IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

CHARACTERIZATION AND COMPUTATIONAL ANALYSIS OF LIPASE INHIBITORS FROM PLANT SOURCES

[1]Santhosh kumar V,[2]Swetha E, [3]Haji mohamed A,[4]Mohammed Suhail S, [5]Pavilan S and [6]Ramya M

[1]Assistant professor and [2]Assistant professor
[1]Department of Biotechnology,
[1]Sri Shakthi Institute of Engineering and Technology , Coimbatore 62, India

Abstract: Obesity is primarily regarded as a disorder of lipid metabolism and the enzymes involved in this process could be selectively targeted to develop anti obesity drugs. The prevalence of obesity is increasing at an alarming rate, but unfortunately only a few medications are currently on the market. Orlistat is an anti-obesity drug which is already available in the market but it has side effects after prolong usage. The anti-obesity drugs extracted from natural compounds will not have any side effects. Since dietary lipids represent the major source of unwanted calories, specifically inhibiting triglyceride (TG) digestion forms a new solution for the reduction of fat absorption. The leaves of the plant samples (cashew, drumstick, fenugreek, keezhanelli, neem) with lipase inhibitory activity were choosen and extracted using Soxhlet extractor using Methanol as solvent. These extracts were analysed for lipase inhibitory activity using para nitrophenyl palmitate as substrate. These extracts with high inhibitory activity of lipase inhibition will be further characterized and studied for enzyme kinetics and computational analysis in future to identify the compound that inhibit the activity of Lipase.

Key words:Plant extracts, anti-lipase activity, para nitrophenyl palmitate

1.INTRODUCTION

GENERAL

Body mass index (BMI), is used to characterize obesity. Obesity is primarily caused by an imbalance between energy intake and expenditure. Worldwide obesity has increased by more than twofold since 1980, according to data from the World Health Organization (WHO). In 2008, there were more than 1.4 billion overweight adults in the world, with 3 times as many women as males. In 2010, there were more than 40 million overweight children under the age of five and in 2012 with atleast 2.8 million adult deaths, increase in weight and obesity were the fifth largest risk factor for fatalities worldwide.

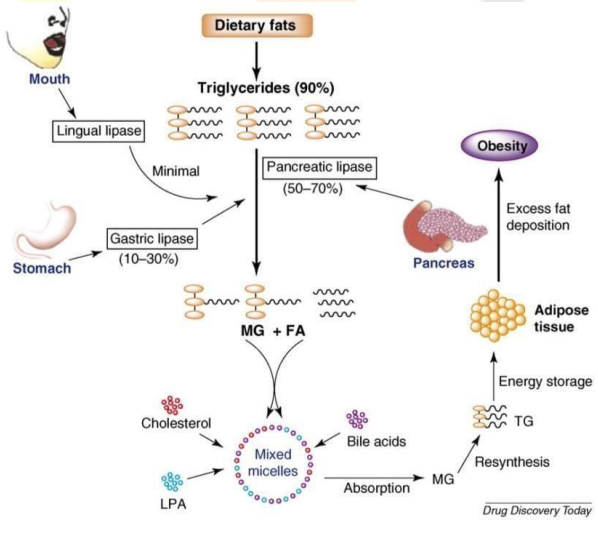
Overweight and obesity are linked to a number of disorders, including increase in blood pressure, non-insulin dependent hyperlipidemia, low blood glucose level and coronary disease. Cardiovascular disease is currently the leading cause of death in Malaysia as a result of various obesity-related disorders growing so quickly. Overweight and obesity were once thought to be the problems specific to high-income countries, but they are now becoming more prevalent in low and middle income nations especially in metropolitan areas.

To maintain homeostatis, lipid metabolism is exquisitely balanced. A range of significant disorders such as Knee pain, increase in blood pressure, increase in blood glucose level and damage of organs and its function can emerge when

balance is lost, including obesity or hyperlipidemia. Consequently many disorders could be prevented or treated by medications that regulate lipid metabolism. Since more and more lipid metabolic pathway enzymes are being discovered and characterized, they offer a wealth of prospective therapeutic targets for obesity and other metabolic diseases. One of the most extensively researched methods for evaluating the potential effectiveness of natural compounds as anti-obesity treatments is PL inhibition. One of the two medications for treating obesity that have received clinical approval, orlistat has been demonstrated to work by inhibiting PL. Although being one of the most popular medications in the world, it has some side effects that affects the intestinal health and the gut health. It is important to find a new anti-obesity drug with no side effects.

Hormone – sensitive lipase (HSL) and the newly discovered adipose triglyceride lipase (ATGL) influence the mobilization of fat stored in adipose tissue, however it is unknown how important each is in the process of lipolysis. We demonstrate that a novel powerful HSL inhibitor does not also inhibit other lipases. The substance showed no effect on residual triglyceride hydrolysis or lipolysis in HSL null mice, but it inhibited catecholamine stimulated lipolysis in mouse adipocytes. The HSL inhibitor completely attenuated catecholamine and natriuretic peptide induced lipolysis in human adipocytes. Glycerol release was decreased but not fatty acid release when fat cells were not stimulated. The main risk factor for type 2 diabetes is obesity, which is defined by an abudance of fat storage.

The fats like phospholipids and triacyglycerol are break down by the enzyme called Lipases in which primary lipolytic enzyme PL (Triacylgycerol acyl hydrolase) which is produced and secreted by the pancreas, is essential for the effective breakdown of triglycerides. The lipolytic products are monoglycerides and long chain saturated and polyunsaturated fatty acids. PL is in charge of hydrolyzing between 50 and 70 percent of all dietary fats. Dietary fats must be hydrolyzed in order to be absorbed because mixed triglycerides (TGs) make up the majority of them (90%) in the body. The food we consume contains fat which is hydrolyzed as fatty acids and monoglycerides about 50 -70 % by the pancreatic lipase which is the important lipolytic enzyme and these are generated during lipid hydrolysis combine with bile salts, cholesterol and lysophosphatidic acid (LPA) to create mixed micelles that are then absorbed into enterocyted where TG resynthesis occurs. Adipocytes use the TGs they store as their main energy source. The physiological role of pancreatic lipase in lipid absorption is shown in Fig-1. Triglyceride hydroloysis and the production of free fatty acids



(FFAs) are the results of adipose tissue lipolysis. Adipose tissue lipolysis is a target for the pharmaceutical sector due to the relationship between elevated circulating FFA levels and the emergence of insulin resistance and the metabolic syndrome. The first widely utilized lipid-lowering substance was nicotinic acid, which works by preventing adipose tissue lipolysis.

Fig- 1 The physiological role of pancreatic lipase in lipid absorption

PROBLEM STATEMENT

Obesity is considered as a serious health hazard which leads to many diseases nowadays. The available anti-obesity approved drugs are causing side effects and this cause major illness to our body. Our aim is to identify naturally available plant sources for inhibiting the pancreatic lipase and further studied for finding naturally available anti-obesity drugs.

OBJECTIVE

- To screen for inhibitors from plant sources.
- To study the computational analysis of molecular interactions.

2.LIPASE

2.1 Pancreatic Lipase

Triacylgycerol acyl hydrolase, the main lipolytic enzyme produced and secreted by the pancreas is crucial for the effective digestion of triglycerides and hydrolyzes between 50 and 70 percent of dietary fats. One of the few medications licensed for use in treating obesity, orlistat, works by inhibiting pancreatic lipase. A third of the fat that is consumed is digested by the lingual lipase that is produced by the serous gland. 10 -40 % of dietary fat is hydrolyzed by gastric lipase, which is released when we eat food. Hence the decrease in PL activity will reduce the absorption of fat and prevents obesity.

The action of pancreatic lipase is primarily responsible for the digestion of dietary triacylglycerols (TAG) in the small intestine of mammals. Obesity develops as a result of fat molecules taken by the body consumed after the process of digestion. Thus, the prevalence of this can be decreased by reducing the degradation of TAG and consequently, its capacity to enter from the intestinal lumen into the body, is halted or reduced. Due to this, a digestive lipase inhibitor may be effective as an anti-obesity medication. The remedy for obesity with available medications such as sibutramine, rimonabant, phentermine diethylpropin, Zonisamide, Topiramate and orlistat is one strategy for reducing obesity. N-formyl-L-leucine-1-[(2S,3S)-3-hexyl-4-oxetanyl] orlistat the non-centrally acring anti-obesity drug methyldodecyl ester (also known as tetrahydrolipstatin) works in the human intestine by reducing the lipases in pancreas and gastric which are responsible for the breakdown of long chain TAG. It decreases dietary fat absorption by around 30% at the recommended therapeutic dose of 120 mg three times per day. The only anti-obesity medication authorized for long-term weight management is this one. Due to unfavorable side effects,sibutramine and rimonabant were recently removed from the market. In high-risk cardiac patients, sibutramine may raise their risk of heart attack and stroke, whilst the latter may result in potentially life- threatening psychiatric illness. As a result, it is very important to produce safe and effective anti-obesity medicine or drug from the extract of natural sources. Although these agents are bad to health, they are less harmful than those that are entirely manufactured. The quest for anti- lipase inhibitors derived from natural sources has been covered in a number of studies. It's feasible that some plants have molecules that will increase the activity of pancreatic lipases even if there haven't been any reports of plants with pro-lipase activity to date.

The two main hormones that in humans stimulate this catabolic pathway are catecholamines and natriuretic peptides. Obese adults and children have shown resistance to catecholamine induced lipolysis in subcutaneous adipose tissue which has been linked to decreased expression of hormone sensitive lipase (HSL), increased antilipolytic properties of 2-adrenoceptors and increased antilipolytic properties of 2-adrenoceptors.Because it is also seen in non-obese first degree relatives of obese people and because there is a correlation between lipolytic capacity and HSL expression in human subcutaneous fat cells, it is conceivable that the HSL deficiency is the most significant contributor.

2.2 Substrate for Lipase

Direct and continuous spectrophotometric assays are made possible by chromogenic analogues such as p-nitrophenyl acyl esters. Lipase is a long-chain palmityl group divided between the activity of lipase and esterase, p-nitrophenyl palmitate is frequently utilised among the many p-nitophenyl acyl esters . When pNPP is hydrolyzed by enzymes, it takes on a yellow hue that can be easily observed at a wavelength of 410mm.

2.3 Lipase Assay

The Lipase activity assay's substrate of choice was a para-nitrophenyl palmitate (pNPP). 0.45g pNPP dissolved in 15ml isopropanol, 0.3g Triton X100 and 135ml 25 mM Tris- HCl were used to make the substrate solution. 2ml of the substrate solution and 0.1 ml of the lipase enzyme solutiom made up the reaction solution mixture. The reaction mixture was heated in a water bath at 37℃for 15 minutes. The addition of 0.15 ml of 0.1 M Na_2CO_3 stopped the reaction. The activity of lipase was measured using Spectrophotometer at 410nm. The hydrolysis of 1 mol of pNPP per minute under analytical circumstances is the one unit of enzyme activity.

2.4 Pancreatic Lipase Structure

Our human pancreatic lipase is said to be aglycoprotein and it has a molecular weight of 46,000 and 449 amino acids. A 16-amino acid short peptide chain was found ti be present at the protein's N-treminus,as indicated by the initial structure predicted from cDNA.The amino acid asparagine (Asn140) is an N-linked and it has glycosylation site. The structure shown in Fig-2 has two domains in which amino acid domain contains sheet core, whereas the C-terminal domain contains sheets.

These two domains are divided by the seven disulfide links of amino acids. The two salt bridges Asp390 and Lys400 connects the procolipase to the C-terminal domain which is present in the pancreatic lipase complex. The hydrophobic residues shapes the three loops by procolipase connected together by the disulfide bonds. The loop ends interact with the substrate interface while being located across from the lipase binding sites. Procolipase does not interact with the N-terminal domain and does not change the way PTL is conformed. Furthermore, Ser152-His263-Asp176 creates the cataylic triad seen in serine proteases,according to site-specific mutagenesis of the cDNA encoding human lipase.

When Serine and histidine are changed to another amino acid, pancreatic lipase's catalytic activity is no longer present, however when Asp176 is changed to glutamate, 80% of the enzyme's activity is still present. The active site was enfolded by many surface loops, which prevented substrate from attaching to the active site. The biggest loop in Fig-2 is a disulfate bridge between Cys238 and Cys262, which forms a lid domain. The -5 loop, amino acids 76-80 and 213-217 and two additional loops were also created. The lipase enzyme, which catalyses water-insoluble substrate has these loops as its distinguishing features. By activating the interface between two surfaces, these loops improves enzyme activity. There are two models one is enzyme model and another is substrate model which explains the method of interfacial action.

The enzyme model describes the lipase's conformation changes are caused by the water-oil contact. The substrate binding to active site is dependent on this conformational shift, more surface loops and lid domain movement. In order to bring colipase into close proximity with the lid domain during the lipase colipase complex,it is also suggested that the lid domain and C-terminal domainpivot. This movement alters the -5 loop and leads to a conformational shift that created an active site for the substrate.In addition to interfacial activation, collipase is also in charge of lipase activity. Lipase is susceptible to inhibition by phospholipids, bile salts and proteins among other things.

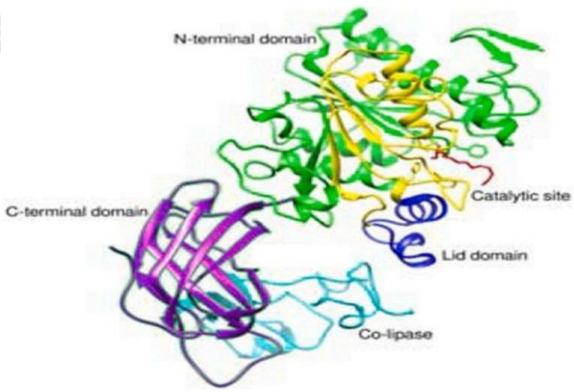


Fig-2 Structure of Pancreatic Lipase

2.5 Mechanism of Lipase in Pancreas

The pancreatic lipase is responsible for the breakdown of fat which is triacylglycerol into diacylglycerol and free fatty acids. Gastric lipase initiates the partial breakdown of ingested fat in the stomach. Lipids when they are emulsified with bile salt together forms a big structure from the big fat compounds. Similar to other esterase enzymes, pancreatic lipase forms a Michaelis- Menten adsorption complex by adhering to the lipid micelle interface. Several native enzymes can be aggregated by this acetylated lipase. Colipase also aids in the activation of the interfacial layer. Serine residue at the catalytic site aids in identifying the contact and as a result regulates lipase adsorption. Adsorption-induced conformational changes in lipase cause the lid domain and surface loops to shift, which opens the pathway for substrate to the catalytic site.Ser152 of the enzyme engages in a nucleophilic attack on the carboxyl group of the ester of triglycerides, demonstrating its significance in maintaining the conformation of this enzyme.

2.6 Clinical listing of Lipase Inhibitors

Orlistat is the only anti-obesity medication that does not act on the central nervous system or enter the bloodstream. It is also the only lipase inhibitor diet treatment that is currently being used in clinical trials. Lipstatin is a powerful irreversible inhibitor of pancreatic lipase that Ballinger isolated from St.streptococci. The FDA granted orlistat, a tetrahydro derivative of lipstain that Roche Corp successfully hydrogenated into a more stable inhibitor known as an anti-obesity medicine in 1997. Fig-2 depicts the orlistat chemical structure. It covalently binds to a serine residue at the lipase active site to have an inhibitory impact and slightly raises blood pressure, fasting blood sugar, total cholesterol and low density lipoprotein levels.

Additionally, orlistat is effective for conditions including cirrhosis, fatty liver, insulin resistance, blood pressure and hyperlipidermia that results in atherosclerosis according to clinical research. A common clinical dose is 120 mg three times a day and this dose can significantly reduce obesity by reducing the rate of fat absorption inhibition by aroung 30%. Currently the inhibitory action of orlistat is primarily used as a reference for comparative investigation in experimental chemical synthesis or natural product screening.

2.7 Molecular structure of Orlistat

According to the most recent studies, orlistat does not influence incretin secretion or hunger, but it does decrease the body's absorption of some lipid nutrients, which in turn affects the body's absorption of some fat

soluble vitamins including A, D and E. In order to prevent aberrant vitamin serum concentrations, typical multivitamins must be supplemented regularly. The essential fatty acids arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are among those whose absorption is also impacted by orlistat. To address the shortage of these fatty acids, fish oil or a specific diet supplementation is required. It is important to note that excessive DHA and EPA supplementation can also result in negative side effects, such as nausea and dizziness and can even alter the original antihypertensive effect of lowering blood pressure and become hypertensive and proinflammatory. The gastrointestinal system is where this lipase inhibitor's main side effects are most common and they frequently appear early in treatment. As treatment is prolonged, these side effects usually get better. Its clinical effects are somewhat constrained by potentially severe abdominal pain. It is yet known whether this medication is safe and and efficient for long-term weight maintenance, affordable therapy and total morbidity and mortality associated with excess body fat. In order to produce medications that inhibit lipase the aforementioned issues must also be resolved.

2.8 Plant sources of Pancreatic Lipase Inhibitor

2.8.1 Peanuts

Peanuts (Arachis hypogaea L.) are generally used for food purposes, either as nuts or as a source of seed oil, peanut shells (hulls, seed coats) which are manufactured in large quantities as byproducts of the peanut industry each year and amount to hundreds of thousands of tonnes, still have no discernible purpose or value. The triacylglycerols are present in seeds of Peanut which is responsible for inhibiting the lipase enzyme in pancreas and the low blood glucose level and low lipid level due to the efforts of peanut seed aqueous extract on rats have also been reported.

2.8.2 Saponins

The roots and rhizomes of many plants are rich in a important constituents namely Saponins.It contains sugars which is attached to steroid or triterpene and carries some biological effects. They are called secondary metabolites and decreases the activity of lipase and helpful for the treatment of reducing obesity.

2.8.3 Grape seed extracts

The extract from the grape seed contains phytochemicals. This phytochemicals reduce the activity of pancreatic lipase and lipoprotein lipase and it helps in reducing the fat absorption and its storage in adipose tissue. Further, GSE was shown to decrease isoproterenol-stimulated lipolysis in 3T3-L1 adipocytes. The flavonoids, procyanidins and their antioxidative metabolites together helps in decreasing the activity if lipase rather than from the activity of a single molecule. The most favourite and consumable by all over the people in world is M.indica is found in many tropical and subtropical climates. The extract from the leaves and stem of Mango were found to be a good PL inhibitors, because they included a range of polyphenols including phenolic acids, phenolic esters, flavan-3-ols and mangiferin. In models of obesity brought on by high-fat diets, these extracts also demonstrated antiobesity properties.

2.8.4 Nelumbo Nucifera

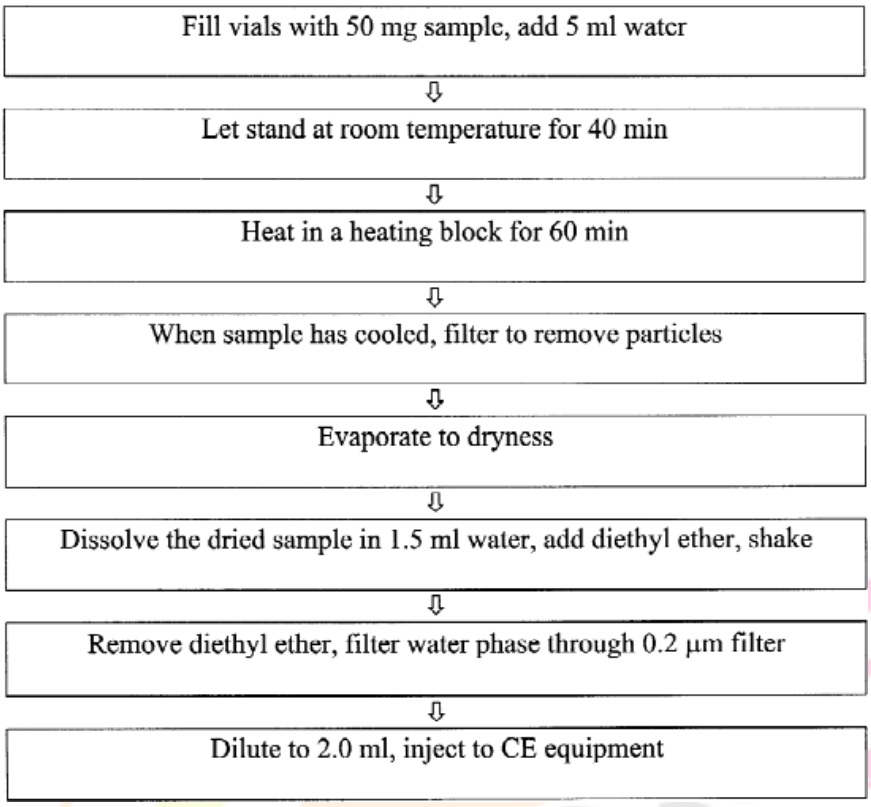
In China, blend tea or N.nucifera leaf extract has lately been utilized to treat obesity. N.nucifera leaf extracts (NNE) has an IC50 value of more than 0.40 mg/ml for inhibiting PL. The phenolic components of the leaves were considered to be responsible for the reduction of activity of enzyme. NNE may also boost thermogenesis by upregulating UCP3 expression. There has previously been information that many plants and their metabolites are pancreatic lipase inhibitors and this information can be used to produce safe and effective anti-obesity medicines. Common medicinal plants were chosen based on their potential to cause hyperlipidemia as in need to find new pancreatic lipase (PL) inhibitors. These were tested in vitro for anti-lipase activity,none of the other plants had been previously screened for anti-lipase activity.In past investigations,different models than those utilized in our study were used to screen plants. Both these plants showed increase in the activity in assay.

2.8.5 Fenugreek seeds

The fenugreek seed powder has the property of reducing the activity of lipase which has been reported by previous studies. But how it reduces the activity of lipase is not defined. This paper discussed about the activity of inhibiting the lipase by different extracts of fenugreek seeds. Initially they have prepared the crude extracts of fenugreek seeds. Petroleum ether, chloroform and 90% ethanol with varying polarity were used for the preparation of extracts and the activity of the extracts on pancreatic lipase was studied. Among all the extracts reviewed, ethanolic extract showed greatest inhibitory efficiency with IC50 value value of greater than 90.

The inhibiting activity due to methanol of the extracts of 98 plants- 60 leaves, 12 fruits, 4 seeds, 15 complete plants, 2 stems, 1 flower and 4 roots – on the porcine pancreatic lipase (PPL) were examined. Since this medication also suppresses pancreatic lipase activity in the duodenum, the inhibition rate of lipase was tabulated as percentage inhibition of lipase against the activity of inhibition of orlistat (anti-obesity drug). This study demonstrates that while many plants can inhibit lipase activity, analyzing the reports of plants with inhibiting property of lipase, some plants increase the activity of enzyme. Some of these plant extracts have a remarkable inhibitory effect (more than 80%), which can be used as anti obesity drugs.

2.9 Hot Water Extraction



2.10 Characterization of Lipase

In today's world, obesity is frequently brought on by a diet high in fat and a lack of exercise because of poor lifestyles. Obesity manifests an excessive fat absorption and accumulation. The primary organs for storing lipids are the liver and white adipose tissue. Non-alcoholic fatty liver and white adipose tissue frequently undergo excessive enlargement as a result of excessive lipid buildup. In adiition to an increased mortality rate, obesity and hyperlipidemia are medical diseases linked to a number of risk factors including insulin resistance, a more severe degree of liver fibrosis and injury reduced glucose tolerance and hypertension.

Studies on people with type 2 diabetes suggest that the onset of hepatic insulin resistance and type 2 diabetes is preceded by intramyocellular lipid accumulation and muscle insulin resistance. The metabolism of body fat is intimately linked to the emergence of obesity. 90% of the diet is made up of mixed triglycerides. The body cannot utilize exogenous fat directly instead it must be hydrolyzed before it can be absorbed. Gastric lipase, pancreatic lipase and tongue lipase are among the lipases found in the digestive system. It's common knowledge that gastric lipase regulates pancreatic lipase secretion and serves as a support mechanism for lipolysis. The most crucial of them is pancreatic lipase which has a direct impact on how well fatty acids are absorbed in the intestine. The major lipase released by the pancreas, pancreatic lipase (PL), hydrolyzes dietary lipase in the gastrointestinal tract, converting the triacylglycerol substrates found in ingested oils to monoglycerides and free fatty acids.

Monoglycerides and free fatty acids are then transported to enterocytes, the cells that line the intestines where they are eventually absorbed. The triglyceride-based lipid is first hydrolyzed by lipase to monoglyceride, glyceryl ester and free fatty acid after the fat-containing food is consumed by the human body. As a result the product has a larger amount of 1,2-glycolide and fatty acids. Although lingual lipase only degrades a very little amount of fat, it can destroy 50 -70% of the fat consumed by newborns and young children. Following hydrolysis by the lipases, gastric lipase (10-30% decomposition) and pancreatic lipase (50-70% decomposition) in the small intestine and digestive system, the

body subsequently produces cholesterol and lipoprotein. The small intestine absorbs lipid-mixed particles like bile acid and resynthesis of triacylglycerol stores energy in the form of adipose tissue.

2.11 Column chromatography

In organic chemistry research facilities, column chromatography is the method that is frequently employed for routine purification of synthetic intermediates. We present a lab experiment for undergraduates that makes use of this method. In the first semester of the sophomore organic laboratory course, experiments using qualitative thin-layer chromatography are frequently conducted. We have employed the separation of acetaminophen, aspirin, phenacetin and caffeine for many years as common analgesics. We have discovered that caffeine derived from tea can be purified using chromatography. With this experiment, it goes well.

If the TLC determinations are made during slow phases of the extraction (for example, while the hot aqueous extract is cooling), other procedures (column chromatography, TLC of analgesics) can be completed in a single 3-hour experimental period. Currently, we spend two lab sessions and include quantitative determination of the same "unknown" analgesie mixture using high performance liquid chromatography.

2.12 Chromatography

We utilized open glass tubes with a diameter of 19 mm and a length of 10 cm that were dragged down at one end to a drip tip. The column's base is filled with a small wad of glass wool that has been tamped down, followed by a sand layer that is 0.5 cm thick. The "silica gel for chromatography" (0.7 gram, 60-200 mesh) is added in a thin stream while being tapped on the column's side to release air bubbles. A second sand layer of 0.5 cm is applied on top of the silica gel once it is settled. The extraction byproduct is added to the column's top. The solvent is allowed to decrease until it reaches the top layer of sand. Rinse the evaporating flask with an 5 ml more of CH_2Cl and rinse well to the column at the bottom. Eluant in test tubes, held on rack, inverted in a column. It's crucial that we maintain order with these tubes. Following 5 ml of pure ethyl acetate, the column is eluted with 5%, 10%, 20% and 40% ethyl acetate.

The column is moved to a new receiving tube after each addition, allowing the solvent level to drop to the level of the top layer of sand. A small amount of air pressure can be supplied to the top of the column if elution is excessively slow. Make sure to release the air pressure before the solvent is applied below the sand level. TLC examines the test tube while comparing it to a sample of caffeine. If this is exercised, several tubes can be displayed on one plate. Marking the spruting softly with the pencil help. A sample should be evaporated to obtain early yellow fractions to determine whether the column has operated properly.

Combine the fractions that contain caffeine in a tapered round-bottom flask, rotary evaporator the solvent and weigh the fluffy white residue. The recommended amount of caffeine is 65 mg. Chromatography using silica gel. It should be noted that with the 1.9cm column, no measures should be made to the bottom of column. The eluting solvent reaches the top layer of sand before the column flow stops.

3. MATERIALS AND METHODS

3.1 Chemicals and Reagents

The lipase from porcine pancreas was purchased from Sigma brand. The substrate para nitrophenyl palmitate, Isopropanol, tris HCL, Triton X 100, sodium carbonate was used

3.2 Plant Sample Preparation

For the extraction of lipase inhibitor, the leaves of cashew, drumstick, fenugreek, keezhanelli, Neem were chosen as the source of lipase inhibitor. The fresh plant leaves were cut into small pieces for extraction.

3.3 Method of Extraction

The chopped leaves of each plant sample were packed in a bag of Whatman filter paper. Then it was subjected to Soxhlet extractor with 1 of Methanol as solvent. The temperature was set to 45-50℃ The extraction time was 7-8 hrs. The extract was then collected and filtered.

3.4 Lipase Inhibitor Assay

The substrate solution was prepared with 0.045g of para nitrophenyl palmitate dissolved in 15 ml of isopropanol in 0.3g Triton X 100 and 135 ml of 25mM Tris HCl buffer. 0.02307g of lipase is mixed in Tris HCl Buffer (pH 8.5) only freshly prepared enzyme solution should be used. 1.0599g of sodium carbonate solution was dissolved in 100ml of distilled water. Only freshly prepared solution should be used. The OD/min and percentage change was calculated as shown in Table- 2.

4. RESULTS AND DISCUSSION

4.1 Effect of Inhibitor – OD/min

Table-1 Cashew Extract

LEVEL OF INHIBITOR	OD/MIN	PERCENTAGE CHANGE /FOLD
T1(0.2)	0.0563	-474.49
T2(0.4)	0.0682	-595.92
T3(0.6)	0.0687	-601.02
T4(0.8)	0.0774	-689.78
T5(1.0)	0.0696	-610.20

From this, at higher concentration 0.2, 0.4, 0.6, 0.8 and 1.0 the cashew extract inhibiting the trypsin by 4.7, 5.9, 6.0, 6.8 and 6.1 times the fold decreases theactivity of enzyme.

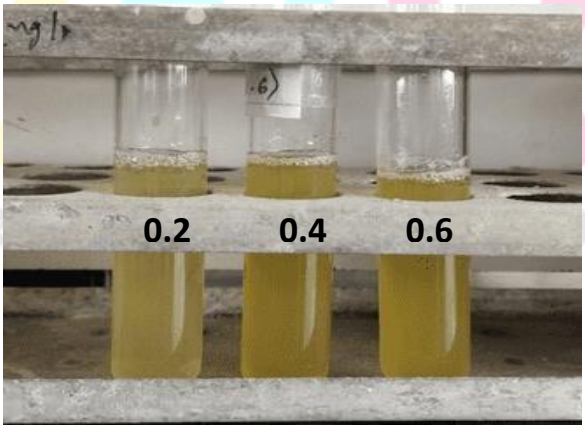


Fig-3 Assay for Cashew Extract

Table-2 Drumstick Extract

LEVEL OF INHIBITOR	OD/MIN	PERCENTAGE CHANGE
T1(0.2)	0.0075	23.46
T2(0.4)	0.0074	24.489
T3(0.6)	0.0554	-465.30
T4(0.8)	0.0621	-533.67
T5(1.0)	0.0237	141.83

From this, at concentration 0.2, 0.4 the drumstick extract inhibiting the trypsinby 23.46%, 24.489% and at higher concentration 0.6, 0.8 and 1.0 (4.6, 5.3, 1.4)times the fold decreases the activity of enzyme.

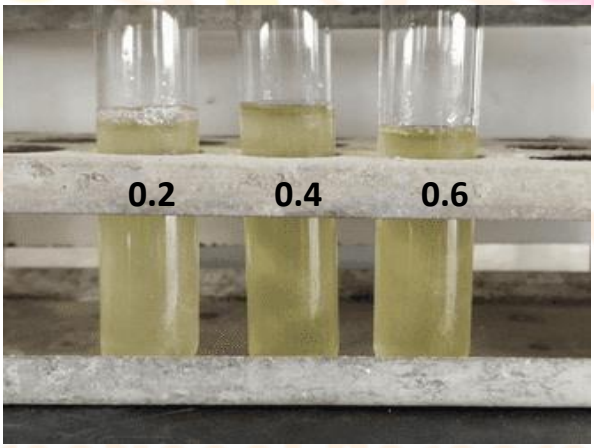


Fig-4 Assay for Drumstick Extract

Table -3 Fenugreek Extract

LEVEL OF INHIBITOR	OD/MIN	PERCENTAGE CHANGE
T1(0.2)	0.0144	-46.93
T2(0.4)	0.020	-104.08
T3(0.6)	0.0021	78.57
T4(0.8)	0.0107	-9.18
T5(1.0)	0.020	-104.08

From this, at concentration 0.2, 0.6 and 0.8 the fenugreek extract inhibiting thetrypsin by 46%, 78% and 9% and at higher concentration 0.4 and 1.0 (1.4 times) the fold decreases the activity of enzyme.

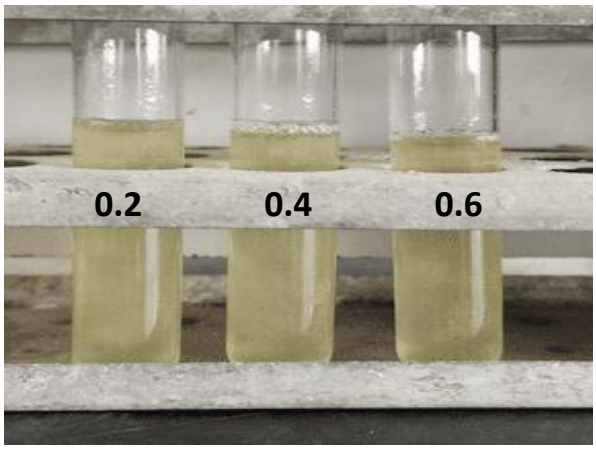


Fig-5 Assay for Fenugreek Extract

Table-4 Keezhanelli Extract

LEVEL OF INHIBITOR	OD/MIN	PERCENTAGE CHANGE
T1(0.2)	0.0802	-718.36
T2(0.4)	0.0776	-691.83
T3(0.6)	0.0877	-794.89
T4(0.8)	0.0701	-615.30
T5(1.0)	0.0883	-801.02

From this, at higher concentration 0.2, 0.4, 0.6, 0.8 and 1.0 the keezhaneli extract inhibiting the trypsin by 7.1, 6.9, 7.9, 6.1 and 8.0 times the folddecreases the activity of enzyme.

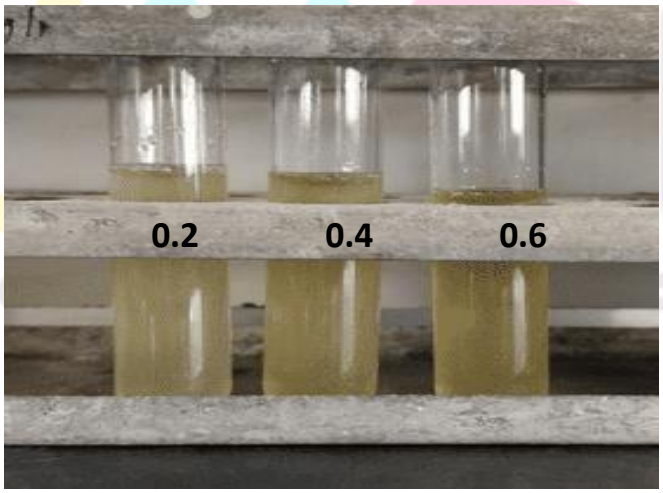


Fig-6 Assay for Keezhanelli Extract

Table-5 Neem Extract

LEVEL OF INHIBITOR	OD/MIN	PERCENTAGE CHANGE/FOLD
T1(0.2)	0.1068	-105.80
T2(0.4)	0.07	-650.65
T3(0.6)	0.0499	-742.56
T4(0.8)	0.048	-805.69
T5(1.0)	0.0461	-548.28

From this, at higher concentration 0.2, 0.4, 0.6, 0.8 and 1.0 the neem extract inhibiting the trypsin by 10.5, 6.5, 7.4, 8.0 and 5.4 times the fold decreases the activity of enzyme.

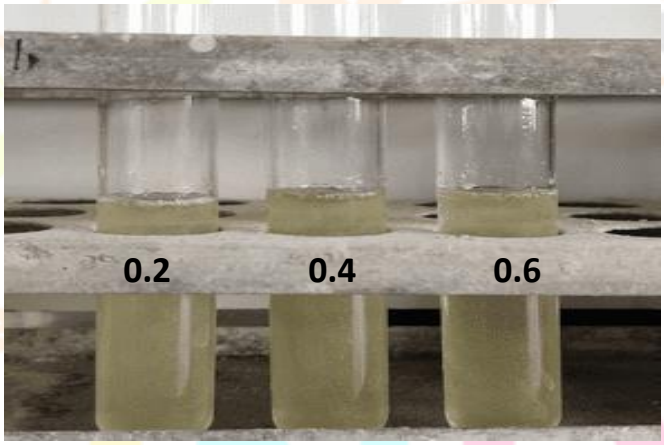


Fig-7 Assay for Neem Extract

4.2 Enzyme Assay without Inhibitor but with Methanol

The enzyme assay was carried out without inhibitor but with methanol to analyze the inhibition activity due to methanol. Approximately 10-15% methanol inhibiting the activity of enzyme which we can use for extraction of samples.

4.3 Extraction PF Inhibitor using Hot water

The five plant samples cashew, drumstick, fenugreek, keezhanelli, neem were subjected to Hot water extraction.

4.3.1 Inhibition of Lipase by Hot water Extracts of Cashew leaves

Table-6 Assay for Cashew Extract

Concentration (mg/ml)	Inhibition/Activation
0.2	94% Inhibition
0.4	86% Inhibition
0.6	78% Inhibition

0.8	75% Inhibition

At 0.2, the Cashew extract inhibited the lipase by 94%, at 0.4 by 86%, at 0.6 by 78%, at 0.8 by 75% the extract activates the enzyme completely.

4.3.2 Inhibition of Lipase by Hot water Extracts of Drumstick leaves

Table-7 Assay for Drumstick Extract

Concentration (mg/ml)	Inhibition/Activation
0.2	49% Inhibition
0.4	43% Inhibition
0.6	11% Inhibition
0.8	14% Inhibition

At 0.2, the drumstick extract inhibited the lipase by 49%, at 0.4 by 43%. The inhibition rate was decreased at increasing concentration.

For every 0.2 ml of extract added a 13.7% loss of inhibitory activity was observed. More studies have to be carried out to understand (a) inhibition at lower levels and (b) activation at higher levels.

4.3.3 Inhibition of Lipase by Hot water Extracts of Fenugreek leaves

Table-8 Assay for Fenugreek Extract

Concentration (mg/ml)	Inhibition/Activation
0.2	49% Inhibition
0.4	54% Inhibition
0.6	86% Inhibition
0.8	156% Inhibition

At 0.2, the fenugreek extract inhibited the lipase by 49%, at 0.4 by 56%. At 0.6 by 86%, at 0.8 the extract activates the enzyme completely.

4.3.4 Inhibition of Lipase by Hot water Extracts of Keezhanelli leaves

Table-9 Assay for Keezhanelli Extract

Concentration (mg/ml)	Inhibition/Activation
0.2	70% Inhibition
0.4	86% Inhibition
0.6	49% Inhibition
0.8	62% Inhibition

At 0.2, the keezhanelli extract inhibited the lipase by 70%, at 0.4 by 86%. The inhibition rate was decreased by increasing concentration.

4.3.5 Inhibition of Lipase by Hot water Extracts of Neem leaves

Table-10 Assay for Neem Extract

Concentration (mg/ml)	Inhibition/Activation
0.2	72% Inhibition
0.4	86% Inhibition
0.6	151% Inhibition
0.8	2.7% Inhibition

At 0.2, the neem extract inhibited the lipase by 72%, at 0.4 by 86%. The inhibition rate was decreased at increasing level of inhibitor.

5. CONCLUSION

In this project the leaves of five plants- cashew, drumstick, fenugreek, keezhanelli, neem are extracted using Soxhlet extractor using methanol as solvent. The extracts were analyzed for the activity of lipase inhibition. The lipase assay was carried out using spectrophotometer at 410nm and the results were tabulated (Table-1 to Table-5). From the analysis of lipase activity of each sample it is concluded that for cashew extract the activity was maximum at 0.4 concentration (69%). For drumstick extract the activity is maximum at 0.2 concentration (89%) and at highest concentration the activity is 69%. For fenugreek extract the activity is maximum at 0.4 concentration (89%) and at 0.2 concentartion the inhibiting activity is 81%. For keezhanelli extract the inhibiting activity is maximum at 0.2 concentration (13%). For neem extract the inhibition activity is maximum at 0.8 concentration (69%) and 67% at 0.4 concentration. Almost all the plant samples are inhibiting lipase. The variations in the inhibiting percentage may be due to some pH changes or instrumental errors. The enzyme assay was carried out without inhibitor but with methanol to analyze the inhibition activity due to methanol. Approximately 10-15% methanol inhibiting the activity of enzyme which we can use for extraction of samples.

6. REFERENCES

1. Chantre, P., & Lairon, D. (2002). Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. *Phytomedicine*, *9*(1), 3-8.

2. Fouad, M. F., Rastam, S., Ward, K. D., & Maziak, W. (2006). Prevalence of obesity and its associated factors in Aleppo, Syria. *Prevention and Control*, *2*(2), 85-94.

3. Hofbauer, K. G. (2002). Molecular pathways to obesity. *International Journal of Obesity*, *26*(2), S18-S27.

4.Shi, Y., & Burn, P. (2004). Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. *Nature reviews Drug discovery*, *3*(8), 695-710.

5. Foster-Schubert, K. E., & Cummings, D. E. (2006). Emerging therapeutic strategies for obesity. *Endocrine reviews*, *27*(7), 779-793.

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