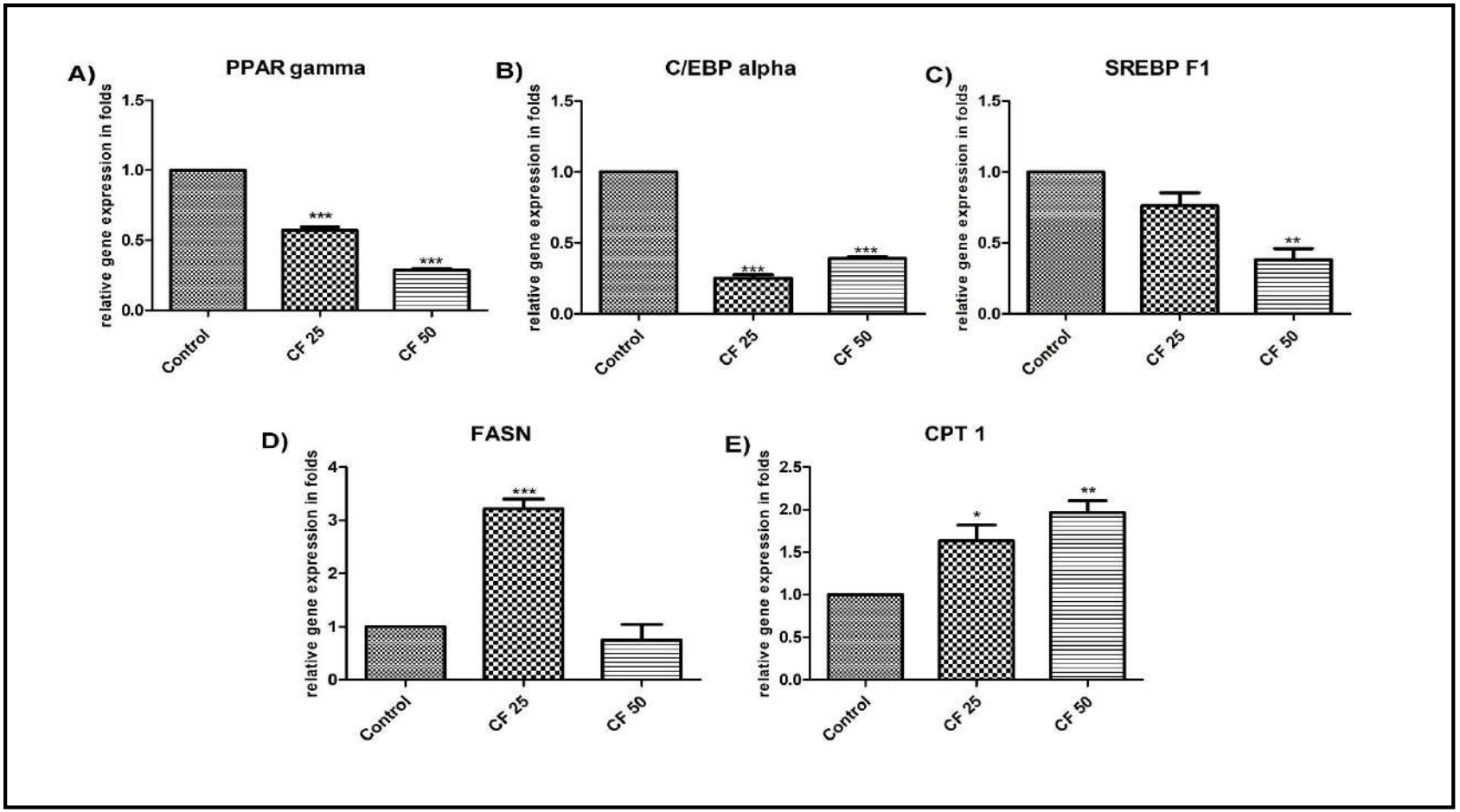


Figure No. 4.23: Effect of Chloroform fraction (CF) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

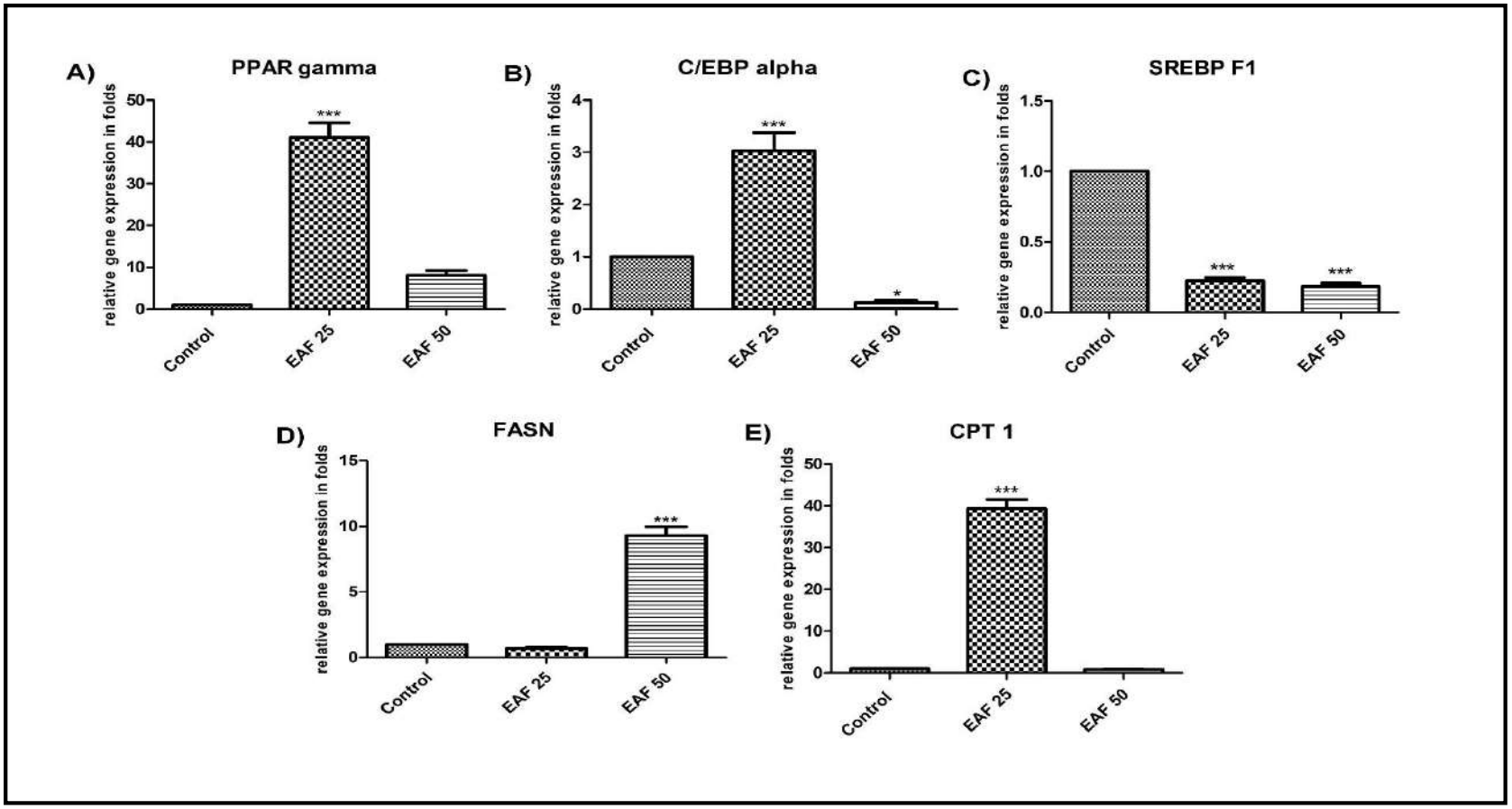


Figure No. 4.24: Effect of ethyl acetate fraction (EAF) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

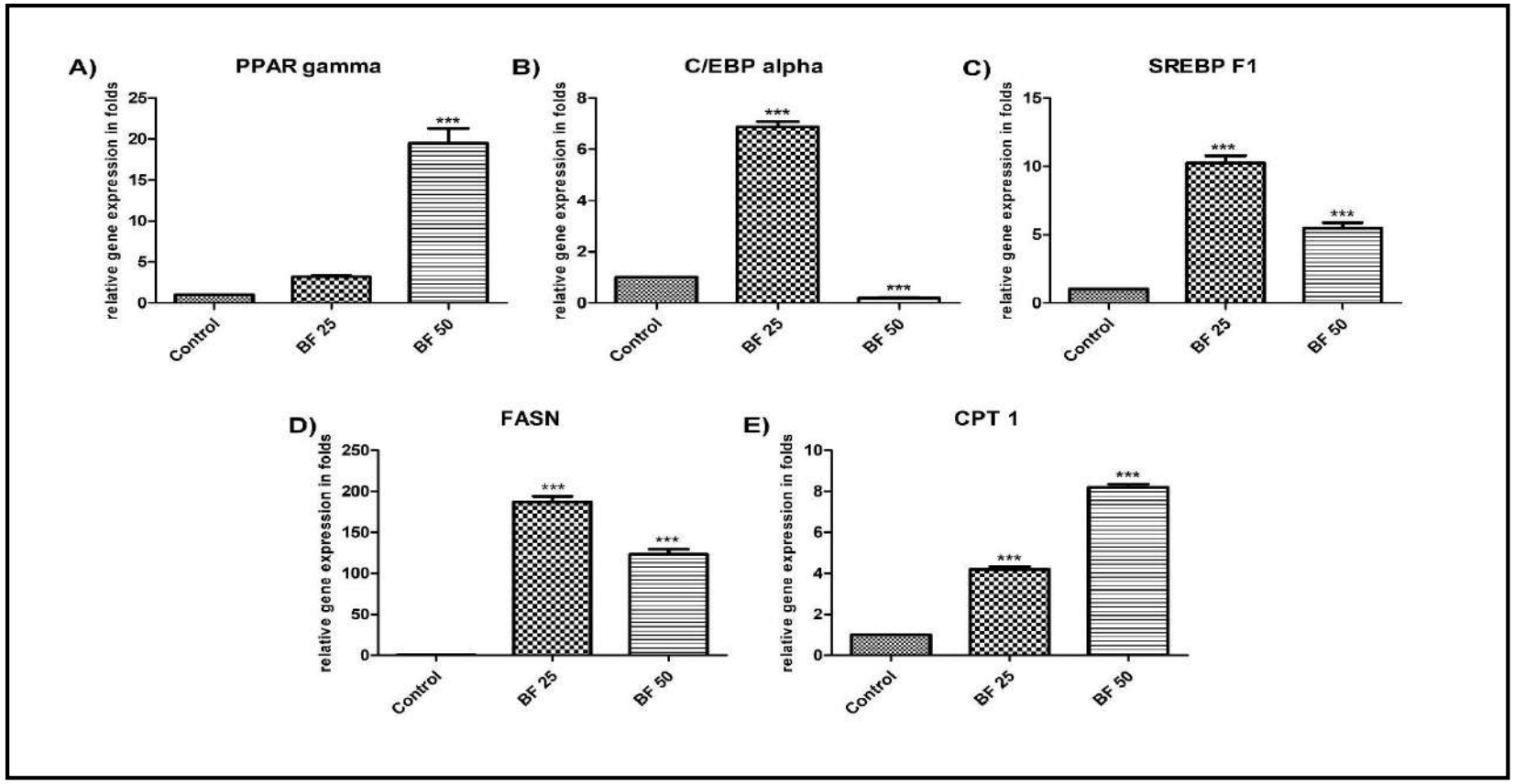


Figure No. 4.25: Effect of n-butanol fraction on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

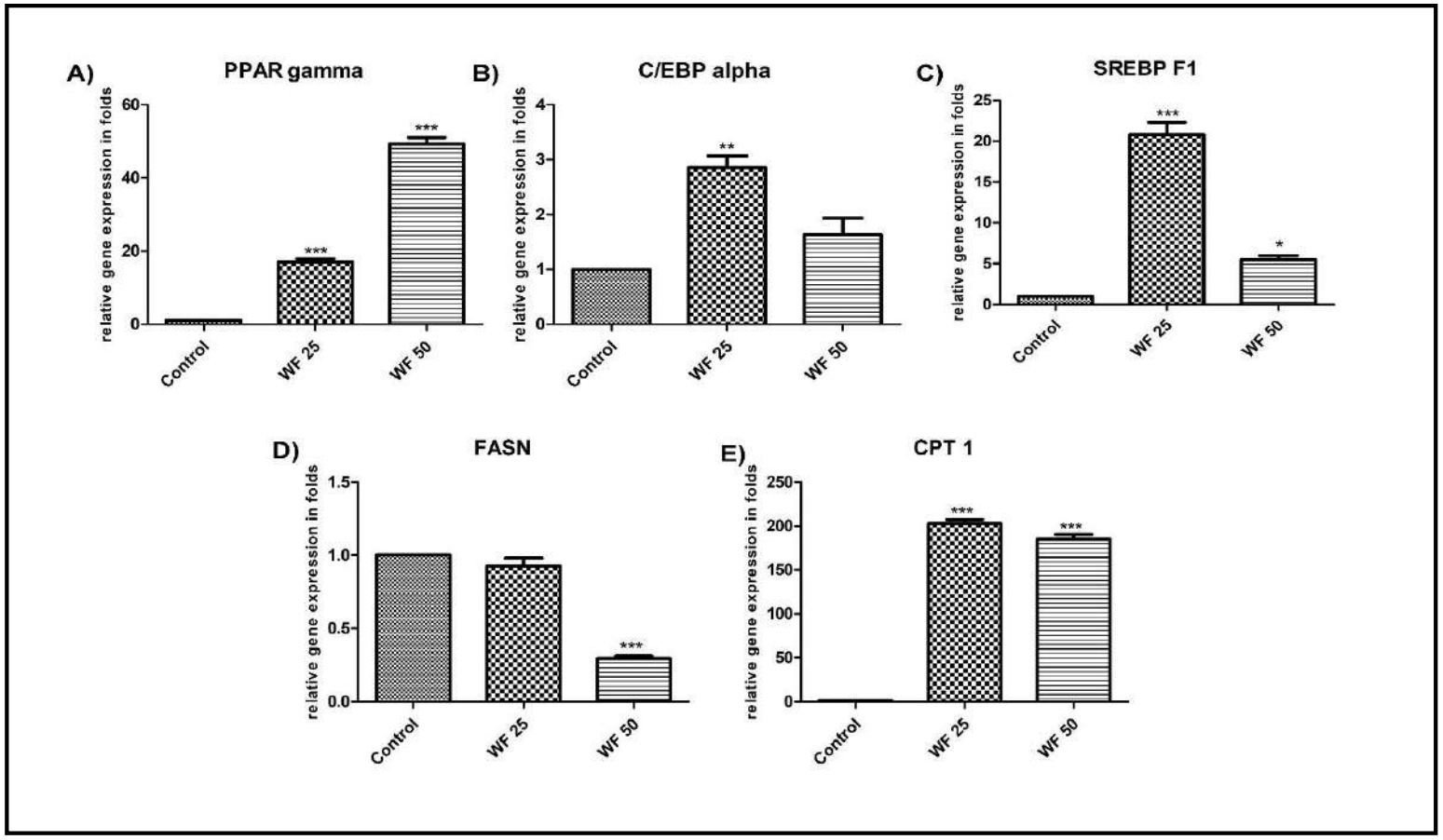


Figure No. 4.26: Effect of water fraction on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

4.4.6. Bioactive compound detection by LCMS:

In LCMS chromatogram of the chloroform fraction of *D. oppositifolia* methanolic whole plant extract shows in **Figure No.4.27**

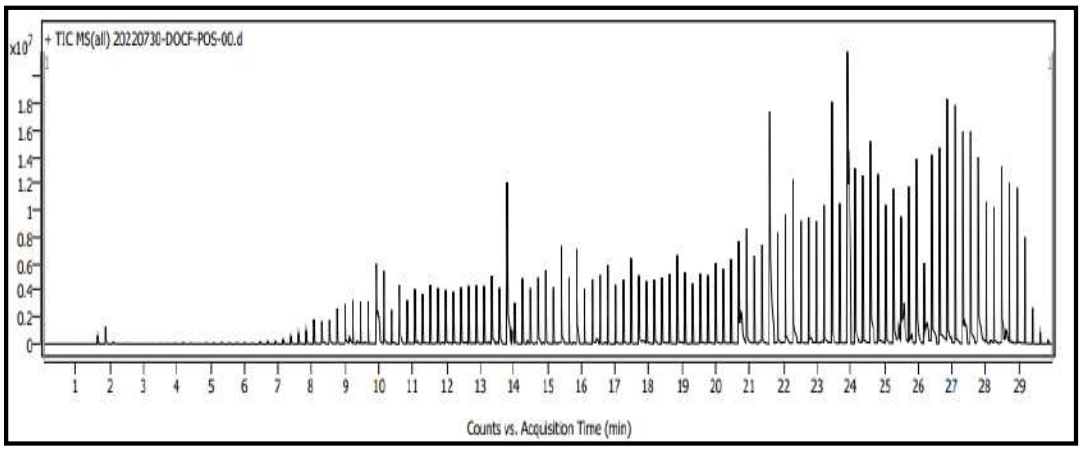


Figure No.4.27. LCMS chromatogram of Chloroform fraction of *D. oppositifolia* methanolic extract

The chloroform fraction contains the 4 anti-obesity metabolites which are identified from the data base and these 4 compounds were previously reported as an anti-obesity effect. Listed below in **Table No. 4.13.**

Table No. 4.13: Anti-obesity metabolites found in chloroform fraction of *D. oppositifolia* methanolic extract

Sr.No.	Compound Name	Class	Formula	RT	m/z	Height
1	Robinetin	Flavonoid	$C_{15}H_{10}O_7$	8.7647	302.0421	21548.5
2	Aloesin	Glycoside	$C_{19}H_{22}O_9$	9.283	417.1153	14347.3
3	Dioscin	Glycoside	$C_{45}H_{72}O_{16}$	19.3088	869.4883	24844.3
4	Oleoyl EthanolAmide	Lipid	$C_{20}H_{39}NO_2$	17.3158	325.2975	2738.5

Chemical structure of all compounds given on page no 90

Batubara *et al.*, (2014) prepared different solvent fractions of *Intsia palembanica* and from the ethyl acetate fraction they extracted probable flavonoids which, including ()-robidanol, (+)-epirobidanol, 4′ dehydroxyrobidanol, fustin, naringenin, robinetin (**Figure No.**

4.28), myricetin, quercetin, and 3,7,3′,5′-tetrahydroxyflavone. These isolated compounds were tested for *In vitro* lipase inhibition activity using substrate 2, 3-dimercapto-1-propanoltributyrate. After screening of compounds seven compounds showed PLE imbibition activity, with IC50 values ranging from 13.7 to 835.0 M. (+)-epirobidanol, robinetin, and naringenin effectively inhibit lipase.

Shin *et al.,* (2011) studied for different *Aloe vera* formulations on Obesity-induced Inflammation in Obese Mice. One of them is the aloesin **(Figure No. 4.29)**. Aloesin inhibited the expression of the adipogenic gene PPARɣ and LXR α genes as compared to control. Obesity-induced inflammatory responses are suppressed by *Aloe* formulations by lowering levels of proinflammatory cytokines, PPARɣ/LXR α, and 11-HSD1, and increasing anti-inflammatory cytokines in WAT and liver, all of which are critical peripheral tissues for insulin responsiveness. The activation of PPARɣ/LXR α has been linked to the therapeutic benefits of *Aloe* formula on obesity-induced insulin resistance and hepatic steatosis.

Kong *et al.,* (2010) studied the effect of processed *Aloe* gel, aloesin, *Aloe* QDM, and an *Aloe* QDM complex on the high fat diet induced mice. The gene expression study exhibited the down regulation of the fatty acid synthase, sterol regulatory element binding protein in liver and white adipose tissue. *Aloe* formula has functional qualities such as regulation of hyperglycemia, hyperlipidemia, blood glucose reduction, and adipogenesis of adipose tissue in HFD-fed mice. Comparison between all the aloe formulas aloesin inhibit the adipogenic genes.

Poudel *et al.,* (2014) examined the effect of marked Dioscin on the 3T3-L1 adipogenesis. Treatment with the dioscin reduce the lipid accumulation and inhibit the adipogenic transcription factors. Increase in the concentration of dioscin down regulates the C/EBPα, β, δ genes. It also reduces the expression of PPARɣ, SREBP1, and FASN genes. They also check the effect of Dioscin on the high fat diet induced mice. They found that dioscin reduces the fat accumulation and weight reduction in the mice by modulating the AMPK/MPK pathway **(Figure No. 4.30)**.

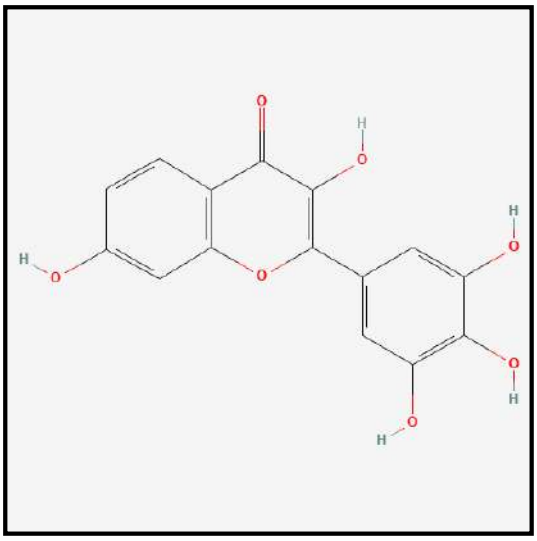


Figure No. 4.28:
Chemical structure of Robinetin

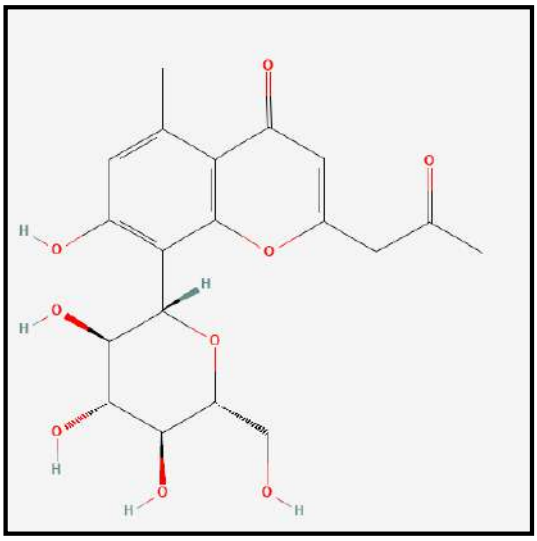


Figure No. 4.29:
Chemical structure of Aleosin

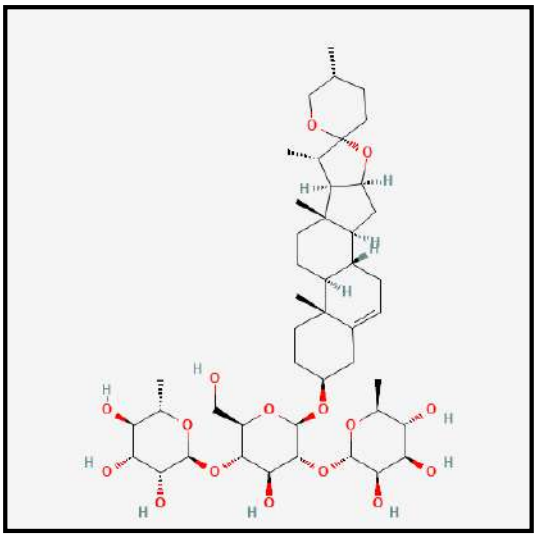


Figure No. 4.30:
Chemical structure of Dioscin

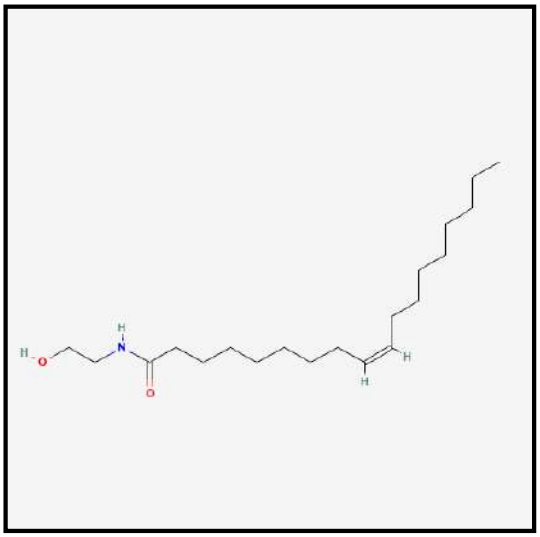


Figure No. 4.31:
Chemical structure of Oleoyl Ethanolamide

Chemical structures acquired from
(https://pubchem.ncbi.nlm.nih.gov)

Dioscin's effects on body weight and serum FFA may aid in the prevention and treatment of cardiovascular disease. As a result, it was suggested that dioscin is useful in controlling the aberrant metabolism of obese mice by restoring the activity of the IRS-1/PI3K/Akt pathway and the PPAR- pathway, therefore reversing the insulin resistance caused by an HFD. Dioscin's lowered body weight and blood FFA levels established it as a viable option for the treatment of various obesity-related illnesses (Li *et al.,* 2019).

Oleoyl Ethanolamide **(Figure No. 4.31)** inhibits food consumption, weight gain and plasma lipid levels in obese Zucker rats which are lacking functioning leptin receptors. Diet-induced obese rats and mice have similar consequences. Subchronic OEA treatment (5 mg kg1, intraperitoneally, i.p., once daily for two weeks) in Zucker rats initiates transcription of PPAR-α and other PPAR-α target genes, including fatty-acid translocase (FAT/CD36), liver fatty-acid binding protein (L-FABP), and uncoupling protein-2 (UCP-2). Furthermore, as measured by Oil red O staining, OEA reduces neutral lipid content in hepatocytes as well as blood cholesterol and triglyceride levels. The findings indicate that OEA modulates lipid metabolism, which may contribute to its anti-obesity benefits (Fu *et al.,* 2005).

Dioscin, aloesin, oleoyl ethanolamide, and robinetin are the four chemicals identified in the chloroform fraction's LCMS MS profile. According to previously published research, these compounds have anti-obesity effect, the presence of these compounds in the chloroform fraction demonstrating anti adipogenic activity.

4.4.7. Effect of extracted Diosgenin on mRNA expression:

Diosgenin was isolated from the dry root powder of *D. oppositifolia* and examined for anti-adipogenic activity on 3T3-L1 pre-adipocytes. Adipogenic genes were down regulated in cells treated with extracted diosgenin. Cells treated with extracted Diosgenin down regulated PPARɣ up to 56% at 25 µg/ml and 69% at 50 µg/ml **(Figure No. 4.32 A),** whereas cells treated with standard Diosgenin PPARɣ down regulated up to 71% and 78% at 25 and 50 µg/ml doses, respectively **(Figure No. 4.33 A).** Wang *et al.,* (2015) investigated the impact of commercial diosgenin on 3T3-L1 adipocytes and mice with a high fat diet. They discovered that increasing the quantities of diosgenin lowers PPARɣ expression and inhibits adipocyte development, as well as reduces adipocyte cell size. In our investigation, the isolated

diosgenin significantly reduced the expression of the PPARϒ gene. C/EBPα expression was moderately up regulated in both cases of diosgenin therapy at 25 µg/ml, 1.6 times **(Figure No. 4.32 B)** and 2.1 **(Figure No. 4.33 B)** times higher than in the control, respectively. However, at greater doses of 50 µg/ml, the C/EBPα gene is down regulated. Standard diosgenin inhibits the gene by up to 47% **(Figure No. 4.32 B),** whereas isolated diosgenin inhibited the gene by 31% **(Figure No. 4.33 B).** When compared to the control, both extracted diosgenin and standard diosgenin diminish the expression of the SREBP 1 gene. Down regulation was detected at 25 and 50 µg/ml of standard diosgenin, with 65 and 76 % down regulation, respectively **(Figure No. 4.33 C).** While isolated diosgenin inhibits the SREBP-1 gene by 85 percent at 25 µg/ml, the effect is reduced to 72 % at 50 µg/ml **(Figure No. 4.32 C).** The expression of the FANS gene was down regulated by Std. Diosgenin up to 67 % at 50 µg/ml, but only 5 percent at 25 µg/ml **(Figure No. 4.33 D).** At 25 µg/ml, isolated diosgenin mildly up regulated the FANS gene by 0.5 times. However, higher concentrations decrease gene expression by up to 40% **(Figure No. 4.32 D).** According to Berndt *et al.*, (2007), increased expression of the fatty acid synthase gene leads to an increase in visceral fat storage in the body. Inhibiting the FASN gene in adipocytes provides a significant increase and may be a viable therapeutic strategy for obesity management. The CPT-1 enzyme is important in the lipolysis of fat. The standard diosgenin promotes CPT-1 gene expression in a concentration-dependent manner. Standard diosgenin at 25 µg/ml increased expression 42 times more than the control. At 50 µg/ml, diosgenin up regulates the gene 103 times more than the control **(Figure No. 4.33 E).**

When cells were treated with extracted diosgenin, the CPT-1 gene level increased up to 223 times higher than the control at 25 µg/ml, but at 50 µg/ml, the CPT-1 level increased up to 147 times higher than the control **(Figure No. 4.32 E).** When compared to the standard diosgenin, the isolated diosgenin provides superior up regulation of CPT-1. The activation of CPT-1 helps in the initiation of fatty acid oxidation on the mitochondrial membrane.

In comparing Standard diosgenin and extracted diosgenin, extracted diosgenin had almost the same impact on adipogenic gene regulation. This difference might be attributed to impurities present in the extracted diosgenin.

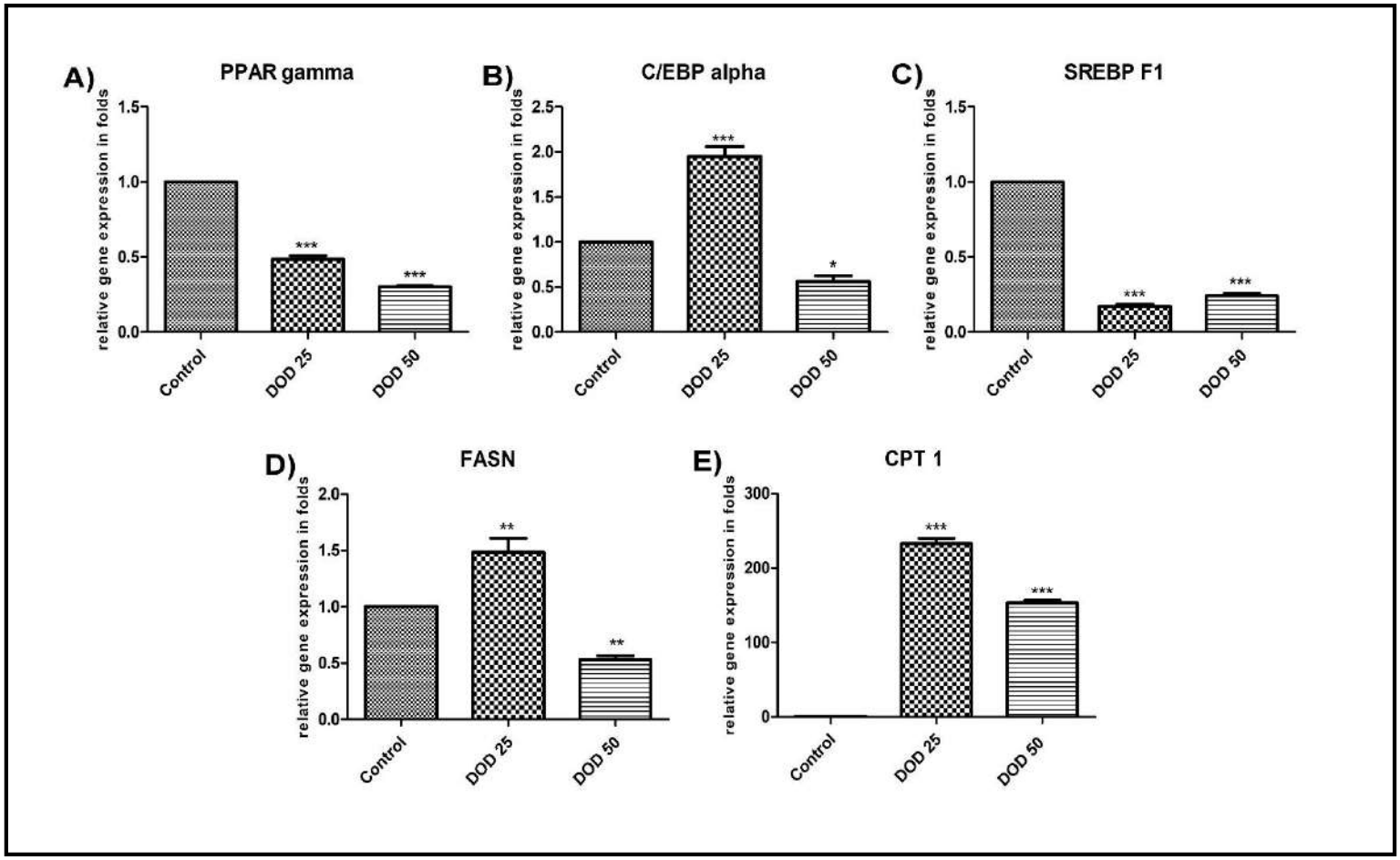


Figure No. 4.32: Effect of extracted Diosgenin (DOD) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

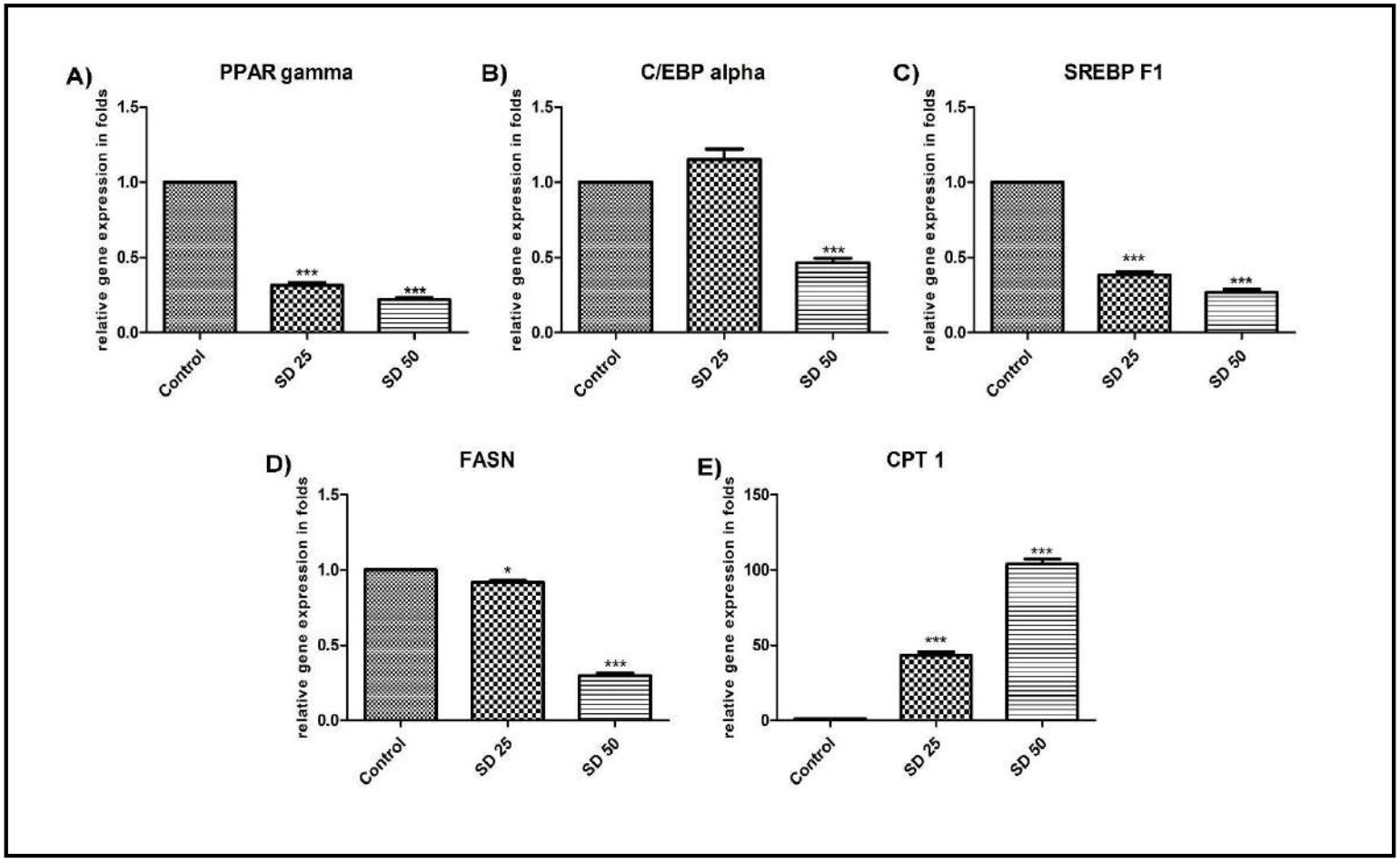


Figure No. 4.33: Effect of standard Diosgenin (SD) on Relative mRNA expression of (a) peroxisome proliferator activated receptor gamma, (b) CCAAT/enhancer-binding proteins α , (c) sterol regulatory element-binding protein-1, and (d) Fatty acid synthase (e) Carnitine palmitoyltransferase-1 *(*P < 0.05)* as compared to control using one way ANOVA followed by Dunnett's Multiple Comparison Test.**

4.4.8 Detection of extracted diosgenin by HPLC

Diosgenin belongs to the class of steroidal saponins. The presence of diosgenin was identified in the current study utilizing the HPLC technique. Based on the chromatogram obtained from the software, a sharp peck of standard diosgenin was obtained at 3.19 min **(Figure No. 4.34).** The diosgenin was extracted from the *D. oppositifolia* root by acid hydrolysis method **(Figure No 4.35)** and it also showed a sharp peck at the same retention time, confirming that the samples contain diosgenin. The amount of diosgenin was calculated by using standard diosgenin i.e.1.226 mg/100gm of dry root powder.

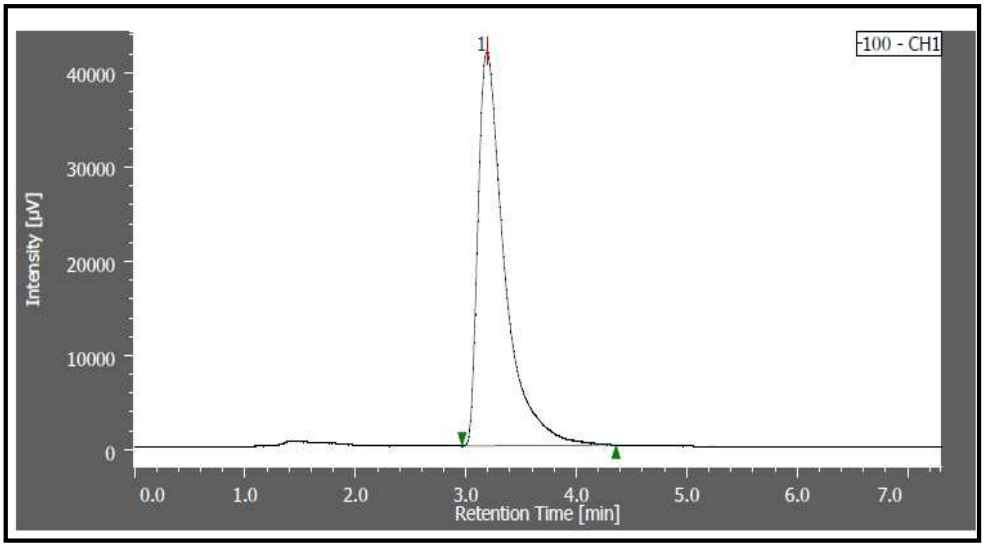


Figure No 4.34 Chromatogram of Standard Diosgenin 100µg/ml

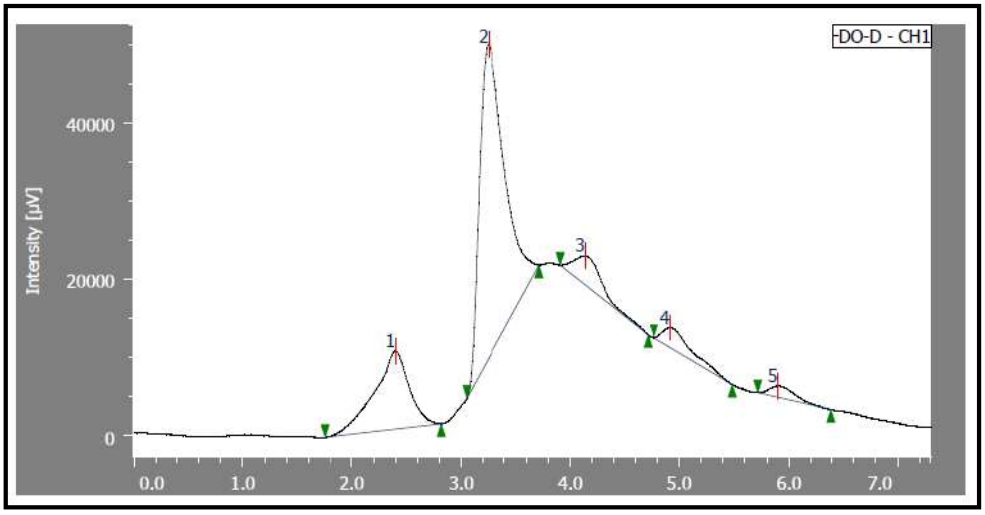


Figure No 4.35 Chromatogram of extracted diosgenin from *D. oppositifolia* Root by using acid hydrolysis

Diosgenin demands special attention because of its biological activity and pharmacological uses like antibacterial, antifungal, anticancer, anti-aging, cardio protective and contraceptive activity. Diosgenin also shows the anti hyperlipidemic activity (Chaudhary *et al.,* 2018). Raina and Misra (2020) estimated the diosgenin content in different *Dioscorea* species by HPTLC method they found that highest content of diosgenin in *D. hispida* and *D. bulbifera* than the other studied species. Niño *et al.*,(2007) collected *D. polygonoides* from different location and diosgenin was extracted in n-hexane by using Soxhlet extractor. Diosgenin was quantified by the HPLC. The concentration of diosgenin was varying 0.02 to 2.64% with the different locations. They suggested that *D. polygonoides* is the good source diosgenin. Yang *et al.,* (2013) extracted diosgenin from *D. Zingiberensis* by using the cellulose enzymolysis and two-phase acid hydrolysis and detected by the RP-HPLC-UV method at 202 nm they have reported that *D. Zingiberensis* root content was 4.46% diosgenin. Edwards *et al.,* (2002) compared diosgenin content in *D. batats* and *D. villosa* the extraction was carried out by homogenizing rhizomes in the methanol followed by overnight stirring in the chloroform: methanol (3:1) mixture after that the diosgenin was detected by HPLC-MS at 210 nm with ortho-phosphoric acid/acetonitrile solvent the observed pick of diosgenin at 28.1 min.

Dioscorea species are a good and natural source of diosgenin. It has multiple biomedical applications. We tested the extracted diosgenin of *D. oppositifolia* root on the 3T3-L1 preadipocytes for antiobesity activity. The extracted diosgenin shows the antiadipogenic activity as exhibited by the standard diosgenin.

4.5 *In vivo* studies:

The effect of *D. oppositifolia* extract on male Wistar rats was studied by creating obesity with a high fat diet. It was shown that consuming plant extracts helps to prevent obesity by lowering body increase in weight and lipid profile

4.5.1 Effect on body weight:

Animals fed a high-fat diet (HFD) have a significant increase in body weight; 22% more body weight than the control group. The animal group feed with the HFD and orlistat exhibited a 24% reduction in body weight as compared to the HFD control group. However, the group

fed with 200mg/kg BW of *D. oppositifolia* whole plant extract along with an HFD showed 23% a reduction in body weight as compared to the HFD group. In case of the animal fed with 400mg/kg BW of *D. oppositifolia* whole plant extracts, exhibited a 40% decrease compared to the HFD control group **(Figure No. 4.36 & 4.37).**

Table No 4.14: Effect of *D. oppositifolia* (DO) extracts on food intake in rats fed along with HFD. Data are expressed as the mean ± SD (n=6), ****p<0.05* vs. HFD control group

Groups	Initial Body weight in gm	Final Body weight in gm
Control	129.33±4.1	261±3.5
HFD	135±3.5	324±4.5
HFD + Orlistat	130.67±2.3	256±3.2
HFD + DO 200mg/kg BW	132.67±3.5	258±2.9
HFD + DO 400 mg/kg BW	131.33±2.9	201±2.6

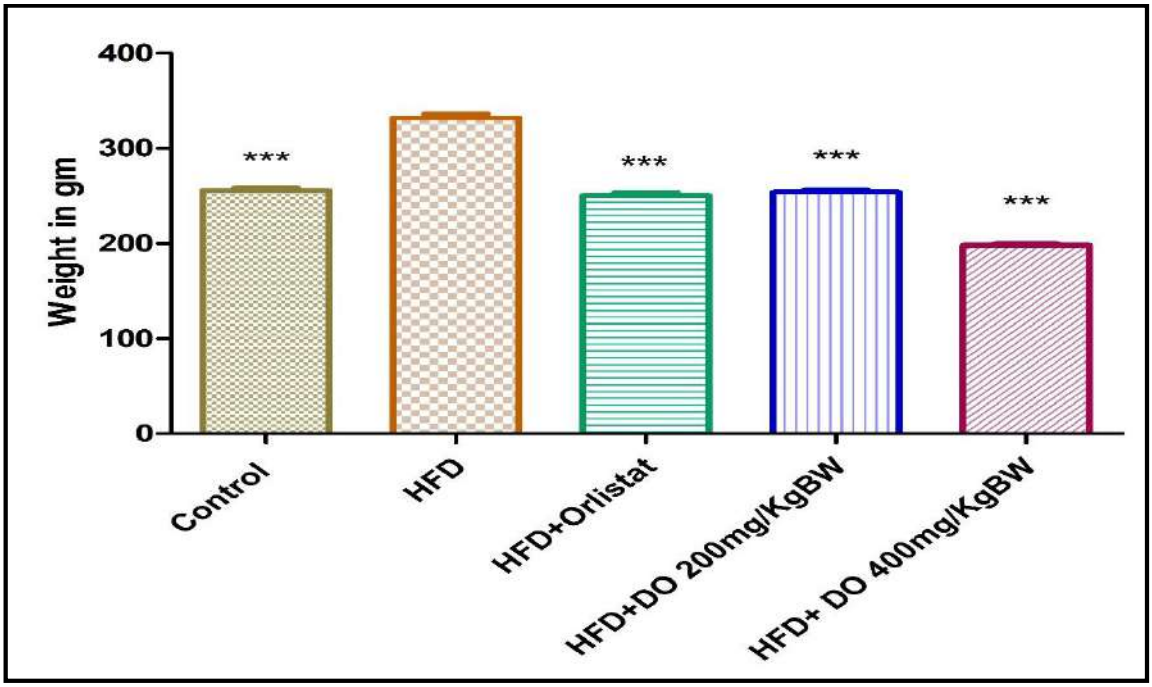


Figure No. 4.36: Effect of *D. oppositifolia* (DO) 200mg/kg/day and *D. oppositifolia* (DO) 400mg/kg/day extracts on food intake in rats fed along with HFD. Data are expressed as the mean ± SD (n=6), ****p<0.05* vs HFD control group

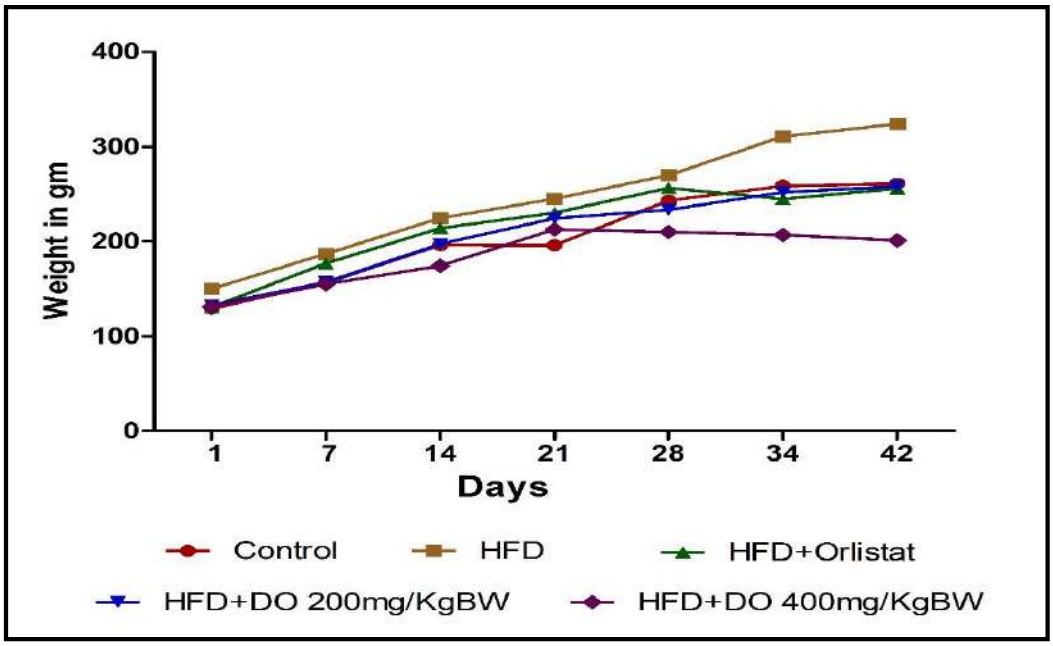


Figure No. 4.37: Effect of High Fat Diet, Orlistat 10mg/kg/day, *D. oppositifolia* (DO) 200mg/kg/day and *D. oppositifolia* (DO) 400mg/kg/day plant extract on weight gain in 6 weeks values are expressed as mean ± SD (n=6)

4.5.2 Effect of *D. oppositifolia* methanolic extract on lipid profile in rat fed with high fat diet:

Serum was separated from the collated blood. Serum lipid content was estimated by commercially available kits. In high fat diet consuming group, the TC, TG, LDL, VLDL levels were elevated than the control group and HDL level was reduced than the control group. The rat group feed with the HFD and 200mg/kg BW of *D. oppositifolia* plant extract the TC, TG, LDL, VLDL levels were reduced as compare to the HFD consuming rat group, but the HDL level was elevated. Whereas the rat group feed with HFD and 400mg/kg BW of *D. oppositifolia* plant extract also exhibit the reduction of TC, TG, LDL and VLDL levels. The orlistat also showing the same effect but plant extracts exhibiting the better results than the orlistat. While comparing in the effect of both the concentration of *D. oppositifolia* extracts, concentration dependent reduction of weight and also the lower the TC, TG, LDL and VLDL levels in the rats were observed. **(Figure No. 4.38A & 4.38B)**

Human studies have revealed that increasing energy intake has been linked with Obesity can be caused by an increase in body weight. This study indicates that the rats treated with

high fat diet had dramatically increased food intake. Whereas the treatment of plant extracts lowered food intake which hypothesises that these plants may have a function in modifying appetite management by reducing food intake.

Dyslipidemia is one of the pathophysiology conditions related with obesity. It results in lower HDL, higher total cholesterol, LDL, and VLDL levels, all of which contribute to the development of cardiovascular disease and atherosclerosis. In this study, *D. oppositifolia* (200 mg/kg b.wt) plant extract administration in conjunction with a high fat diet significantly reduced LDL, VLDL, and total cholesterol levels as compared to the HFD group. *D. oppositifolia* Plant extract supplementation at had superior results than the *D. pentaphylla* plant extracts group.

Jeong *et al.,* (2014) reported that the *D. oppositifolia* n-BuOH extract efficiently reduced fat accumulation in high-fat diet-induced obese mice. This butanolic extracts contains 3,5-dimethoxyphenanthrene-2,7-diol and (3R,5R)-3,5- dihydroxy-1,7-bis(4-hydroxyphenyl)-3,5-heptanediol as major components. This helps to reduce weight in mice body.

Kwon *et al.,* (2003) studied the effect of the methanolic extract of *Dioscorea nipponica* on SD rats. The SD rats feed with the extracts lower the TC, TG, LDL and VLDL and increase the HDL levels in the rats with weight loss. Also, they extracted the Dioscin and Diosgenin and studied the lipase inhibitory activity with IC_{50} 20µg/ml and 28 µg/ml respectively.

Ikete and Chinko (2022) reported that the rat feed with the high fat diet and hydromethanolic extracts of *D. bulbifera* reduces the TC, TG, LDL and VLDL and increase the HDL levels in the rats and lower the weight as compare to the control.

The effects of resistant starch (RS) produced from purple yam (*Dioscorea alata* L.) on lipid metabolism and gut microbiota in hyperlipidemic hamsters were studied. High dosage of RS administration in hamsters was shown to be more efficient in controlling body weight and adipose tissue mass, with increased HDL, concentration and decreased TG, TC, and LDL concentrations. Furthermore, HR changed the makeup of the gut community by increasing the presence and abundance of *Bifidobacteria, Lactobacillus, Coprococcus*, and *Allobaculum* while lowering the relative abundances of Parabacteroides and Dorea. Probiotics like *Bifidobacteria* and *Lactobacillus* were shown to be significantly higher and substantially linked with blood lipid levels. These findings showed that using RS derived from purple yam

might improve lipid metabolism in conjunction with gut microbiota modification, which could give guidelines for future treatment (Li *et al.*, 2019).

The *D. oppositifolia* whole plant extract decreas body weight in a diseased rat model. Treatment with *D. oppositifolia* lowers body weight, TC, TG, LDL, VLDL, and increases HDL levels, suggesting that it may be a viable approach to treating obesity and associated disorders.

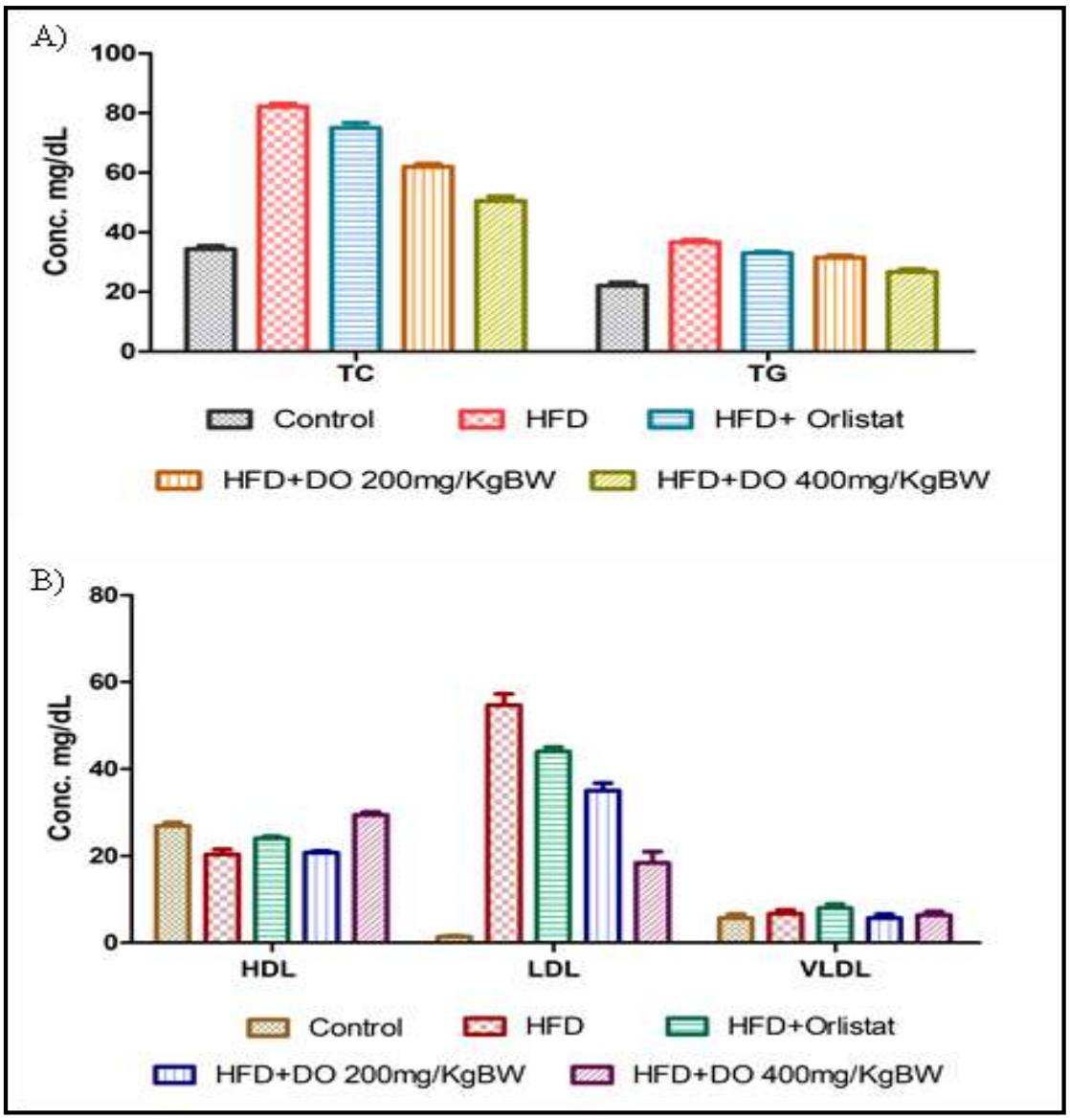


Figure No. 4.38: A and B Effect of Orlistat 10mg/kg/day, *D. oppositifolia* (DO) 200mg/kg/day and *D. oppositifolia* (DO) 400mg/kg/day plant extract on lipid profile in rats fed along with HFD. Data are expressed as the mean ± SD (n=6).

5. SUMMARY AND CONCLUSIONS

Obesity is the major problem now a days. Obesity is linked to many disease like cancer, diabetes, osteoporosis, and cardiovascular disease. Control of the obesity leads to overcome problem of the linked disease. The present study was planned to explore the anti-obesity efficacy of the *Dioscorea* species to avoid increase of obesity. The outcome of present study will possibly help develop a food/beverage with anti-obesity properties.

Collection of *Dioscorea* species:

The four *Dioscorea* species were collected from the Kolhapur district Maharashtra India. Namely *D. alata, D. bulbifera D. pentaphylla* and *D. oppositifolia.* Collected plant germplasm was maintained in botanical garden, and the herbariums of all species deposited to herbarium Department of Botany, Shivaji University Kolhapur. Plant material were dried and used for the preparation of the methanolic and aqueous extracts.

Chemo-profiling of *Dioscorea* extract:

The primary phytochemical analysis was carried out by different colorimetric methods. The total phenolic, total flavonoids, total alkaloids, total terpenoids and total saponin were studied. The methanolic extract of *D. oppositifolia* showing the highest content of the all the phytochemicals than the other extracts. The antioxidant activity studied by the free radicals scavenging by DPPH and ABTS assay *D. oppositifolia* methanolic extracts showing the highest % inhibition in both the assay. It also has the ferrous reducing antioxidant power.

Screening of the different *Dioscorea* extract for the anti-obesity potential:

The prepared extracts were screened by using the 2 different methods for the antiobesity activity i.e. lipase inhibition, effect of extracts on the lipid accumulation in the 3T3-L1 cells and regulation of the adipogenic genes.

In lipase inhibition study all the extracts of the *Dioscorea* shows the significant inhibition. The both methanolic and aqueous extracts of the *D. pentaphylla* shows the above 70% inhibition. *D. alata, D. bulbifera,* and *D. oppositifolia* exhibit the above 50% lipase inhibition activity.

In vitro anti-obesity analysis was performed on 3T3-L1 preadipocyte cell line, which serves as an *In vitro* model system to research anti-obesity because it develops from pre-adipocyte to mature adipocyte, which is specialized in lipid droplet formation. The toxicity of

the plant extracts was studied by using the MTT assay and from the IC 50 concentration the dose of treatment was decided for the further study. From the results obtained from the Oil O red staining both aqueous and methanolic extracts of the *D. oppositifolia* reduces by 1.8 and 2.2 times the lipid accumulation in the 3T3-L1 cells as compared with Control induced group.

The 3T3-L1 cells were treated with methanolic and aqueous extracts of *D. oppositifolia* and *D. pentaphylla* during the differentiation. *D. oppositifolia* methanolic extract suppresses expression of PPAR ϒ, C/EBPα, SREBP-1 and FASN concentration dependently. This results in the inhibition of adipogenesis in 3T3-L1 cells. It also increase the expression of the CPT-1 gene that helps to enhances lipolysis of accumulated lipids. Aqueous extract of *D. oppositifolia* down regulate the expression PPARϒ, C/EBPα and SREBP1. At lower concentration of extracts the fatty acid synthase down regulate and at the higher concentration it unregulated the FANS gene. It up regulate the CPT-1 gene that helps in the lipolysis of the accumulated lipids. In case of the *D. pentaphylla* methanolic and aqueous extracts up regulate the PPAR ϒ, C/EBPα, SREBP1, FASN, gene and down regulate the CPT-1 gene. So the *D. oppositifolia* methanolic extract used for the further study.

The LCMS analysis of the *D. pentaphylla* and *D. oppositifolia* methanolic extracts were carried from the obtained data. Group of compound was identified from the human metabolomics database and Pub chem database. The compound having an anti-obesity property previously studied were identified 11 compound shows the anti-obesity activity.

This significant down-regulation of PPARγ gene may be due to the cumulative action of phytochemicals from the extracts. This reduction of adipogenic transcription factors may aid in regulating adipocyte differentiation and lowering adipocyte lipid accumulation.

Screening of anti-obesity metabolites from *D. oppositifolia*:

From the above study the *D. oppositifolia* methanolic extract used for the liquid-liquid extraction by using the hexane, chloroform, ethyl acetate and n-butanol. Fraction were collected and solvents were evaporated reaming reside after evaporation was dissolved in to the DMSO, Also the diosgenin was extracted from the root powder of *D. oppositifolia* by using the acid hydrolysis method.

The effect of all fractions and extracted diosgenin were studied during the differentiation of 3T3-L1 cells. In lipid accumulation study there was no significant changes were seen in cells after treatment with all fractions and extracted diosgenin. For that, the impact of all the fractions explored by gene expression study.

In comparing the effects of each fraction, the chloroform fraction prevents the expression of all examined adipogenic genes more than the other four fractions while also stimulating the activity of the CPT-1 gene. The PPARϒ, C/EBPα, SREBP1, and FASN genes are up regulated by hexane fractions. In contrast, down regulates the CPT 1 gene. Treatment with the Ethyl acetate fraction raises PPARϒ, C/EBPα, and FASN levels. SREBP 1 gene expression was lower than in the control group. The CPT-1 is down regulated at greater concentrations of ethyl acetate and up regulated at lower concentrations. At the studied concentrations, cells treated with n-butanol and water fraction up regulate all adipogenic genes. At greater concentrations of the n-butanol fraction, C/EBPα was down regulated.

LCMS-QTOF analysis was used to profile the metabolites in the chloroform fraction. Robinetin, Aloesin, Dioscin, and Oleoyl Ethanol Amide were identified in the chloroform fraction of *D. oppositifolia.* The inclusion of these substances in the fraction demonstrating antiadipogenic gene regulate.

Dioscorea species are a good and natural source of diosgenin. It has multiple biomedical applications. We tested the extracted diosgenin of *D. oppositifolia* root on the 3T3-L1 preadipocytes for antiobesity activity. The effect of the extracted diosgenin was compared with the effect of standard diosgenin on the adipogenesis were studied. The PPARϒ, and SREBP 1 was down regulated by the extracted diosgenin at tested concentration the expression levels of the C/EBPα, and FASN was slightly up regulated at lower concentration of extracted diosgenin but at higher concentration down regulation were observed. Whereas the CPT -1 gene was up regulated at both the concentration. The extracted diosgenin shows the antiadipogenic activity as exhibited by the standard diosgenin.

The extracted diosgenin was confirmed by using the HPLC with the standard diosgenin. In both sample and standard diosgenin shows the pick at same retention time. Hence it confirmed that the sample contains the diosgenin.

***In vivo* studies:**

The *D. oppositifolia* whole plant extract decreases body weight in a diseased rat model. Treatment with *D. oppositifolia* lowers body weight, TC, TG, LDL, VLDL, and increases HDL levels as compare to the High fat induced rat group, suggesting that it may be a viable approach to treating obesity and associated disorders.

The key conclusion from the above study is that all the *Dioscorea* species used in the study have lipase inhibition properties. *D. oppositifolia* whole plant extracts have a good source of phytochemicals and antioxidants than other three species. Considering the results obtained from the gene expression study both methanolic and aqueous whole plant extracts of *D. oppositifolia* have an ability to affect adipogenesis at the gene level. Treatment with *D. oppositifolia* whole plant extract to the high-fat diet-induced rats exhibited a lowering of the TC, TG, LDL, VLDL, and body weight as compared to the control group. The chloroform fraction and extracted Diosgenin from the *D. oppositifolia* root shows the virtuous down regulation of the adipogenic genes in 3T3-L1 cells. In LCMS profiling of the chloroform fraction of *D. oppositifolia*, robinetin, aloesin, diocin, and oleoyl ethanol amide were found which has anti-obesity activity, and down regulate the adipogenic genes. So, *D. oppositifolia* whole plant can be a potential candidate for the treatment of obesity and related problems.

BIBLIOGRAPHY

Abdali, D., Samson, S. E., & Grover, A. K. (2015). How effective are antioxidant supplements in obesity and diabetes?. *Medical principles and practice: international journal of the Kuwait University, Health Science Centre*, *24* (3), 201–215.

Abdul, R. H., Saari N., Abas F., Ismail A., Mumtaz M. W., & Abdul H. A. (2016). Anti-obesity and antioxidant activities of selected medicinal plants and phytochemical profiling of bioactive compounds. *International Journal of Food Properties,* 20 (11), 2616–2629.

Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of pharmacy & bioallied sciences*, 12(1), 1–10.

Adeosun, O.M, Arotupin D.J, Toba O.A, & Adebayo A.A. (2016). Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (Air Potatoe) tubers and peels against some pathogenic bacteria. *The Journal of Phytopharmacology* 2016;5(1):15-26.

Ado, M. A., Abas, F., Mohammed, A. S., & Ghazali, H. M. (2013). Anti- and pro-lipase activity of selected medicinal, herbal and aquatic plants, and structure elucidation of an anti-lipase compound. *Molecules (Basel, Switzerland)*, 18(12), 14651–14669.

Aguilar, S. L., Romero, C. O., Gonzalez C. M., & Tortoriello,J., (2012). Effect of *Bursera grandiflora* on body weight and lipemia in obese mice. *Boletín latinoamericano y del Caribe de plantas medicinales y aromáticas ;* 11(2): 138-146.

Ahmad, Q. I., Ahmad, C. B., & Ahmad, S. M. (2010). Childhood obesity. *Indian journal of endocrinology and metabolism*, 14(1), 19–25.

Ahmadian, M., Suh, J. M., Hah, N., Liddle, C., Atkins, A. R., Downes, M., & Evans, R. M. (2013). PPARγ signalling and metabolism: the good, the bad and the future. *Nature medicine*, 19(5), 557–566.

Ahn, E. K., Lee, J. A., Seo, D. W., Hong, S. S., & Oh, J. S. (2012). 1β-Hydroxy-2-oxopomolic acid isolated from *Agrimonia pilosa* extract inhibits adipogenesis in 3T3-L1 cells. *Biological & pharmaceutical bulletin*, *35*(5), 643–649.

Ahn, J. H., Kim, E. S., Lee, C., Kim, S., Cho, S. H., Hwang, B. Y., & Lee, M. K. (2013). Chemical constituents from *Nelumbo nucifera* leaves and their anti-obesity effects. *Bioorganic & medicinal chemistry letters*, *23*(12), 3604–3608.

Ahn, J., Lee, H., Jung, C. H., Choi, W. H., & Ha, T. Y. (2017). Zerumbone ameliorates high-fat diet-induced adiposity by restoring AMPK-regulated lipogenesis and microRNA-146b/SIRT1-mediated adipogenesis. *Oncotarget*, 8(23), 36984–36995.

Alexander, J. and Coursey, D. G. 1969. The domestication of the yams.In: Ucko, P. J. and Dimbleby, G. W. edit. The domestication and exploitation of plants and animals, pp. 405–425. Gerald Duckworth, London.

Ali-Shtayeh, M. S., Jamous, R. M., Abu-Zaitoun, S. Y., Khasati, A. I., & Kalbouneh, S. R. (2019). Biological Properties and Bioactive Components of *Mentha spicata* L. Essential Oil: Focus on Potential Benefits in the Treatment of Obesity, Alzheimer's disease, Dermatophytosis, and Drug-Resistant Infections. *Evidence-based complementary and alternative medicine: eCAM, 2019*, 3834265.

An, S., Han, J. I., Kim, M. J., Park, J. S., Han, J. M., Baek, N. I., Chung, H. G., Choi, M. S., Lee, K. T., & Jeong, T. S. (2010). Ethanolic extracts of *Brassica campestris* spp. rapa roots prevent high-fat diet-induced obesity via beta (3)-adrenergic regulation of white adipocyte lipolytic activity. *Journal of medicinal food*, 13(2), 406–414.

Arçari, D. P., Bartchewsky, W., dos Santos, T. W., Oliveira, K. A., Funck, A., Pedrazzoli, J., de Souza, M. F., Saad, M. J., Bastos, D. H., Gambero, A., Carvalho, P., & Ribeiro, M. L. (2009). Antiobesity effects of yerba maté extract *(Ilex paraguariensis*) in high-fat diet-induced obese mice. *Obesity (Silver Spring, Md.)*, *17*(12), 2127–2133.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants (Basel, Switzerland)*, *8*(4), 96.

Asdaq S. M. B., and C. Malsawmtluangi. (2015). "Evaluation of Anti-Obesity Potential of Cashew Nut (*Anacardium Occidentale*) In Animals" *Indian Journal of Pharmaceutical Science & Research* 5, no. 1: 42-50

Avadhani, M. N. M., Selvaraj, C. I., Rajasekharan, P. E. & Tharachand, C. (2013) The Sweetness and Bitterness of Sweet Flag [*Acorus calamus* L.] – A Review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4: 598-610.

Bansal, V.K., Malaviya, T., & Sharma, P.K. (2014). Phytochemical, Pharmacological Profile and Commercial Utility of Tropically Distributed Plant Bauhinia *Variegata.variegata*. *Global Journal of Pharmacology* 8: 196-205.

Batubara, I., Kuspradini, H., Muddathir, A. M., & Mitsunaga, T. (2014). *Intsia palembanica* wood extracts and its isolated compounds as *Propionibacterium acnes* lipase inhibitor. *Journal of Wood Science*, 60(2), 169–174.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70–76.

Berndt, J., Kovacs, P., Ruschke, K., Klöting, N., Fasshauer, M., Schön, M. R., Körner, A., Stumvoll, M., & Blüher, M. (2007). Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Diabetologia*, 50(7), 1472–1480.

Bhandari, M. R., & Kawabata, J. (2004). Organic acid, phenolic content and antioxidant activity of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chemistry*, 88(2), 163–168.

Bhattacharjee R, M Gedil, A Sartie, E Otoo, D Dumet, H Kikuno, P Lava Kumar, and R Asiedu (2011) *Dioscorea.* Wild Crop Relatives: Genomic and Breeding Resources, Industrial Crops (Ed.) Kole C. pp. 71–96.

Blois, M. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* 181, 1199–1200.

Borzelleca JF and Lane RW (2008). The Art, the Science, and the Seduction of Toxicology: an Evolutionary Development. In: Principles and methods of toxicology (5th ed.) (Eds.) Hayes AW. *Taylor and Francis. Philadelphia* pp. 13.

Bounihi, A., Bitam, A., Bouazza, A., Yargui, L., & Koceir, E. A. (2017). Fruit vinegars attenuate cardiac injury via anti-inflammatory and anti-adiposity actions in high-fat diet-induced obese rats. *Pharmaceutical biology*, *55*(1), 43–52.

Bradford P.G., (2013). Curcumin and obesity. *Biofactors* 39: 78-87.

Brown R., (1810). Prodromus Florae Novae. *Hollandiae* pp. 294–295.

Brown, J., (1999). Lipotrim diet in general practice. *The British Journal of General Practice*, *49*(439), 147.

Calderon-Dominguez, M., Sebastián, D., Fucho, R., Weber, M., Mir, J. F., García-Casarrubios, E., Obregón, M. J., Zorzano, A., Valverde, Á. M., Serra, D., & Herrero, L. (2016). Carnitine Palmitoyltransferase 1 Increases Lipolysis, UCP1 Protein Expression and Mitochondrial Activity in Brown Adipocytes. *PloS one*, 11(7), e0159399.

Chang, C. L., Lin, C. S., & Lai, G. H. (2012). Phytochemical characteristics, free radical scavenging activities, and neuroprotection of five medicinal plant extracts. *Evidence-based complementary and alternative medicine:* eCAM, 2012, 984295.

Chaudhary, S. A., Chaudhary, P. S., Syed, B. A., Misra, R., Bagali, P. G., Vitalini, S., & Iriti, M. (2018). Validation of a method for diosgenin extraction from fenugreek (*Trigonella foenum-graecum* L.). *Acta Scientiarum Polonorum Technologia Alimentaria*, 17(4), 377-385.

Choi, J. S., Kim, J. H., Ali, M. Y., Min, B. S., Kim, G. D., & Jung, H. A. (2014). *Coptis chinensis* alkaloids exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBP-α and PPAR-γ. *Fitoterapia*, *98*, 199–208.

Cornago D. F., Rowena Grace O. R., and Inacrist M. G. (2011) Philippine Yam (*Dioscorea* spp.) Tubers Phenolic Content and Antioxidant Capacity Philippine. *Journal of Science* 140 (2): 145-152.

Coursey DG (1967) Yams; An account of the Nature, Origins, Cultivation and Utilisation of the Useful Member of the *Dioscoreaceae*. Longmans, London.

Devlin, M. J., Yanovski, S. Z., & Wilson, G. T. (2000). Obesity: what mental health professionals need to know. *The American journal of psychiatry*, *157*(6), 854–866.

Du H., (2010). Antiobesity and hypolipidemic effects of lotus leaf hot water extract with taurine supplementation in rats fed a high fat diet. *Journal of Biomedical Science* 17: S42.

Dudhia, Z., Louw, J., Muller, C., Joubert, E., de Beer, D., Kinnear, C., & Pheiffer, C. (2013). *Cyclopia maculata* and *Cyclopia subternata (*honeybush tea) inhibits adipogenesis in 3T3-L1 pre-adipocytes. *Phytomedicine: international journal of phytotherapy and phytopharmacology*, *20*(5), 401–408.

Dzomba P, and Musekiwa C. (2014). Anti-obesity and antioxidant activity of dietary flavonoids from *Dioscorea steriscus* tubers., *Journal of Coastal Life Medicine*, Volume 2, Number 6, 2014, pp. 465-470(6)

Edison S., M.Unnikrishnan, B.Vimala, Santha V.Pillai, M.N.Sheela, M.T. Sreekumari and K.Abraham. 2006. Biodiversity of Tropical Tuber Crops in India. NBA Scientific Bulletin Number - 7, National Biodiversity Authority, Chennai, TamilNadu, India, p60.

Edwards, A. L., Jenkins, R. L., Davenport, L. J., & Duke, J. A. (2002). Presence of diosgenin in *Dioscorea batatas* (Dioscoreaceae). *Economic Botany*, 56(2), 204–206.

Ekeleme-Egedigwe, C. A., Ijeh, I. I., & Okafor, P. N. (2017). Modulatory effects of dietary supplementation by *Vernonia amygdalina* on high-fat-diet-induced obesity in Wistar rats. *Acta scientiarum polonorum. Technologia alimentaria*, *16*(4), 431–442.

El Ayed, M., Kadri, S., Smine, S., Elkahoui, S., Limam, F., & Aouani, E. (2017). Protective effects of grape seed and skin extract against high-fat-diet-induced lipotoxicity in rat lung. *Lipids in health and disease*, *16*(1), 174.

Ezeabara, C. A., and Anona, R. O. (2018) Comparative Analyses of Phytochemical and Nutritional Compositions of Four Species of *Dioscorea*. *Acta Scientific Nutritional Health*. Volume 2 Issue 7.

Ezure, T., & Amano, S. (2011). *Rubus suavissimus* S. Lee extract increases early adipogenesis in 3T3-L1 preadipocytes. *Journal of natural medicines*, *65*(2), 247–253.

Farkas, G. J., Gorgey, A. S., Dolbow, D. R., Berg, A. S., & Gater, D. R. (2018). The influence of level of spinal cord injury on adipose tissue and its relationship to inflammatory adipokines and cardiometabolic profiles. *The journal of spinal cord medicine*, *41*(4), 407–415.

Farombi, E. O., Britton, G., & Emerole, G. O. (2000). Evaluation of the antioxidant activity and partial characterisation of extracts from browned yam flour diet. *Food Research International*, *33*(6), 493-499.

Ferré P. (2004). The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes*, 53 Suppl 1, S43–S50.

Forbes A, D Henley and D Henley (2013) 'Pedanius Dioscorides' *In*: Health and Well Being: A Medieval Guide. Chiang Mai, *Cognoscenti Books*, Thailand.

Forman L. and Bridson D. (1989) *The herbarium Handbook*, Royal Botanical Gardens,

Freitas J., Luciano M., and Eduardo B. de Almeida Jr. (2017) "Medicinal plants for the treatment of obesity: ethnopharmacological approach and chemical and biological studies. *American Journal of Translational Research* 9, no. 5 (2017): 2050 -2064.

Freytag, S. O., Paielli, D. L., & Gilbert, J. D. (1994). Ectopic expression of the CCAAT/enhancer-binding protein alpha promotes the adipogenic program in a variety of mouse fibroblastic cells. *Genes & development*, *8*(14), 1654–1663.

Frühbeck, G., Becerril, S., Sáinz, N., Garrastachu, P., & García-Velloso, M. J. (2009). BAT: a new target for human obesity?. *Trends in pharmacological sciences*, *30*(8), 387–396.

Fu, J., Oveisi, F., Gaetani, S., Lin, E., & Piomelli, D. (2005). Oleoylethanolamide, an endogenous PPAR-alpha agonist, lowers body weight and hyperlipidemia in obese rats. *Neuropharmacology*, *48*(8), 1147–1153.

Gadde, K. M., Martin, C. K., Berthoud, H. R., & Heymsfield, S. B. (2018). Obesity: Pathophysiology and Management. *Journal of the American College of Cardiology*, *71*(1), 69–84.

Gao, Q. H., Wu, C. S., & Wang, M. (2013). The jujube (*Ziziphus jujuba* Mill.) fruit: a review of current knowledge of fruit composition and health benefits. *Journal of agricultural and food chemistry*, *61*(14), 3351–3363.

Gesta, S., Tseng, Y. H., & Kahn, C. R. (2007). Developmental origin of fat: tracking obesity to its source. *Cell*, 131(2), 242–256.

Ghosh, S., Derle, A., Ahire, M., More, P., Jagtap, S., Phadatare, S. D., Patil, A. B., Jabgunde, A. M., Sharma, G. K., Shinde, V. S., Pardesi, K., Dhavale, D. D., & Chopade, B. A. (2013). Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. *PloS one*, 8(12), e82529.

Gosmann, G., Barlette, A. G., Dhamer, T., Arçari, D. P., Santos, J. C., de Camargo, E. R., Acedo, S., Gambero, A., Gnoatto, S. C., & Ribeiro, M. L. (2012). Phenolic compounds from maté (*Ilex paraguariensis*) inhibit adipogenesis in 3T3-L1 preadipocytes. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *67*(2), 156–161.

Grasa-López, A., Miliar-García, Á., Quevedo-Corona, L., Paniagua-Castro, N., Escalona-Cardoso, G., Reyes-Maldonado, E., & Jaramillo-Flores, M. E. (2016). *Undaria pinnatifida* and Fucoxanthin Ameliorate Lipogenesis and ers of Both Inflammation and Cardiovascular Dysfunction in an Animal Model of Diet-Induced Obesity. *Marine drugs*, 14(8), 148.

Gupta, P., Goyal, R., Chauhan, Y., & Sharma, P. L. (2013). Possible modulation of FAS and PTP-1B signaling in ameliorative potential of *Bombax ceiba* against high fat diet induced obesity. *BMC complementary and alternative medicine*, *13*, 281.

Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta medica*, *29*(2), 116–122.

Ho, J. N., Son, M. E., Lim, W. C., Lim, S. T., & Cho, H. Y. (2012). Anti-obesity effects of germinated brown rice extract through down-regulation of lipogenic genes in high fat diet-induced obese mice. *Bioscience, biotechnology, and biochemistry*, *76*(6), 1068–1074.

Ho, J.-N., Park, S.-J., Choue, R., & Lee, J. (2013). Standardized Ethanol Extract of *Curcuma longa* L. Fermented by *Aspergillus oryzae* Promotes Lipolysis via Activation of cAMP-Dependent PKA in 3T3-L1 Adipocytes. *Journal of Food Biochemistry,* 37: 595-603.

Hornel J (1934) Indonesian influence on East Afrixcan culture. J R Anthrop. Inst. 64: 3058–3332.

Hsu, C. (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. *Food Chemistry, 83(1), 85–92.*

Hsu, H.-M., Chen, W.-Y., Pan, P.-H., & Mao, F. C. (2014). *Vitis thunbergii* supplementation demonstrates an anti-obesity effect in developing obese mice. *European Journal of Integrative Medicine, 6(5), 581–587.*

Hua, S., Li, Y., Su, L., & Liu, X. (2016). Diosgenin ameliorates gestational diabetes through inhibition of sterol regulatory element-binding protein-1. *Biomedicine & Pharmacotherapy,* 84, 1460–1465.

Ignjatovic, V., Ogru, E., Heffernan, M., Libinaki, R., Lim, Y., & Ng, F. (2000). Studies on the use of *"slimax",* a chinese herbal mixture, in the treatment of human obesity. *Pharmaceutical Biology, 38(1), 30–35.*

Ikete, P., & Chinko, B. (2022). Inhibition of Serum Lipase as a Mechanism of Action for Anti-Obesity Potentials of *Dioscorea bulbifera* Extracts on Wistar Rats. *Asian Journal of Health Sciences*, *8*(1), ID29.

Ilavenil, S., Kim, d., Vijayakumar, M., Srigopalram, S., Roh, S. G., Arasu, M. V., Lee, J. S., & Choi, K. C. (2016). Potential role of marine algae extract on 3T3-L1 cell proliferation and differentiation: an in vitro approach. *Biological research*, *49*(1), 38.

Jeon, J. R., Lee, J. S., Lee, C. H., Kim, J. Y., Kim, S. D., & Nam, D. H. (2006). Effect of ethanol extract of dried Chinese yam (*Dioscorea batatas*) flour containing dioscin on gastrointestinal function in rat model. *Archives of pharmacal research*, *29*(5), 348–353.

Jeong, E. J., Jegal, J., Ahn, J., Kim, J., & Yang, M. H. (2016). Anti-obesity Effect of *Dioscorea oppositifolia* Extract in High-Fat Diet-Induced Obese Mice and Its Chemical Characterization. *Biological & pharmaceutical bulletin*, *39*(3), 409–414.

Jeong, Y. S., Hong, J. H., Cho, K. H., & Jung, H. K. (2012). Grape skin extract reduces adipogenesis- and lipogenesis-related gene expression in 3T3-L1 adipocytes through the peroxisome proliferator-activated receptor-γ signaling pathway. *Nutrition research (New York, N.Y.)*, *32*(7), 514–521.

Jeong, Y. S., Jung, H. K., Cho, K.-H., Youn, K.-S., & Hong, J.-H. (2011). Anti-obesity effect of grape skin extract in 3T3-L1 adipocytes. *Food Science and Biotechnology, 20(3), 635–642.*

Ju, J. H., Yoon, H. S., Park, H. J., Kim, M. Y., Shin, H. K., Park, K. Y., Yang, J. O., Sohn, M. S., & Do, M. S. (2011). Anti-obesity and antioxidative effects of purple sweet potato extract in 3T3-L1 adipocytes in vitro. *Journal of medicinal food*, *14*(10), 1097–1106.

Juge-Aubry, C. E., Henrichot, E., & Meier, C. A. (2005). Adipose tissue: a regulator of inflammation. *Best practice & research. Clinical endocrinology & metabolism*, *19*(4), 547–566.

Kajimura, S., & Saito, M. (2014). A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. *Annual review of physiology*, *76*, 225–249.

Kang, H. J., Seo, H. A., Go, Y., Oh, C. J., Jeoung, N. H., Park, K. G., & Lee, I. K. (2013). Dimethylfumarate suppresses adipogenic differentiation in 3T3-L1 preadipocytes through inhibition of STAT3 activity. *PloS one*, *8*(4), e61411.

Kang, H., & Koppula, S. (2014). *Houttuynia cordata* Attenuates Lipid Accumulation via Activation of AMP-Activated Protein Kinase Signaling Pathway in HepG2 Cells. *The American Journal of Chinese Medicine, 42(03), 651–664.*

Kang, J. S., Lee, W. K., Lee, C. W., Yoon, W. K., Kim, N., Park, S. K., Lee, H. S., Park, H. K., Han, S. B., Yun, J., Lee, K., Lee, K. H., Park, S. K., & Kim, H. M. (2011). Improvement of high-fat diet-induced obesity by a mixture of red grape extract, soy isoflavone and L-carnitine: implications in cardiovascular and non-alcoholic fatty liver diseases. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *49*(9), 2453–2458.

Kang, Y. H., Lee, Y.-S., Kim, K. K., Kim, D. J., Kim, T. W., & Choe, M. (2013). Study on antioxidative, antidiabetic and antiobesity activity of solvent fractions of *Smilax china L*. leaf extract. *Journal of Nutrition and Health, 46(5), 401.*

Karak, P. (2019). Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research*, *10*(4), 1567-1574.

Karmase, A., Birari, R., & Bhutani, K. K. (2013). Evaluation of anti-obesity effect of *Aegle marmelos* leaves. *Phytomedicine, 20(10), 805–812.*

Karmase, A., Jagtap, S., & Bhutani, K. K. (2013). Anti adipogenic activity of *Aegle marmelos* Correa. *Phytomedicine, 20(14), 1267–1271.*

Kaur, C., & Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*, *37*(2), 153-161.

Kaushal, S. K., Shailesh, K., Brijendra, S., Saurabh, B., Nisha, P., Ishan, P. K., & Prakash, B. S. (2019). Targeting fatty acid synthase protein by molecular docking studies of naturally occurring ganoderic acid analogues acting as anti-obesity molecule. *Research Journal of Biotechnology Vol*, *14*, 7.

Kazemipoor, M., Radzi, C. W., Hajifaraji, M., Haerian, B. S., Mosaddegh, M. H., & Cordell, G. A. (2013). Antiobesity effect of caraway extract on overweight and obese women: a randomized, triple-blind, placebo-controlled clinical trial. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 928582.

Khalid, M., & Siddiqui, H. H. (2012). Evaluation of weight reduction and anti–cholesterol activity of Punarnava root extract against high fat diets induced obesity in experimental rodent. *Asian Pacific Journal of Tropical Biomedicine, 2(3), S1323–S1328.*

Kim, G. S., Park, H. J., Woo, J. H., Kim, M. K., Koh, P. O., Min, W., Ko, Y. G., Kim, C. H., Won, C. K., & Cho, J. H. (2012). *Citrus aurantium* flavonoids inhibit adipogenesis through the Akt signaling pathway in 3T3-L1 cells. *BMC complementary and alternative medicine*, *12*, 31.

Kim, H. R., Kim, J. M., Kim, M. S., Hwang, J. K., Yang, S. H., Kim, H. J., Lee, D. S., Oh, H., Kim, Y. C., Ryu, D. G., Lee, Y. R., & Kwon, K. B. (2014). Inhibitory effects of *Pericarpium zanthoxyli* extract on adipocyte differentiation. *International journal of molecular medicine*, *33*(5), 1140–1146.

Kim, H., Bartley, G. E., Arvik, T., Lipson, R., Nah, S.-Y., Seo, K., & Yokoyama, W. (2014). Dietary Supplementation of Chardonnay Grape Seed Flour Reduces Plasma Cholesterol Concentration, Hepatic Steatosis, and Abdominal Fat Content in High-Fat Diet-Induced Obese Hamsters. *Journal of Agricultural and Food Chemistry*, 62(8), 1919–1925.

Kim, H.J., Hwang, J.T., Kim, M.J., Yang, H.J., Sung, M.J., Kim, S.H., Park, S., Gu, E.J., Park, Y. and Kwon, D.Y., (2014). The inhibitory effect of saponin derived from Cheonggukjang on adipocyte differentiation In vitro. *Food Science and Biotechnology*, 23(4), 1273–1278.

Kim, O. Y., Lee, S. M., Do, H., Moon, J., Lee, K. H., Cha, Y. J., & Shin, M. J. (2012). Influence of quercetin-rich onion peel extracts on adipokine expression in the visceral adipose tissue of rats. *Phytotherapy Research*, 26(3), 432-437.

Kim, Y. M., Lee, E. W., Eom, S. H., & Kim, T. H. (2014). Pancreatic lipase inhibitory stilbenoids from the roots of *Vitis vinifera. International journal of food sciences and nutrition*, *65*(1), 97–100.

Kim, Y. S., Lee, Y. M., Kim, H., Kim, J., Jang, D. S., Kim, J. H., & Kim, J. S. (2010). Anti-obesity effect of *Morus bombycis* root extract: anti-lipase activity and lipolytic effect. *Journal of ethnopharmacology*, *130*(3), 621–624.

Kong, C.-S., & Seo, Y. (2012). Antiadipogenic activity of isohamnetin 3-O-β-D-glucopyranoside from *Salicornia herbacea*. *Immunopharmacology and Immunotoxicology, 34(6), 907–911.*

Kong, H-S., Lee, S-W., Shin, S-M., Kwon, J-H., Jo, T-H.,Shin, E-J., Shim, K-S., Park, Y-I., Lee, C-K., & Kim, K-J., (2010). Down-regulation of adipogenesis and hyperglycemia in diet-induced obesity mouse model by Aloe QDM. *Biomolecules & Therapeutics*, *18*(3), 336-342.

Kubota, H., Morii, R., Kojima-Yuasa, A., Huang, X., Yano, Y., & Matsui-Yuasa, I. (2009). Effect of *Zizyphus jujuba* extract on the inhibition of adipogenesis in 3T3-L1 preadipocytes. *The American journal of Chinese medicine*, *37*(3), 597–608.

Kumar, S., & Alagawadi, K. R. (2013). Anti-obesity effects of galangin, a pancreatic lipase inhibitor in cafeteria diet fed female rats. *Pharmaceutical biology*, *51*(5), 607–613.

Kumar, S., Alagawadi, K. R., & Rao, M. R. (2011). Effect of *Argyreia speciosa* root extract on cafeteria diet-induced obesity in rats. *Indian journal of pharmacology*, *43*(2), 163–167.

Kwon, C. S., Sohn, H. Y., Kim, S. H., Kim, J. H., Son, K. H., Lee, J. S., Lim, J. K., & Kim, J. S. (2003). Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. *Bioscience, biotechnology, and biochemistry*, *67*(7), 1451–1456.

Laliotis, G. P., Bizelis, I., & Rogdakis, E. (2010). Comparative Approach of the de novo Fatty Acid Synthesis (Lipogenesis) between Ruminant and Non Ruminant Mammalian Species: From Biochemical Level to the Main Regulatory Lipogenic Genes. *Current genomics*, *11*(3), 168–183.

Lee, J. J., Park, M. R., Kim, A. R., & Lee, M. Y. (2011). Effects of ramie leaves on improvement of lipid metabolism and antiobesity effect in rats fed a high fat/high cholesterol diet. *Korean Journal of Food Science and Technology*, *43*(1), 83-90.

Lee, J., Kim, D., Choi, J., Choi, H., Ryu, J. H., Jeong, J., Park, E. J., Kim, S. H., & Kim, S. (2012). Dehydrodiconiferyl alcohol isolated from *Cucurbita moschata* shows anti-adipogenic and anti-lipogenic effects in 3T3-L1 cells and primary mouse embryonic fibroblasts. *The Journal of biological chemistry*, *287*(12), 8839–8851.

Lee, M., Song, J. Y., Chin, Y.-W., & Sung, S. H. (2013). Anti-adipogenic diarylheptanoids from *Alnus hirsuta F*. sibirica on 3T3-L1 cells. *Bioorganic & Medicinal Chemistry Letters, 23(7), 2069–2073.*

Lei, F., Zhang, X. N., Wang, W., Xing, D. M., Xie, W. D., Su, H., & Du, L. J. (2007). Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. *International journal of obesity (2005)*, *31*(6), 1023–1029.

Li, H., Yu, L., & Zhao, C. (2019). Dioscin attenuates high-fat diet-induced insulin resistance of adipose tissue through the IRS-1/PI3K/Akt signaling pathway. *Molecular medicine reports*, *19*(2), 1230–1237.

Li, T., Teng, H., , An, F., , Huang, Q., , Chen, L., , & Song, H., (2019). The beneficial effects of purple yam (*Dioscorea alata* L.) resistant starch on hyperlipidemia in high-fat-fed hamsters. *Food & function*, *10*(5), 2642–2650.

Liang, C. H., Chan, L. P., Chou, T. H., Chiang, F. Y., Yen, C. M., Chen, P. J., Ding, H. Y., & Lin, R. J. (2013). Brazilein from *Caesalpinia sappan* L. Antioxidant Inhibits Adipocyte Differentiation and Induces Apoptosis through Caspase-3 Activity and Anthelmintic Activities against *Hymenolepis nana* and *Anisakis simplex*. *Evidence-based complementary and alternative medicine : eCAM*, *2013*, 864892.

Lieberman, S. (2004). A New Potential Weapon for Fighting Obesity: Forskolin—the Active Diterpene in Coleus. *Alternative and Complementary Therapies, 10(6), 330–333.*

Lindley, J. (1853). The vegetable kingdom, or, The structure, classification, and uses of plants (3rd ed., with corrections and additional genera.). Bradbury and Evans.

Linnaeus C (1737) Flora Zeylanica. Laurentius Salvius, Stockholm 170–172.

Liu, R.H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *The American journal of clinical nutrition, 78 3 Suppl*, 517S-520S .

Liu, Y., Li, H., Fan, Y., Man, S., Liu, Z., Gao, W., & Wang, T. (2016). Antioxidant and Antitumor Activities of the Extracts from Chinese Yam (*Dioscorea opposite* Thunb.) Flesh and Peel and the Effective Compounds. *Journal of food science*, *81*(6), H1553–H1564.

Louise, E.O., Marju, O.M., Lena, W.O., Kajsa, S.H., Lars, S., Leif, G., Bjorn, C., Lena, M.S. C., Bob, O., (2008). CCAAT/enhancer binding protein (C/EBP) in adipose tissue regulates genes in lipid and glucose metabolism and a genetic variation in C/EBP is associated with serum levels of triglycerides. *The Journal of Clinical Endocrinology & Metabolism*. 93 (12), 4880–4886.

Lowe, M. E. (1997). Structure and Function of Pancreatic Lipase and Colipase. *Annual Review of Nutrition, 17(1), 141–158.*

Luximon-Ramma, A., Bahorun, T., Soobrattee, M. A., & Aruoma, O. I. (2002). Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of Cassia fistula. *Journal of agricultural and food chemistry*, 50(18), 5042-5047.

Mabberley DJ (2008) Mabberly's Plant Book – A Portable Dictionary of Plants, Their Classification and Uses. Third Edition, *Cambridge University Press* pp 276–277.

Manna, P., & Jain, S. K. (2015). Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metabolic syndrome and related disorders*, *13*(10), 423–444.

Marrelli, M., Loizzo, M. R., Nicoletti, M., Menichini, F., & Conforti, F. (2013). Inhibition of key enzymes linked to obesity by preparations from Mediterranean dietary plants: effects on α-amylase and pancreatic lipase activities. *Plant foods for human nutrition*, *68*(4), 340-346.

Matsusue, K., Peters, J. M., & Gonzalez, F. J. (2004). PPARβ/δ potentiates PPARγ-stimulated adipocyte differentiation. *The FASEB Journal, 18(12), 1477–1479.*

Merola, N., Castillo, J., Benavente-García, O., Ros, G., & Nieto, G. (2017). The Effect of Consumption of Citrus Fruit and Olive Leaf Extract on Lipid Metabolism. *Nutrients*, *9*(10), 1062.

Miyata, M., Koyama, T., & Yazawa, K. (2010). Water extract of *Houttuynia cordata* Thunb. leaves exerts anti-obesity effects by inhibiting fatty acid and glycerol absorption. *Journal of nutritional science and vitaminology*, *56*(2), 150–156.

Mohamed, G. A., Ibrahim, S. R. M., Elkhayat, E. S., & El Dine, R. S. (2014). Natural anti-obesity agents. *Bulletin of Faculty of Pharmacy, Cairo University, 52(2), 269–284.*

Moon, J., Do, H.-J., Kim, O. Y., & Shin, M.-J. (2013). Antiobesity effects of quercetin-rich onion peel extract on the differentiation of 3T3-L1 preadipocytes and the adipogenesis in high fat-fed rats. *Food and Chemical Toxicology, 58, 347–354.*

Moro, C. O., & Basile, G. (2000). Obesity and medicinal plants. *Fitoterapia, 71 Suppl 1*, S73–S82.

Morton, G. J., Meek, T. H., & Schwartz, M. W. (2014). Neurobiology of food intake in health and disease. *Nature reviews. Neuroscience, 15*(6), 367–378.

Mostafa, U. E., & Labban, L. (2013). The effect of *Zizyphus jujube* on serum lipid profile and some anthropometric measurements. *Pakistan Journal of Nutrition, 12*(6), 538-543.

Mukherjee, M. (2003). Human digestive and metabolic lipases—a brief review. *Journal of Molecular Catalysis B: Enzymatic, 22(5-6), 369–376.*

Munshi, R. P., Joshi, S. G., & Rane, B. N. (2014). Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. *Indian journal of pharmacology, 46*(3), 270–276.

Narayana, D. B. A., Katayar, C. K., & Brindavanam, N. B. (1998). Original system: search, research or re-search. *IDMA Bulletin, 29*(17), 413-416.

Ngondi, J. L., Etoundi, B. C., Nyangono, C. B., Mbofung, C. M., & Oben, J. E. (2009). IGOB131, a novel seed extract of the West African plant *Irvingia gabonensis*, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. *Lipids in health and disease, 8*, 7.

Niño, J., Jiménez, D. A., Mosquera, O. M., & Correa, Y. M. (2007). Diosgenin quantification by HPLC in a *Dioscorea polygonoides* tuber collection from colombian flora. *Journal of the Brazilian Chemical Society, 18(5), 1073–1076.*

Nugara, R. N., Inafuku, M., Iwasaki, H., & Oku, H. (2014). Partially purified *Peucedanum japonicum* Thunb extracts exert anti-obesity effects in vitro. *Nutrition (Burbank, Los Angeles County, Calif.), 30*(5), 575–583.

Nugara, R. N., Inafuku, M., Takara, K., Iwasaki, H., & Oku, H. (2014). Pteryxin: A coumarin in *Peucedanum japonicum* Thunb leaves exerts antiobesity activity through modulation of adipogenic gene network. *Nutrition, 30(10), 1177–1184.*

O'Neill, H. M., Holloway, G. P., & Steinberg, G. R. (2013). AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: Implications for obesity. *Molecular and Cellular Endocrinology, 366(2), 135–151.*

Odimegwu, J. I., Odukoya, O., Yadav, R. K., Chanotiya, C. S., Ogbonnia, S., & Sangwan, N. S. (2013*).* A New Source of Elemol Rich Essential Oil and Existence of Multicellular Oil Glands in Leaves of the *Dioscorea* Species. *The Scientific World Journal, 2013, 1–6.*

Ogbuagu, M.N., (2008). Nutritive and Anti-Nutritive Composition of the Wild (In-Edible) Species of *Dioscorea bulbifera* (Potato Yam) and *Dioscorea dumentorum* (Bitter Yam). *Journal of Food Technology*, 6: 224-226.

Oh, J., JUNG, S. R., LEE, Y. J., Park, K. W., Kim, S. Y., & Han, J. (2013). Antioxidant and antiobesity activities of seed extract from campbell early grape as a functional ingredient. *Journal of Food Processing and Preservation*, 37(4), 291-298.

Oh, S.D., Kim, M., Min, B.I., Choi, G.S., Kim, S.K., Bae, H., Kang, C., Kim, D.G., Park, B.J. and Kim, C.K., (2014). Effect of *Achyranthes bidentata* Blume on 3T3-L1 adipogenesis and rats fed with a high-fat diet. *Evidence-Based Complementary and Alternative Medicine,* 2014.

Osbaldeston, T. A., & Wood, R. P. (2000). Dioscorides: De materia medica.

Park, H. J., Chung, B. Y., Lee, M. K., Song, Y., Lee, S. S., Chu, G. M., Kang, S. N., Song, Y. M., Kim, G. S., & Cho, J. H. (2012). Centipede grass exerts anti-adipogenic activity through inhibition of C/EBPβ, C/EBPα, and PPARγ expression and the AKT signaling pathway in 3T3-L1 adipocytes. *BMC complementary and alternative medicine*, *12*, 230.

Park, J., Jeon, Y. D., Kim, H. L., Lim, H., Jung, Y., Youn, D. H., Jeong, M. Y., Kim, H. J., Kim, S. H., Kim, S. J., Hong, S. H., & Um, J. Y. (2013). Interaction of *Veratrum nigrum* with *Panax ginseng* against Obesity: A Sang-ban Relationship. *Evidence-based complementary and alternative medicine: eCAM*, *2013*, 732126.

Park, J., Jin, K. S., Lee, J. Y., Kwon, H. J., & Kim, B. W. (2013). Anti-oxidative and anti-obesity activities of *Tetrapanax papyriferus* and *Siegesbeckia pubescens* extracts and their synergistic anti-obesity effects. *Microbiology and Biotechnology Letters*, *41*(3), 341-349.

Park, J., Jin, K. S., Lee, J. Y., Kwon, H. J., & Kim, B. W. (2014). Anti-oxidative and anti-obesity effects of *Amomum Cardamomum* L. extract. *Microbiology and Biotechnology Letters*, *42*(3), 249-257.

Park, M., Sharma, A., & Lee, H. J. (2019). Anti-adipogenic effects of delphinidin-3-O-β-glucoside in 3T3-L1 preadipocytes and primary white adipocytes. *Molecules*, *24*(10), 1848.

Patil, D. N., Yadav, S. R., Patil, S., Bapat, V. A., & Jadhav, J. P. (2020). Multidimensional studies of *Pancratium parvum* Dalzell against acetylcholinesterase: a potential enzyme for Alzheimer's management. *Journal of the American College of Nutrition*, *39*(7), 601-618.

Patra, S., Nithya, S., Srinithya, B., & Meenakshi, S. M. (2015). Review of medicinal plants for anti-obesity activity. *Translational Biomedicine*, *6*(3), 0-0.

Poornima, G. N., & Ravishankar, R. V. (2009). Evaluation of phytonutrients and vitamin contents in a wild yam, *Dioscorea belophylla* (Prain) Haines. *African Journal of Biotechnology*, *8*(6).

Poudel, B., Lim, S. W., Ki, H. H., Nepali, S., Lee, Y. M., & Kim, D. K. (2014). Dioscin inhibits adipogenesis through the AMPK/MAPK pathway in 3T3-L1 cells and modulates fat accumulation in obese mice. *International Journal of Molecular Medicine*, *34*(5), 1401-1408.

Rahim, A. T. M., Takahashi, Y., & Yamaki, K. (2015). Mode of pancreatic lipase inhibition activity in vitro by some flavonoids and non-flavonoid polyphenols. *Food Research International*, *75*, 289-294.

Raina, A. P., & Misra, R. (2019). Evaluation of diosgenin, a bioactive compound from natural source of *Dioscorea* species: A wild edible tuber plant. *Journal of Pharmacognosy and Phytochemistry*, *9*(1), 1120-1124.

Ramírez, N.M., Toledo, R.C.L., Moreira, M.E.C., Martino, H.S.D., dos Anjos Benjamin, L., de Queiroz, J.H., Ribeiro, A.Q. and Ribeiro, S.M.R., (2017). Anti-obesity effects of tea from *Mangifera indica* L. leaves of the Ubá variety in high-fat diet-induced obese rats. *Biomedicine & Pharmacotherapy*, *91*, 938-945.

Rayalam, S., Della-Fera, M. A., & Baile, C. A. (2008). Phytochemicals and regulation of the adipocyte life cycle. *The Journal of nutritional biochemistry*, *19*(11), 717-726.

Redinger, R. N. (2009). Fat storage and the biology of energy expenditure. *Translational Research*, *154*(2), 52-60.

Resende, P. E., Verza, S. G., Kaiser, S., Gomes, L. F., Kucharski, L. C., & Ortega, G. G. (2012). The activity of mate saponins (*Ilex paraguariensis*) in intra-abdominal and epididymal fat, and glucose oxidation in male Wistar rats. *Journal of ethnopharmacology*, *144*(3), 735–740.

Rezaee, F., & Dashty, M. (2013). Role of adipose tissue in metabolic system disorders adipose tissue is the initiator of metabolic diseases. *Journal of Diabetes and Metabolic Disorders*, *13*(2).

Roh, C., & Jung, U. (2012). *Nepeta japonica* Maximowicz extract from natural products inhibits lipid accumulation. *Journal of the Science of Food and Agriculture*, *92*(10), 2195-2199.

Romano, A., Coccurello, R., Giacovazzo, G., Bedse, G., Moles, A., & Gaetani, S. (2014). Oleoylethanolamide: a novel potential pharmacological alternative to cannabinoid antagonists for the control of appetite. *BioMed Research International*, *2014*.

Rosidah, Yam, M., Sadikun, A., & Asmawi, M. (2008). Antioxidant potential of Gynura procumbens. *Pharmaceutical Biology*, *46*(9), 616-625.

Sakthidevi, G., & Mohan, V. R. (2013). Total phenolic, flavonoid contents and in vitro antioxidant activity of Dioscorea alata L. tuber. *Journal of Pharmaceutical Sciences and Research*, *5*(5), 115.

Sam, S., & Mazzone, T. (2014). Adipose tissue changes in obesity and the impact on metabolic function. *Translational Research*, *164*(4), 284-292.

Sari, D. R. T., Safitri, A., Cairns, J. R. K., & Fatchiyah, F. (2020, October). Virtual screening of black rice anthocyanins as antiobesity through inhibiting TLR4 and JNK pathway. *Journal of Physics: Conference Series* (Vol. 1665, No. 1, p. 012024).

Sarker, S. D., & Nahar, L. (2012). An introduction to natural products isolation. *Natural products isolation*, 1-25.

Shah, H. J., & Lele, S. S. (2012). Extraction of diosgenin, a bioactive compound from natural source Dioscorea alata Var purpurae. *Journal of Analytical & Bioanalytical Techniues*, *3*(4), 1-3.

Shikov, A. N., Pozharitskaya, O. N., Makarova, M. N., Makarov, V. G., & Wagner, H. (2014). *Bergenia crassifolia* (L.) Fritsch–Pharmacology and phytochemistry. *Phytomedicine*, *21*(12), 1534-1542.

Shin, E., Shim, K. S., Kong, H., Lee, S., Shin, S., Kwon, J., Jo, T. H., Park, Y. I., Lee, C. K., & Kim, K. (2011). Dietary aloe improves insulin sensitivity via the suppression of obesity-induced inflammation in obese mice. *Immune Network*, *11*(1), 59-67.

Singh, A. K., Patki, P. S., & Mitra, S. K. (2008). Evaluation of clinical efficacy of ayurslim on body weight, body mass index, lipid profile and skin fold thickness: a phase IV clinical trial. *The Antiseptic*, *105*(5), 241-243.

Singh, B. P., Vij, S., & Hati, S. (2014). Functional significance of bioactive peptides derived from soybean. *Peptides*, *54*, 171-179.

Singh, D. K., Srivastava, B., & Sahu, A. (2004). Spectrophotometric determination of Rauwolfia alkaloids: estimation of reserpine in pharmaceuticals. *Analytical sciences*, 20(3), 571-573.

Slanc, P., Doljak, B., Kreft, S., Lunder, M., Janeš, D., & Štrukelj, B. (2009). Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *23*(6), 874-877.

Son, I. S., Kim, J. H., Sohn, H. Y., Son, K. H., Kim, J. S., & Kwon, C. S. (2007). Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea spp*.), on high-cholesterol fed rats. *Bioscience, biotechnology, and biochemistry*, *71*(12), 3063-3071.

Song, H., Shen, X., Chu, Q., & Zheng, X. (2021). *Vaccinium bracteatum* Thunb. fruit extract reduces high-fat diet-induced obesity with modulation of the gut microbiota in obese mice. *Journal of Food Biochemistry*, *45*(7), e13808.

Song, M. Y., Lv, N., Kim, E. K., Kwon, K. S., Yoo, Y. B., Kim, J. H., ... & Kwon, K. B. (2009). Antiobesity activity of aqueous extracts of Rhizoma *Dioscoreae Tokoronis* on high-fat diet-induced obesity in mice. *Journal of medicinal food*, *12*(2), 304-309.

Song, M., Yang, G., Hoa, T. Q., Hieu, H. D., Amin, A., Choe, W., Kang, I., Kim, S. S., & Ha, J. (2020). Anti-obesity Effect of Fermented Persimmon Extracts via Activation of AMP-Activated Protein Kinase. *Biological & pharmaceutical bulletin*, *43*(3), 440–449.

Sonibare, M. A., & Abegunde, R. B. (2012). In vitro antimicrobial and antioxidant analysis of *Dioscorea dumetorum* (Kunth) Pax and *Dioscorea hirtiflora* (Linn.) and their bioactive metabolites from Nigeria. *Journal of Applied Biosciences*, *51*, 3583-3590.

Stoeckman, A. K., & Towle, H. C. (2002). The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. *Journal of Biological Chemistry*, *277*(30), 27029-27035.

Stohs, S. J., Preuss, H. G., & Shara, M. (2012). A review of the human clinical studies involving *Citrus aurantium* (bitter orange) extract and its primary protoalkaloid p-synephrine. *International journal of medical sciences*, *9*(7), 527.

Swinburn, B. A., Sacks, G., Hall, K. D., McPherson, K., Finegood, D. T., Moodie, M. L., & Gortmaker, S. L. (2011). The global obesity pandemic: shaped by global drivers and local environments. *The Lancet*, *378*(9793), 804-814.

Tamaru, S., Ohmachi, K., Miyata, Y., Tanaka, T., Kubayasi, T., Nagata, Y., & Tanaka, K. (2013). Hypotriglyceridemic potential of fermented mixed tea made with third-crop green tea leaves and Camellia (*Camellia japonica*) leaves in Sprague–Dawley rats. *Journal of agricultural and food chemistry*, *61*(24), 5817-5823.

Thapyai, C. (2004). *Taxonomic Revision of Dioscoreaceae in Thailand*. Kasetsart University, Graduate SchoolThesis

Toromanyan, E., Aslanyan, G., Amroyan, E., Gabrielyan, E., & Panossian, A. (2007). Efficacy of Slim339® in reducing body weight of overweight and obese human subjects. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(12), 1177-1181.

Totani, N., Tateishi, S., Takimoto, T., Maeda, Y., & Sasaki, H. (2011). Gallic acid glycerol ester promotes weight-loss in rats. *Journal of oleo science*, *60*(9), 457-462.

Trayhurn P. (2007). Adipocyte biology. *Obesity reviews: an official journal of the International Association for the Study of Obesity*, 8 Suppl 1, 41–44.

Tzeng, T. F., Lu, H. J., Liou, S. S., Chang, C. J., & Liu, I. M. (2014). Lipid-lowering effects of zerumbone, a natural cyclic sesquiterpene of Zingiber zerumbet Smith, in high-fat diet-induced hyperlipidemic hamsters. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, *69*, 132–139.

Udani, J., Hardy, M., & Madsen, D. C. (2004). Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2™ brand proprietary fractionated white bean extract. *Alternative medicine review*, *9*(1), 63-69.

Uemura, T., Goto, T., Kang, M. S., Mizoguchi, N., Hirai, S., Lee, J. Y., Nakano, Y., Shono, J., Hoshino, S., Taketani, K., Tsuge, N., Narukami, T., Makishima, M., Takahashi, N., & Kawada, T. (2011). Diosgenin, the main aglycon of fenugreek, inhibits LXRα activity in HepG2 cells and decreases plasma and hepatic triglycerides in obese diabetic mice. *The Journal of nutrition*, *141*(1), 17–23.

Velusami, C. C., Agarwal, A., & Mookambeswaran, V. (2013). Effect of Nelumbo nucifera petal extracts on lipase, adipogenesis, adipolysis, and central receptors of obesity. *Evidence-Based Complementary and Alternative Medicine, 2013*.

Wafer, R., Tandon, P., & Minchin, J. E. (2017). The role of peroxisome proliferator-activated receptor gamma (PPARG) in adipogenesis: Applying knowledge from the fish aquaculture industry to biomedical research. *Frontiers in endocrinology*, *8*, 102..

Wang, X., Liu, J., Long, Z., Sun, Q., Liu, Y., Wang, L., Zhang, X., & Hai, C. (2015). Effect of diosgenin on metabolic dysfunction: Role of ERβ in the regulation of PPARγ. *Toxicology and applied pharmacology*, *289*(2), 286–296.

Watanabe, T., Hata, K., Hiwatashi, K., Hori, K., Suzuki, N., & Itoh, H. (2010). Suppression of murine preadipocyte differentiation and reduction of visceral fat accumulation by a *Petasites japonicus* ethanol extract in mice fed a high-fat diet. *Bioscience, biotechnology, and biochemistry*, *74*(3), 499-503.

Wolfe, K. L., & Liu, R. H. (2003). Apple peels as a value-added food ingredient. *Journal of Agricultural and Food Chemistry*, *51*(6), 1676-1683.

Wozniak, S. E., Gee, L. L., Wachtel, M. S., & Frezza, E. E. (2009). Adipose tissue: the new endocrine organ? A review article. *Digestive diseases and sciences*, *54*(9), 1847-1856.

Yamamoto, Y., & Oue, E. (2006). Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. *Bioscience, biotechnology, and biochemistry*, *70*(4), 933-939.

Yang, H., Chen, B., Wang, X., Chue, P., Shen, Y., Xia, G., & Jia, X. (2013). Rapid quantitative analysis of diosgenin in the tubers of *Dioscorea zingiberensis* C.H. Wright by coupling cellulose enzymolysis and two-phase acid hydrolysis in tandem with HPLC-UV. *Natural Product Research,* 27(20), 1933–1935.

Yang, J., Martinson, T. E., & Liu, R. H. (2009). Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry*, *116*(1), 332-339.

Yang, M. H., Chin, Y. W., Chae, H. S., Yoon, K. D., & Kim, J. (2014). Anti-adipogenic constituents from *Dioscorea opposita* in 3T3-L1 cells. *Biological and Pharmaceutical Bulletin*, *37*(10), 1683-1688.

Yazici-Tutunis, S., Gurel-Gurevin, E., Ustunova, S., Demirci-Tansel, C., & Mericli, F. (2016). Possible effects of *Phillyrea latifolia* on weight loss in rats fed a high-energy diet. *Pharmaceutical Biology*, *54*(10), 1991-1997.

You, J. S., Lee, Y. J., Kim, K. S., Kim, S. H., & Chang, K. J. (2014). Anti-obesity and hypolipidaemic effects of *Nelumbo nucifera* seed ethanol extract in human pre-adipocytes and rats fed a high-fat diet. *Journal of the Science of Food and Agriculture*, *94*(3), 568-575.

Zhang, X. H., Huang, B., Choi, S. K., & Seo, J. S. (2012). Anti-obesity effect of resveratrol-amplified grape skin extracts on 3T3-L1 adipocytes differentiation. *Nutrition research and practice*, *6*(4), 286-293.

Zheng, C. D., Duan, Y. Q., Gao, J. M., & Ruan, Z. G. (2010). Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs. *Journal of the Chinese Medical Association*, *73*(6), 319-324.

Zhu, J., Jin, J., Ding, J., Li, S., Cen, P., Wang, K., Wang, H., & Xia, J. (2018). Ganoderic Acid A improves high fat diet-induced obesity, lipid accumulation and insulin sensitivity through regulating SREBP pathway. *Chemico-biological interactions*, *290*, 77–87.

STATEMENT I

The present investigation deal with **"Studies on Antiobesity Metabolites from *Dioscorea* Species"**. In the present investigation an attempt has been made to study screening of *Dioscorea* species against the obesity and find the biological active metabolites showing antiobesity activity. Further this work has not been submitted for the award of any degree or diploma in any institute.

Dr. M. S. Nimbalkar **Mr. Ruturaj Sudhakar Patil**

Guide **Candidate**

STATEMENT II

The present investigation embodies **"Studies on Antiobesity Metabolites from *Dioscorea* Species".** The work reports a new investigation. The source from which information is gathered have been listed in the last part of the thesis- "Bibliography". The current issue of Journal, review articles, textbook and monograph have been extensively referred and correlated. Every attempt has been made to keep the reference work as updated as possible.

Dr. M. S. Nimbalkar **Mr. Ruturaj Sudhakar Patil**

Guide **Candidate**

STUDIES ON ANTI-OBESITY METABOLITES FROM *DIOSCOREA* SPECIES

A

THESIS SUBMITTED TO

SHIVAJI UNIVERSITY, KOLHAPUR

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

UNDER THE FACULTY OF SCIENCE AND TECHNOLOGY

BY

MR. PATIL RUTURAJ SUDHAKAR
M. Sc.

UNDER THE GUIDANCE OF

DR. MANSINGRAJ S. NIMBALKAR
M. Sc., Ph. D.

DEPARTMENT OF BOTANY,
SHIVAJI UNIVERSITY,
KOLHAPUR - 416 004
MAHARASHTRA, INDIA

2022

Chapter "80 Recommendations"

1. Recommendation:

Obesity is the major problem now days. Obesity is linked to many diseases like cancer, debits, osteoporosis, and cardiovascular disease. Control of the obesity leads to overcome the problem of the linked disease. The present study has been planned to explore the anti-obesity efficacy of the *Dioscorea* species to avoid increase of obesity. The outcome of present study will possibly help develop a food/beverage with anti-obesity properties.

2. Conclusion:

The key conclusion from the above study is that all the *Dioscorea* species used in the study have lipase inhibition properties. *D. oppositifolia* whole plant extracts have a good source of phytochemicals and antioxidants than other three species. Considering the results obtained from the gene expression study both methanolic and aqueous whole plant extracts of *D. oppositifolia* have an ability to affect adipogenesis at the gene level. Treatment with *D. oppositifolia* whole plant extract to the high-fat diet-induced rats exhibited a lowering of the TC, TG, LDL, VLDL, and body weight as compared to the control group. The chloroform fraction and extracted Diosgenin from the *D. oppositifolia* root shows the virtuous down regulation of the adipogenic genes in 3T3-L1 cells. In LCMS profiling of the chloroform fraction of *D. oppositifolia*, robinetin, aloesin, diocin, and oleoyl ethanol amide were found which has anti-obesity activity, and down regulate the adipogenic genes. So, *D. oppositifolia* whole plant can be a potential candidate for the treatment of obesity and related problems.

3. Summery

Collection of *Dioscorea* species:

The four *Dioscorea* species were collected from the Kolhapur district Maharashtra India. Namely *D. alata, D. bulbifera D. pentaphylla* and *D. oppositifolia.* Collected plant germplasm was mentioned in botanical garden, Department of Botany. Also the herbariums of all species deposited to herbarium Department of Botany, Shivaji University Kolhapur.

Chemo-profiling of *Dioscorea* extract:

The primary phytochemical analysis was carried out by different colorimetric methods. The total phenolic, total flavonoids, total alkaloids, total terpenoids and total saponin were studied the methanolic extract of *D. oppositifolia* showing the highest content of the all the phytochemicals than the other extracts. The antioxidant activity studied by the

free radicals scavenging by DPPH and ABTS assay *D. oppositifolia* methanolic extracts showing the highest % inhibition in both the assay. It also has the ferrous reducing antioxidant power.

Screening of the different *Dioscorea* extract for the anti-obesity potential:

Plant material were dried and used for the preparation of the methanolic and aqueous extracts of the plant. The prepared extracts were screened by using the 2 different methods i.e. lipase inhibition, effect of extracts on the lipid accumulation in the 3T3-L1 cells and regulation of the adipogenic genes.

In lipase inhibition study all the extracts of the *Dioscorea* shows the significant inhibition. The both methanolic and aqueous extracts of the *D. pentaphylla* shows the above 70% inhibition. *D. alata, D. bulbifera,* and *D. oppositifolia* exhibit the above 50% lipase inhibition activity.

In vitro anti-obesity analysis was performed on 3T3-L1 preadipocyte cell line, which serves as an *In vitro* model system to research anti-obesity because it develops from pre-adipocyte to mature adipocyte, which is specialized in lipid droplet formation. The toxicity of the plant extracts was studied by using the MTT assay and from the IC 50 concentration the dose of treatment was decided for the further study. From the results obtained from the Oil O red staining both aqueous and methanolic extracts of the *D. oppositifolia* reduces by 1.8 and 2.2 times the lipid accumulation in the 3T3-L1 cells as compared with Control induced group.

The 3T3-L1 cells were treated with methanolic and aqueous extracts of *D. oppositifolia* and *D. pentaphylla* during the differentiation. *D. oppositifolia* methanolic extract suppresses expression of PPAR Ɣ, C/EBPα, SRBP1 and FAS concentration dependently. This results in the inhibition of adipogenesis in 3T3-L1 cells. It also increase the expression of the CPT-1 gene that helps to enhances lipolysis of accumulated lipids. Aqueous extract of *D. oppositifolia* down regulate the expression PPAR Ɣ, C/EBPα and SRBP1. At lower concentration of extracts the fatty acid synthase down regulate and at the higher concentration it unregulated the FANS gene. It up regulate the CPT 1 gene that helps in the lipolysis of the accumulated lipids. In case of the *D. pentaphylla* methanolic and aqueous extracts up regulate the PPAR Ɣ, C/EBPα, SRBP1, FASN, gene and down regulate the CPT 1 gene. So the *D. oppositifolia* methanolic extract used for the further study.

The LCMS analysis of the *D. pentaphylla* and *D. oppositifolia* methanolic extracts were carried from the obtained data. Group of compound was identified from the human metabolomics database and Pub chem database. The compound having an anti-obesity property previously studied were identified 11 compound shows the anti-obesity activity.

This significant down-regulation of PPARγ gene may be due to the cumulative action of phytochemicals from the extracts. This reduction of adipogenic transcription factors may aid in regulating adipocyte differentiation and lowering adipocyte lipid accumulation.

Screening of anti-obesity metabolites from *D. oppositifolia*:

From the above study the *D. oppositifolia* methanolic extract used for the liquid-liquid extraction by using the hexane, chloroform, ethyl acetate and n-butanol. Fraction were collected and solvents were evaporated reaming reside after evaporation was dissolved in to the DMSO, Also the diosgenin was extracted from the root powder of *D. oppositifolia* by using the acid hydrolysis method.

The effect of all fractions and extracted diosgenin were studied during the differentiation of 3T3-L1 cells. In lipid accumulation study there was no significant changes were seen in cells after treatment with all fractions and extracted diosgenin. For that, the impact of all the fractions explored by gene expression study.

In comparing the effects of each fraction, the chloroform fraction prevents the expression of all examined adipogenic genes more than the other four fractions while also stimulating the activity of the CPT-1 gene. The PPARɣ, C/EBPα, SREBP1, and FASN genes are up regulated by hexane fractions. In contrast, down regulates the CPT 1 gene. Treatment with the Ethyl acetate fraction raises PPARɣ, C/EBPα, and FASN levels. SREBP 1 gene expression was lower than in the control group. The CPT-1 is down regulated at greater concentrations of ethyl acetate and up regulated at lower concentrations. At the studied concentrations, cells treated with n-butanol and water fraction up regulate all adipogenic genes. At greater concentrations of the n-butanol fraction, C/EBPα was down regulated.

LCMS-QTOF analysis was used to profile the metabolites in the chloroform fraction. Robinetin, Aloesin, Dioscin, and Oleoyl Ethanol Amide were identified in the chloroform

fraction of *D. oppositifolia.* The inclusion of these substances in the fraction demonstrating antiadipogenic gene regulate.

Dioscorea species are a good and natural source of diosgenin. It has multiple biomedical applications. We tested the extracted diosgenin of *D. oppositifolia* root on the 3T3-L1 preadipocytes for antiobesity activity. The effect of the extracted diosgenin was compared with the effect of standard diosgenin on the adipogenesis were studied. The PPARϒ, and SREBP 1 was down regulated by the extracted diosgenin at tested concentration the expression levels of the C/EBPα, and FASN was slightly up regulated at lower concentration of extracted diosgenin but at higher concentration down regulation were observed. Whereas the CPT -1 gene was up regulated at both the concentration. The extracted diosgenin shows the antiadipogenic activity as exhibited by the standard diosgenin.

The extracted diosgenin was confirmed by using the HPLC with the standard diosgenin. In both sample and standard diosgenin shows the pick at same retention time. Hence it confirmed that the sample contains the diosgenin.

Effect of *D. oppositifolia* on male Wistar rat:

The *D. oppositifolia* whole plant extract decreases body weight in a diseased rat model. Treatment with *D. oppositifolia* lowers body weight, TC, TG, LDL, VLDL, and increases HDL levels as compare to the High fat induced rat group, suggesting that it may be a viable approach to treating obesity and associated disorders.

4. Future Findings:

Future research will focus on isolating the primary anti-obesity ingredient from *D. oppositifolia* and evaluating the effect of isolated compound on an animal model. By using plant tissue culture techniques, try to increase the synthesis of anti-obesity metabolites like dioscin and diosgenin in the plant. Preparation of herbal mixture using entire plant extracts against to obesity and overweight.