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Research Article

In Vitro Anti-obesity, Antioxidant and Anti-Inflammatory Studies on the Selected Medicinal Plants

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ABSTRACT
Obesity is a significant risk factor for increased morbidity and mortality from cardiovascular disease and diabetes and it is also associated with many other medical conditions including cancer, liver and kidney diseases, sleep apnea, and depression. The inhibition of dietary fat absorption is a logical target for managing obesity and pancreatic lipase is a key enzyme involved in triglyceride absorption in the small intestine. Inhibitors of intestine lipases are suggested to function as antiobesity agents. Recently, studies have been intensified for new lipase inhibitors in natural resources with minimal adverse effect. In this view, attempts are made in the present study to evaluate antiobesity, antioxidant and anti-inflammatory activities of four medicinal plants namely *Acorus calamus, Alpinia galanga, Cinnamomum zeylanicum and Piper cubeba.* From the results obtained, it can be concluded that total phenol content was more in *Piper cubeba,* lipase inhibitory activity was high in *Acorus calamus,* DPPH radical scavenging activity was more in *Cinnamomum zeylanicum,* inhibition of lipid peroxidation and stabilization of RBC membrane was maximum for the extract of *Alpinia galanga.* Hence, preparation of herbal formulation using the investigated plant drugs will leads to the development of effective anti-obese drug.

Keywords: Indian medicinal plants, Anti-obesity, Antioxidant, Anti-inflammatory, lipase inhibitory activity.

INTRODUCTION

Obesity is becoming one of the greatest threats to global health in this century, with more than 1.5 billion overweight adults and at least 400 million of clinically obese subjects[1]. Due to these increasing obesity rates, the World Health Organization (WHO) has prompted to consider it as the epidemic of XXI century and to promote strategies to prevent and control its progress[2]. The development of obesity is characterized by a chronic imbalance between energy intake and energy expenditure[3], and it is often ascribed to changing lifestyles and inadequate dietary habits. Also, decreased energy expenditure is often associated with an inherited low basal metabolic rate, physical in activity, and low capacity for fat oxidation[4]. To reduce body weight and adiposity, a change in lifestyle habits is still the crucial cornerstone[5]. Physical activity might be helpful in the prevention of obesity by elevating the average daily metabolic rate and increasing energy expenditure[3]. Unfortunately, this clinical approach is not long-term lasting, and weight regain is often seen.

Epidemiological studies have shown a direct relation between the incidence of overweight/obesity and dietary fat consumption[4]. Humans are frequently exposed to fat rich foods, which are usually associated with a high-energy intake[6]. Thus, those foods with a high-energy and dietary fat content are considered to promote body fat storage and weight gain in humans[7]. One explanation is that, in commercially available food items, the percentage of energy derived from fat is highly correlated with energy density. Given that fat contains 9 kcal/g compared with 4 kcal/g for carbohydrates and proteins, foods rich in fat are often high in energy density. Thus, when a similar volume of food is consumed, energy intake will be higher in high-fat diets compared with low-fat diets[3].

On the other hand, independently of an increased energy intake, specific dietary constituents may promote the development of obesity. This statement means that even consuming an equal amount of energy, the diet composition is important, especially the balance between nutrients[8]. Thus, a macronutrient profile (high-protein, high-carbohydrate, and high-lipid diets) can affect diet-induced thermogenesis, the oxidation pathway, energy intake, gene expression, or the level of some hormones[9]. Following a high-fat diet, the diet-induced thermogenesis is lower than following high-protein and carbohydrate diets, and also fat is more effectively absorbed from the gastrointestinal tract than carbohydrates, which translates into lower energy expenditure when following a high-fat diet[6]. So, high-fat diets produce a metabolically more efficient state, at least in part because of the lower

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postprandial thermogenic effect of lipids in comparison with carbohydrates[10].

Furthermore, the consumption of a high-fat diet has the capacity to modulate the gastrointestinal responses to ingested fat and thereby, may lead to impairments in appetite regulation that favour the development of obesity. Dietary fat usually implies an increase in energy consumption because it has a lower potential for inducing satiety than carbohydrates and protein[4]. Hence, high-fat diets may play an important role in the increased prevalence of obesity and can be a triggering factor in the development of hyperglycemia and hyperinsulinemia[11]. Moreover, the intake of dietary fats is usually accompanied by a higher intake of refined sweet carbohydrates (fast food, desserts), where the high intake of sucrose promotes weight gain, visceral adiposity, and the development of diseases that are related with obesity, such as diabetes and cardiovascular diseases[12]. Therefore, low-fat diets often are prescribed in the prevention and treatment of overweight and obesity because a reduction in dietary lipids without restriction of total energy intake could cause weight loss[6].

Recent studies indicate that fat digestion is a prerequisite for the effects of fat on gastric emptying, gastrointestinal hormone secretion, appetite, and energy intake[4]. An increasing number of gastrointestinal enzymes involved in nutrient digestion are being identified and characterized, representing a rich pool of potential therapeutic targets for obesity and other metabolic disorders[13]. Especially interesting are those enzymes that are related with dietary fat, which includes pre-duodenal lipases (lingual and gastric lipases), pancreatic lipase (PL), cholesterol-ester lipase, and bile-salt stimulated lipase[14]. Most dietary fat is ingested as triglycerides (90–95%), and their hydrolysis starts in the mouth, then goes on through the stomach by an acid stable gastric lipase, and continues in the duodenum through the synergistic actions of gastric and colipase-dependent pancreatic lipases (PL), leading to the formation of monoglycerides and free fatty acids (FFA). FFA are absorbed by the enterocyte to synthesize new triglyceride molecules, which are transported to the different organs via lipoproteins, especially chylomicrons, after a meal[14].

Pancreatic lipase plays a key role in the efficient digestion of triglycerides[15]. It is secreted into the duodenum through the duct system of the pancreas and is responsible for the hydrolysis of 50–70% of total dietary fats[13]. This enzyme has been widely used for the determination of the potential efficacy of natural products as antiobesity agents[16]. Orlistat is currently the only clinically approved drug for obesity management in Europe. This molecule acts by inhibiting PL activity and the reduction of triglyceride absorption, and its long-term administration accompanying an energy restricted diet, results in weight loss[17]. Reduction on intestinal lipid digestion has been related to a decrease in the intra-abdominal fat content[5]. Thus, this compound is associated with a small, but statistically significant weight loss of about 3% more than diet alone in overweight and obese people[1]. In addition to losing weight, Orlistat within a prescribed limit has been shown to be safe and more effective than diet alone in modifying some of the risk of coronary artery disease and other obesity-related comorbidities. The most commonly reported adverse effects of Orlistat are a range of gastrointestinal side effects, including steathorrhsea, bloating, oily spotting, fecal urgency, and fecal incontinence, as well as hepatic adverse effects[18]. These adverse effects are similar to those observed for other lipase inhibitors tested in phase II studies, such as Cetilistat[19]. On the other hand, the inhibition of fat absorption could be accompanied by fat-soluble vitamin deficiencies, which could be prevented by the vitamin supplementation strategy, as other authors have recommended when vitamin deficiency occurs in patients undergoing Orlistat therapy[20]. Hence the interest is in the search for new natural substances that show potent inhibitory activity against PL and have fewer side effects than the current ones.

Drugs that prevent weight regain appear necessary in obesity treatment[5]. Thus, the development of natural products for the treatment of obesity is a challenging task, which can be launched faster and cheaper than conventional single-entity pharmaceuticals[21]. Many medicinal plants may provide safe, natural, and cost-effective alternatives to synthetic drugs[13]. Currently, one of the most important strategies in the treatment of obesity includes development of inhibitors of nutrient digstion and absorption. For example, acarbose is an antidiabetic drug that inhibits glycoside hydrolases, thus preventing the digestion of complex carbohydrates and decreasing postprandial hyperglycemia[22]. Similar compounds with alpha-amylase inhibiting activity that can be used for diabetes control are being isolated from different plants. The list includes valoneaic acid dilactone[23], obtained from banaba (*Lagerstroemia speciosa*), the ethanol extract obtained from chestnut astringent skin[24], or the purified pancreatic alpha-amylase inhibitor isolated from white beans (*Phaseolus vulgar*), which is able to reduce glycemia in both nondiabetic and diabetic rats[25].

In this context, since dietary lipids represent the major source of unwanted calories, the inhibition of fat digestion is an interesting approach for reducing fat absorption[26]. Orlistat is the only authorized antiobesity drug in Europe and has been shown to act through inhibition of pancreatic lipase (PL), which is a key enzyme for the digestion of dietary triglycerides[27]. Orlistat is the saturated derivative of lipstatin, an inhibitor of PL isolated from the bacterium *Streptomyces toxytricini*[28]. This molecule exerts a modest weight lowering effect when accompanying a suitable dietary advice. Thus, in a recent meta-analysis[18], the mean BMI change with Orlistat (120 mg three times daily) was a reduction of 0.83 kg m^{-2} (95% CI: 0.47–1.19) compared with placebo. Accompanying this antiobesity action, Orlistat is also able to modestly reduce blood pressure, improve oral glucose tolerance and prevent the onset of type 2 diabetes[29]. Now, extracts from hundreds of species of medicinal plants, vegetables, and fruits[30] as well as products from microorganisms[13], fungi[31] and marine algae[32] are being screened for potential lipase inhibitory

activity. Ideally, these treatments will be viewed as adjuncts to behavioral and lifestyle changes aimed at maintenance of weight loss and improved health[21].

Knowledge of herbs has been handed down from generation to generation for thousands of years. Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants, above all other agents, have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive. In the recent past there has been a tremendous increase in use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. In view of this, in the present study, attempts are made to evaluate the anti obesity, antioxidant and anti-inflammatory potentials of four medicinal plants namely *Acorus calamus, Alpinia galanga, Cinnamomum zeylanicum* and *Piper cubeba*, through *in vitro* experiments.

MATERIALS AND METHODS

Collection of plant materials

Fresh samples of *Acorus calamus* rhizome, *Alpinia galanga* rhizome, *Cinnamomum zeylanicem* bark, *Piper cubeba* fruit were purchased from local herbal market at Mannargudi, Thiruvaur Dt, Tamilnadu, India, which were carefully indentified with the regional floras. The medicinal plant materials were cut into pieces and washed thoroughly 2-3 times with running water and once with sterile distilled water, than the plant materials were air-dried blotter under shade.

Preparation of the extract

Aqueous extracts of selected plant materials were prepared according to the methodology of Indian Pharmacopoeia[33]. The shade dried plant materials were subjected to pulverization to get coarse powder. The powdered materials were subjected to aqueous extraction separately with water. These extract were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40º-50º). The aqueous extracts of medicinal plants were put in air tight containers separately and stored in refrigerator till the time of use.

Estimation of total phenol content

The total phenolic concentration of aqueous extract was estimated according to the modified method of Singleton et al.[34]. Extract (10 µl) was taken in a 96 well microplate and 25 µl of Folin reagent and 230 µl of 4.4% of Na_2CO_3 were added and incubated for 30 min in dark place. Then the absorbance was measured at 750 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). A calibration curve was prepared using standard gallic acid (100 – 1000 mg/L, R^2 = 0.9978) and used to express the results as gallic acid equivalents (GAE).

Assessment of antioxidant activity

The antioxidant activity was analyzed using DPPH free radical scavenging assay[35]. Extracts (10 µl) were taken in the 96 well microplate and 200 µl of DPPH solution (2.5 mg/100 ml) and incubated for 30 min in dark place. Then the absorbance was measured at 515 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). The radical scavenging activity of tested samples was calculated using the formula (Antioxidant activity = Abs control – Abs test / Abs control x 100) and expressed on percentage basis.

Lipid peroxidation inhibition assay

The MDA levels of plant extracts treated liver homogenate was analyzed according to the method of Yen and Hsieh[36]. Thin liver slice sample (0.2 g) was weighed and treated with 2 ml of PBS and 0.5 ml of extract for 2 h. Then the sample was homogenized with 2 ml of PBS and the liver homogenate (0.5 ml) was mixed with 1 ml of TBA and heated in a boiling water bath for 30 min and cooled to room temperature. The samples are subsequently centrifuged at 3000 rpm for 10 min and the absorbance of the supernatant was read at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane as a precursor of MDA and the TBARS values were then calculated using the standard curve and expressed as mg MDA equivalents per 100 g of sample.

Assessment of anti-inflammatory activity

The anti-inflammatory activity was evaluated using RBC membrane stabilization method[37]. Blood sample (2 ml) was collected from volunteer in a heparinized tube and washed with PBS twice and centifuged at 3000 rpm for 10 min (Centrifuge Make: Eppendorf, Model 5810-R). Then RBC was suspended in phosphate buffer and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml hypotonic solution and incubated for 30 min at room temperature. Then the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance was read at 560 nm using Micro plate reader (Make: Biotek, Model: Epoch). Based on the absorbance of extract and control, the membrane stabilization effect was calculated and expressed on percentage basis.

Pancreatic lipase inhibitory activity

The lipase inhibition activity of plant extract was determined as per the method proposed by Kim et al.[38]. In this assay, the porcine pancreatic lipase activity was measured using p-nitrophenyl butyrate (NPB) as a substrate. Lipase solution (1 mg/mL) was prepared in a 0.1 mM potassium phosphate buffer (pH 6.0). To determine the lipase inhibitory activity, 1 ml of extract were pre-incubated with 1 ml of lipase for 10 min at 37°C. The reaction was then started by adding 0.1 mL NPB substrate. After incubation at 37°C for 15 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer and the percentage of inhibitory activity was calculated.

RESULTS AND DISCUSSION

Obesity is a serious health problem. It is one of the most common and swiftly increasing diseases in today's world. Until the 20th century obesity was uncommon, however in 1997, the World Health Organization (WHO) formally recognized obesity as a global epidemic. Further, it has categorized obesity as one among the top ten health problems. An alarming rate of obesity prevalence has been observed globally over the past thirty years independent of

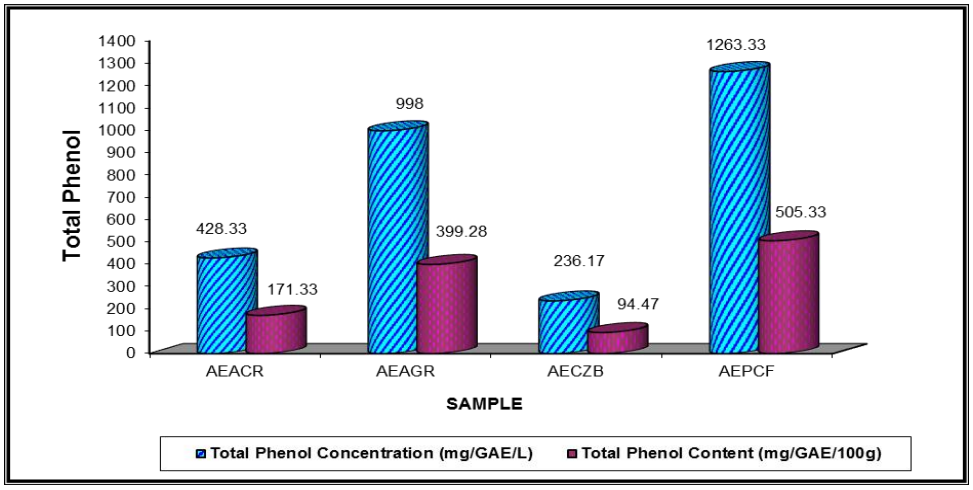

Figure 1: Total phenolic content of selected medicinal plants
AEACR: Aqueous extract of *Acorus calamus* rhizome, AEAGR: Aqueous extract of *Alpinia galanga* rhizome, AECZB: Aqueous extract of *Cinnamomum zeylanicem* bark, AEPCF: Aqueous extract of *Piper cubeba* fruit.

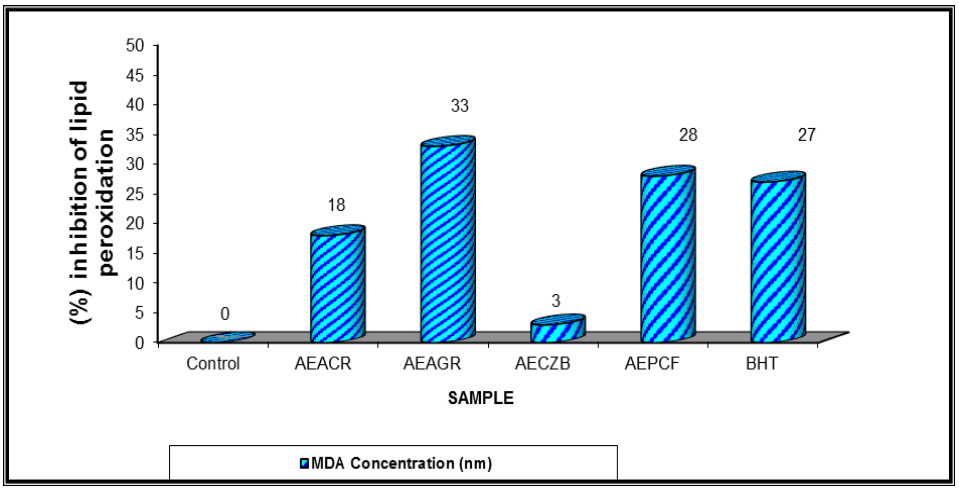

Figure 2: Inhibition of lipid peroxidation by the selected medicinal plants
AEACR: Aqueous extract of *Acorus calamus* rhizome, AEAGR: Aqueous extract of *Alpinia galanga* rhizome, AECZB: Aqueous extract of *Cinnamomum zeylanicem* bark, AEPCF: Aqueous extract of *Piper cubeba* fruit.

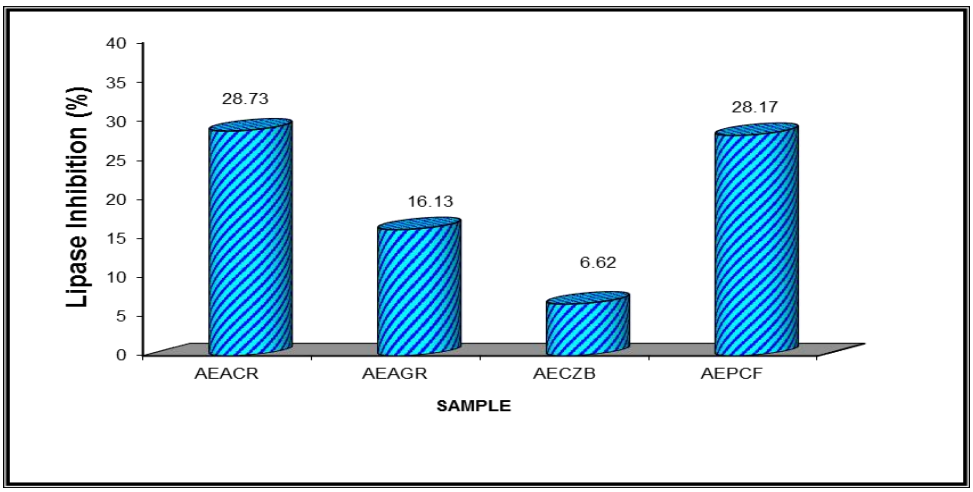

Figure 3: Pancreatic lipase inhibition by selected medicinal plants
AEACR: Aqueous extract of *Acorus calamus* rhizome, AEAGR: Aqueous extract of *Alpinia galanga* rhizome, AECZB: Aqueous extract of *Cinnamomum zeylanicem* bark, AEPCF: Aqueous extract of *Piper cubeba* fruit.

age, sex, race, socio-economic status, profession etc., creating a serious health concern and also becoming an epidemic of 21st century. It is estimated that there were about one billion of overweight individuals and more than 300 million who are obese. Obesity is mainly associated with modern life style, for this the modern medicine has little to offer mainly because of their non-availability and adverse side effects. Also the major concern is the problem of regaining weight upon cessation of treatment of the existing drugs. Therefore, it is necessary to reinforce an effective alternative therapy to relieve this metabolic disorder.

Total phenolic content

Phenolics include simple phenols, phenolic acid (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans, and lignins. These compounds are among the most widely occurring secondary metabolites in the plant kingdom, acting mainly as phytoalexins, attractants for pollinators, contributors to plant pigmentation, antioxidants, and protective agents against UV light, among others[39]. Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry. The Folin-Ciocalteu method is described in several pharmacopoeias[40]. The reaction forms a blue chromophore constituted by a phosphotungstic-phosphomolybdenum complex[39], where the maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds. Many studies have discussed the use of the Folin-Ciocalteau reagent to determine polyphenols.

Table 2 shows the results of Total phenol content of the aqueous extract of selected medicinal plants. In the present study, total phenol content was found to be maximum in the extract of *Piper cubeba* (505.33 mg GAE / 100 g). Total phenol content of *Alpinia galanga* was found to be 399.28 mg/GAE/100 g. Among the four plants, *Cinnamomum zeylanicum* possess lesser phenol content (94.47%).

DPPH Radical scavenging activity

The radical scavenging activity of different extracts was tested using methanolic solution of the stable free radical DPPH. Unlike laboratory generated free radical such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally fades/ disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attract on the DPPH molecules) and convert them to a colorless product (2,2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine) resulting in a decrease in absorbance at 518 nm[41].

Table 3 shows the results of DPPH radical scavenging activity of the aqueous extract of medicinal plants at various concentrations. The percentage of DPPH radical scavenging activity is found to be maximum at a concentration of 10 mg/mol of the plant extract. The free radical scavenging activity of the plant extract and the results are expressed as percentage of the ratio of decrease in absorbance at 518 nm to the absorbance of DPPH solution activity. In the present study DPPH radical scavenging activity of the four plants was evaluated at 5 different concentrations. All the selected plants exhibited good antioxidant activity in a dose dependent manner.

Lipid peroxide scavenging activity

Free radical induced lipid peroxidation has been associated with a number of disease process. The lipid peroxidation of the cell membrane has been associated with a number of pathologic phenomena such as cancer, diabetes mellitus, and inflammatory diseases. Table 4 shows the results of lipid peroxide scavenging activity of the aqueous extract of medicinal plants. The activity of the extract was compared with standard antioxidant BHT. In the present study, inhibition of lipid peroxidation was shown by *Alpinia galanga* (33%) least activity was recorded for *Cinnamomum zelanicum* (3%). The antioxidant activity of *Alpinia galanga and Piper cubeba* was found to be greater than the activity of standard antioxidant BHT.

Anti-inflammatory activity

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological function. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and sed the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers[42]. Natural products contributed significantly towards the

Table 1: Antioxidant activity of selected medicinal plants

S.No	Sample	Concentration (mg/ml)				
		10	5	2.50	1.24	0.63
1.	AEACR	66.59 ± 0.83	59.55 ± 0.83	43.49 ± 1.33	35.87 ± 2.16	22.04 ± 2.49
2.	AEAGR	80.89 ± 1.16	82.36 ± 0.25	75.79 ± 5.39	47.25 ± 2.65	25.97 ± 3.90
3.	AECZB	90.04 ± 0.83	84.17 ± 0.83	78.66 ± 1.33	71.04 ± 2.16	57.21 ± 2.16
4.	AEPCF	74.21 ± 1.66	68.23 ± 3.15	59.32 ± 4.48	47.25 ± 2.65	25.97 ± 3.90

AEACR: Aqueous extract of *Acorus calamus* rhizome, AEAGR: Aqueous extract of *Alpinia galanga* rhizome, AECZB: Aqueous extract of *Cinnamomum zeylanicem* bark, AEPCF: Aqueous extract of *Piper cubeba* fruit.

Table 2: Anti-inflammatory activity of selected medicinal plants

S.No	Sample	Concentration mg/ml				
		10	5	2.50	1.25	0.63
1.	AEACR	2.43 ± 0.22	-	-	-	-
2.	AEAGR	90.20 ± 0.09	82.22±0.57	76.31±5.27	63.00±0.39	48.00±0.12
3.	AECZB	6.62 ± 0.6	-	-	-	-
4.	AEPCF	2.35 ± 0.11	-	-	-	-

AEACR: Aqueous extract of *Acorus calamus* rhizome, AEAGR: Aqueous extract of *Alpinia galanga* rhizome, AECZB: Aqueous extract of *Cinnamomum zeylanicem* bark, AEPCF: Aqueous extract of *Piper cubeba* fruit.

development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse, and low cost. There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated[43].

Table 5 shows the results of inhibition of haemolysis and percentage of RBC membrane stabilization by the aqueous extract of medicinal plants at various concentrations. Among the four plants, *Alpinia galanga* was found to have maximum stabilization activity (90.20%) at a concentration of 10 mg/ml. All the other three have shown only negligible activity in stabilization of RBC membrane.

Inhibition of pancreatic lipase

Tremendous health concerns have been raised over a dramatic increase in the prevalence of obesity and related metabolic disorders. Majorly considered as a life style disorders of developed countries, obesity is prevailing at alarming speed in developing countries is because of industrialization, fast food intake, decrease in physical activity[44]. According to World Health Organization, 65 % of the world's population live in countries where over-weight and obesity kills more people than underweight. More than 1.4 million adults (age 20 and older) were overweight in 2008. Among them, over 200 million men and nearly 300 million women were obese[45]. A vast range of health problems co-exist with a weight problem and dysfunction of lipid homeostasis. This interlinked network of metabolic disorders and co-morbidities involve serious consequences in cardiovascular anomalies (heart failure, hypertension, pulmonary embolism etc.), endocrine imbalance (insulin imbalance, glucose intolerance, hypo-thyroidism etc.), arthiritis, urinary incontinence, gastrointestinal complications. Apart from obesity and related metabolic disorders disturb life style physically, financially and psychologically. Psychological effect like social discrimination, depression, physical inability etc. separates person from society[46].

The deeper understanding of the process of lipid homeostasis i.e. absorption, metabolism, storage, deposition and oxidation has presented a wide verity of enzymatic target involved. Dietary fats are mainly regarded as mixed triglyceride, which undergo a complex serious of biochemical reactions before absorption in the gastrointestinal tract. Pancreatic, endothelial, hepatic, lipoprotein lipases are members of the human lipase super family and possess structural similarity. Other tissues like lung, kidney, skeletal muscles, adipose tissue and placenta also secrete lipase enzymes. Pancreatic acinar cells secrete pancreatic lipase (triacylglycerol acyl hydrolase EC 3.1.1.3), an important enzyme of pancreatic juice responsible for digestion of dietary trigylcerides in the small intestine.

Gastric and lingual lipases are responsible for partial hydrolysis of dietary triacylglycerol into free acids and diacylglycerol. This partial digestion in stomach forms large fat molecule which undergoes emulsification with bile salts to form small droplets of fat. A physical property of emulsion influences the efficiency of digestion. In the emulsion, dietary triglycerides and diglycerides in the centre of droplet followed by a mixture of polar lipids, phospholipids, cholesterol, and free fatty acids and later coated with oligosaccharides, denatured proteins and bile salts, this forms very complex structure. The pancreatic lipase interacts with emulsion droplet which continuously change its physical properties as products formed, leaves the surface during the process of hydrolysis. Complete hydrolysis process results into free fatty acid, monoacylglycerols, diacylglycerols binds with cholesterol, bile salts, fat soluble vitamin and lysophosphatidic acid to form mixed micelles which can be absorbed by enterocytes. Pancreatic lipase uses a pancreatic protein colipase, as cofactor, to facilitate lipolytic activity. Phosphatidyl choline inhibits lipase-substrate complex. Colipase reverses to interact with the scarce surface of the substrate and stabilizes its conformation[47, 48].

In the present study, four plants namely *Acorus calamus*, *Alpinia galanga*, *Cinnamomum zeylanicum* and *Piper cubeba* have been evaluated for lipid lowering activity through percentage inhibition of pancreatic lipase. Table 1 shows the results of pancreatic Lipase inhibition of the aqueous extract of selected medicinal plants at various concentrations. From the data of the results obtained, maximum percentage of lipase inhibition was shown by *Acorus calamus* (28.73 %). The aqueous extract of *Cinnamomum zeylanicum* has shown minimum activity (6.62 %).

Pancreatic lipase inhibition is the most widely studied mechanism for the identification of potential anti obesity agents. Only one blockbuster drug, Orlistat approved by FDA and available for the obesity treatment apart from the

centrally acting antiobesity drugs, is acting through the pancreatic lipase inhibition. Discovery of orlistat was done from the naturally occurring molecule lipstatin. The success of naturally occurring compounds for treatment of obesity has influenced the research for the identification of newer pancreatic lipase inhibitors that lack unpleasant side effects. Till now, many plant extracts and isolated compounds were identified for the pancreatic lipase inhibition. Other than, many microbial products and isolated compounds, basic protamines, ε-polylysine[49], polysaccharides like chitosan[50], dietary fibers from wheat bran and cholestyramine[51], soya proteins[52], and synthetic compounds etc. have been studied for inhibitory potential against pancreatic lipase. However, plant microbial origin and reported for the pancreatic lipase inhibition.

CONCLUSION

Knowledge of herbs has been handed down from generation to generation for thousands of years. Herbal drugs constitute a major part in all traditional systems of medicines. Herbal medicine is a triumph of popular therapeutic diversity. Plants, above all other agents, have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive. In the recent past there has been a tremendous increase in use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. In view of this, in the present study, attempts are made to evaluate the anti obesity, antioxidant and anti-inflammatory potentials of four medicinal plants namely *Acorus calamus, Alpinia galanga, Cinnamomum zeylanicum* and *Piper cubeba* through *in vitro* experiments. From the results obtained, it can be concluded that total phenol content was more in *Piper cubeba*, lipase inhibitory activity was high in *Acorus calamus.* Antioxidant activity was high in *Cinnamomum zeylanicum* while inhibition of lipid peroxidation and anti-inflammatory activity were maximum in the extract of *Alpinia galanga*. Further in depth studies should be carried out to potentiate the use of these plants individually as antiobese, antioxidant and anti-inflammatory agents or formulation of effective drugs.

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REFERENCES

1. Drew B, Dixon A, Dixon J. Obesity management: update on orlistat. Vascular Health Risk and Management 2007; 3: 817-821.
2. Brug J, Crawford D. The obesity pandemic. Is it bad or worse? European Journal of Public Health 2009; 19: 570-571.
3. Schrauwen P, Westerterp KR. The role of high-fat diets and physical activity in the regulation of body weight. British Journal of Nutrition 2000; 84: 417-427.
4. Little T, Horowitz M, Feinle-Bisset C. Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. American Journal of Clinical Nutrition 2007; 86: 531-541.
5. Rubio M, Gargallo M, Millán A, Moreno B. Drugs in the treatment of obesity: sibutramine, orlistat and rimonabant. Public Health and Nutrition 2007; 10: 1200-1205.
6. Astrup A. The role of dietary fat in the prevention and treatment of obesity. Efficacy and safey of low-fat diets. International Journal of obesity 2001; 25: 46-50.
7. Moreno D, Ilic N, Poulev A, Brasaemle D, Fried S, Raskin I. Inhibitory effects of grape seed extract on lipases. Nutrition 2003; 19: 876-879.
8. Lomba A, Martinez JA, Garcia-Daz D, Paternain L, Marti A, Campion J, Milagro F. Weight gain induced by an isocaloric pair-fed high fat diet: a nutriepigenetic study on FASN and NDUFB6 gene promoters. Molecular Genetics and Metabolism 2010; 101: 273-278.
9. Hermsdorff HHM, Volp ACP, Bressan J. Macronutrient profile affects diet-induced thermogenesis and energy intake. Archieves in Latinoam Nutrition 2007; 57: 33-42.
10. Mobbs C, Mastaitis J, Yen K, Schwartz J, Mohan V, Poplawski M. Low-carbohydrate diets cause obesity, low-carbohydrate diets reverse obesity: a metabolic mechanism resolving the paradox. Appetite 2007; 48: 135-138.
11. Jager S, Trojan H, Kopp T, Laszczyk M, Scheffler A. Pentacyclic triterpene distribution in various plants – rich sources for a new group of multi-potent plant extracts. Molecules 2009; 14: 2016-2031.
12. Stevenson E, Astbury N, Simpson E, Taylor M, Macdonald I. Fat oxidation during exercise and satiety during recovery are increased following a low-glycemic index breakfast in sedentary women. Journal of Nutrition 2009; 139: 890-897.
13. Birari R, Bhutani K. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discov Today 2007; 12: 879-889.
14. Armand M. Lipases and lipolysis the human digestive tract: Where do we stand? Current Opinion in Clinical Nutrition Metabolism and Care 2007; 10: 156-164.
15. Lowe M. The triglyceride lipases of the pancreas. Journal of Lipid Research 2002; 43: 2007-2016.
16. Sugiyama H, Akazome Y, Shoji T, Yamaguchi A, Yasue M, Kanda T. Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. Journal of Agricultural and Food Chemistry 2007; 55: 4604-4609.
17. Neovius M, Johansson K, Rssner S. Head-to-head studies evaluating efficacy of pharmacotherapy for

obesity: A systematic review and meta-analysis. Obesity Reviews 2008; 9: 420-427.
18. Viner RM, Hsia Y, Tomsic T, Wong ICK. Efficacy and safety of anti-obesity drugs in children and adolescents: systematic review and meta-analysis. Obesity Reviews 2010; 11: 593-602.
19. Kopelman P, Bryson A, Hickling R, Rissanen A, Rossner S, Toubro S. Cetilistat (ATL-962), a novel lipase inhibitor: a 12-week randomized, placebo-controlled study of weight reduction in obese patients. International Journal of Obesity 2007; 31: 494-499.
20. Melia AT, Koss-Twardy SG, Zhi J. The effect of orlistat, an inhibitor of dietary fat absorption, on the absorption of vitamins A and E in healthy volunteers. Journal of Clinical Pharmacology 1996; 36: 647-653.
21. Moreno D, Ilic N, Poulev A, Raskin I. Effects of *Arachis hypogaea* nutshell extract on lipid metabolic enzymes and obesity parameters. Life Science 2006; 78: 2797-2803.
22. Yamagishi S, Matsui T, Ueda S, Fukami K, Okuda S. Clinical utility of acarbose, an alpha-glucosidase inhibitor in cardiometabolic disorders. Current Drug Metabolism 2009; 10: 159-163.
23. Hosoyama H, Sugimoto A, Suzuki Y, Sakane I, Kakuda T. Isolation and quantitative analysis of the alpha-amylase inhibitor in *Lagerstroemia speciosa* (L.) Pers. (Banaba). Yakugaku Zasshi 2003; 123: 599-605.
24. Tsujita T, Takaku T, Suzuki T. Chestnut astringent skin extract, an alpha-amylase inhibitor, retards carbohydrate absorption in rats and humans. Journal of Nutritional Science and Vitaminology 2008; 54: 82-88.
25. Tormo MA, Gil-Exojo I, de Tejada AR, Campillo JE. White bean amylase inhibitor administered orally reduces glycaemia in type 2 diabetic rats. British Journal of Nutrition 2006; 96: 539-544.
26. Bray G, Ryan D. Drug treatment of the overweight patient. Gastroenterology 2007; 132: 2239-2252.
27. McClendon K, Riche D, Uwaifo G. Orlistat: current status in clinical therapeutics. Expert Opinions in Drug Safety 2009; 8: 727-744.
28. Weibel EK, Hadvary P, Hochuli E, Kupfer E, Lengsfeld H. Lipstatin, an inhibitor of pancreatic lipase, produced by *Streptomyces toxytricini*. I. Producing organism, fermentation, isolation and biological activity. Journal of Antibiotics 1987; 40: 1081-1085.
29. Heymsfield SB, Segal KR, Hauptman J, Lucas CP, Boldrin MN, Rissanen A. Effects of weight loss with orlistat on glucose tolerance and progression to type 2 diabetes in obese adults. Archieves in Internal Medicine 2000; 160: 1321-1326.
30. Slanc P, Doljak B, Kreft S, Lunder M, Janes D, Strukelj B. Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. Phytotherapy Research 2009; 23: 874-877.
31. Slanc P, Doljak B, Mlinaric A, Strukelj B. Screening of wood damaging fungi and macrofungi for inhibitors of pancreatic lipase. Phytotherapy Research 2004; 18: 758-762.
32. Bitou N, Ninomiya M, Tsujita T, Okuda H. Screening of lipase inhibitors from marine algae. Lipids 1999; 34: 441-445.
33. Anonymous. Pharmacopoeia of India, Vol. 2, Edn 4, Published by Ministry of Health, Government of India, New Delhi, 1996, pp. 53–55.
34. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 1998; 299: 152-178.
35. Sanchez-Moreno C, Larrauri JA, Saura-Calixto FA. A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture 1998; 76: 270-276.
36. Yen GC, Hsieh CL. Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) towards various lipid peroxidation models in vitro. Journal of Agricultural and Food Chemistry 1998; 46: 3952-3957.
37. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti- inflammatory activity of methonal extract of *Oxalis corniculate* Linn. International Journal of Pharma and Pharmacological Sciences 2010; 2 (1): 146-155.
38. Kim YS, Lee YM, Kim H, Kim J, Jang DK, Kim JH, Kim JS. Anti-obesity effect of *Morus bombycis* root extract: Anti-lipase activity and lipolytic effect. Journal of Ethnopharmacology 2010; 130: 621-624.
39. Gottlieb OR, Borin MR. Medicinal products: Regulation of biosynthesis in space and time. Mem. Inst. Oswaldo. Cruz. 2000; 95: 115-120.
40. Farmacopeia Brasileira, 5th ed.; Agencia Nacional de Vigilancia Sanitaria: Brasilia, Brazil, 2010.
41. Yamaguchi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical scavenging activity of foods by using 2,2-diphenyl 1- 1-picryl hydrazyl. Bioscience, Biotechnology and Biochemistry 2002; 62: 1201-1204.
42. Tripathi S. Essential of medical pharmacology 7th Ed. New Delhi, Jaypee brother's medicinal publishers Private Ltd 2008, pp. 122-128.
43. Sangita C, Priyanka C, Protapaditya D, Sanjib B. Evaluation of *in vitro* anti- inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine 2012; 12: 178-180.
44. Cairns E. Obesity: the fat lady sings? Drug Discovery Today 2005; 10: 305–307.
45. WHO. Obesity and overweight. Geneva, Fact sheet No 311. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/ 2014; pp. 1341.
46. Aronne LJ. Classification of obesity and assessment of obesity-related health risks. Obesity 2002; 10: 105S-115S.
47. Shi Y, Burn P. Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. Nature Reviews on Drug Discovery 2004; 3: 695-710.
48. Mukherjee M. Human digestive and metabolic lipases-a brief review. Journal of Molecular Catalysis B: Enzymology 2003; 22: 369-376.

49. Tsujita T, Matsuura Y, Okuda H. Studies on the inhibition of pancreatic and carboxylester lipases by protamine. Journal of Lipid Research 1996; 37: 1481-1487.
50. Sumiyoshi M, Kimura Y. Low molecular weight chitosan inhibits obesity induced by feeding a high-fat diet long-term in mice. Journal of Pharmacy and Pharmacology 2006; 58: 201-207.
51. Lairon D, Lafont H, Vigne JL, Nalbone G, Lonardi J, Hauton JC. Effects of dietary fibers and cholestyramine on the activity of pancreatic lipase *in vitro*. American Journal of Clinical Nutrition 1985; 42: 629-638.
52. Roy DM, Schneeman BO. Effect of soy protein, casein and trypsin inhibitor on cholesterol, bile acids and pancreatic enzymes in mice. Journal of Nutrition 1981; 111: 878-885