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IN-VITRO ANTI-OBESITY ASSAY OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *CAMELLIA SINENSIS* LEAVES

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ABSTRACT

Obesity is one of the main public health problems in developed countries. It is considered to be a risk factor associated with the genesis or development of major chronic diseases, including cardiovascular disease, diabetes, and cancer. Many botanicals may provide safe, natural, and cost-effective alternatives to synthetic drugs. The results of this study show promise for the potential use of these plant materials as a lipase inhibitor in the treatment of obesity. The present study tested the hypothesis that the selected herbs/extracts can inhibit the enzymatic activity of LPL in vitro. Based on the literature review *Camellia sinensis* leaves was selected for the present study. Methanolic (MCS) and aqueous (ACS) extracts of *Camellia sinensis* leaves were prepared by soxhlet and hot reflux extraction methods respectively. These extracts were then examined for their *in-vitro* lipase inhibitory activity at a concentration level of 0.5, 1 and 2 g/l and their percentage inhibitory effect were reported and statistically analyzed. Results of the in-vitro lipase activity reveals that percentage inhibition of MCS was found to be more than that of ACS at all tested concentration levels. Among the methanolic extracts MCS 2 g/l showed maximum inhibition of pancreatic lipase enzyme i.e. 80%. Hence this extract can be considered as most effective extract for anti-obesity activity.

INTRODUCTION: Obesity is undoubtedly one of the most significant health care problems in the developed world today. The prevalence of obesity has substantially increased in the past 30 years in India. In many developed countries, more than half of the population is obese or overweight. In 2005, the World Health Organization estimated that there were 400 million obese adults worldwide, and predicted that the figure would increase to 700 million by 2015 [1].

Physiologically, obesity is a disarray of energy balance and primarily considered as a disorder of lipid metabolism [2]. A growing number of enzymes involved in lipid metabolic pathways are being identified and characterized. They represent a rich pool of potential therapeutic targets for obesity [3, 4]. Inhibition of PL (triacylgycerol acyl hydrolase), the principal lipolytic enzyme, synthesized and secreted by is one of the approaches for the development of newer antiobesity drugs. Tetrahydrolipstatin (Orlistat), a commercial anti-obesity drug, is a known pancreatic lipase inhibitor [6, 7].

Previously, we have given an account of the reported plants with antiobesity properties [8] and the various PL inhibitors reported from these natural sources [9]. Recently, much interest has been shifted on plant flavonoids that might be beneficial in reducing the risk of obesity [10]. Dietary catechins and anthocyanins significantly decrease the weight of abdominal adipose tissues [11, 12]. Accordingly, investigation on the metabolic effects of plant flavonoids might lead to more effective strategies for the treatment of obesity.

The health hazards like diabetes, obesity and metabolic related disorders are related to the dietary habits and most of the nutraceutical on the market focuses on these areas. Anthocyanin-rich berries or derived extracts, procyanidins rich grape seed, bilberry and cranberry extract are well known for their antioxidant and lipid lowering ability [13].

On a global scale, obesity has reached epidemic proportions and is a major contributor to the global burden of chronic disease and disability. Currently, more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese.

A variety of natural products, including crude extracts and isolated compounds from plants, can induce body weight reduction and prevent diet-induced obesity. Therefore, they have been widely used in treating obesity [14, 15, 16]. A wealth of information indicates numerous bioactive components from nature are potentially useful in obesity treatments.

Green tea is brewed from the unfermented dried leaves of the plant, *Camellia sinensis*. Like other natural products, the leaves of this plant contain an array of phytochemicals that vary in concentration by the harvest season, age of the plant, climate, environmental conditions and processing conditions[17, 18].

The three most common types of tea are green, oolong and black (others include yellow, white, compressed and flavoured teas). All use the same leaves of the same plant. Green tea is steamed (Japanese method) or roasted (Chinese method) very soon after picking to stop the oxidation process. Further distinctions are made to denote the size of the leaves used (the youngest, smallest leaves are generally held to have the highest quality flavour), and the region of origin. The leaves have been used intraditional Chinese medicine (TCM), and other medical systems to treat asthma, bronchodilator, angina pectoris, peripheral vascular disease, and coronary artery disease, antibacterial activity.

MATERIALS AND METHOD:

Collection, identification and processing of plant material: Leaves of green tea (*Camellia Sinensis*) were collected by farms of Ooty, Tamilnadu, India, in the month of November, 2009. It was identified and authenticated from National Institute of Herbal Science (PARC), Chennai, India. Certificate no. is PARC/2009/121.

The collected leaves were thoroughly washed with water to remove the adherent impurities. Dried leaves (shade dried) were pulverized using grinder and passed through sieve mesh size 60. The coarse powder thus prepared was subjected to extraction.

Extraction:

Alcoholic extraction: Powdered leaves were extracted with methanol by Soxhlet method for 4 hrs. The extract was filtered and concentrated with the help of rotavapor under reduced pressure and evaporated to a semisolid mass on a water bath.

Aqueous extraction: Powdered sample was taken and distilled water was added and refluxed for 1 hour. The extraction was performed three times. Contents were filtered and concentrated using rotavapor under reduced pressure and evaporated to a semisolid mass on a water bath.

Both extracts were dried at a temperature not more than 40°C in a hot air oven [19].

Phytochemical Analysis: The extracts were subjected for preliminary screening of phytochemicals such as alkaloids (Dragendorff's test), flavonoids (Shinoda test), catechins (Phloroglucinol test), tannins ($FeCl_3$ test), saponins (Foam test), sterols (Salkowski test), glycosides (Molisch test), and phenols (Folin's test) [20].

Enzymatic assay of lipase from Human Pancreas: The Extracts were further characterized by in-vitro lipase activity to access their anti-obesity potential. Measurement of pancreatic lipase activity *in-vitro* Lipase activity was determined by measuring the rate of release of oleic acid from triolein.

Continuous Spectrophotometric Rate Determination: A suspension of triolein (80 mg), phosphatidylcholine (10 g) and taurocholic acid (5 mg) in 9 ml 0.1M *N-Tris* (hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.0) containing 0.1M NaCl was sonicated for 5 min. Than this sonicated substrate suspension (0.1 ml) was incubated with 0.05 ml (final concentration 5 units per tube) pancreatic lipase and

0.1 ml of various concentrations of sample solutions for 30 min at 37 ºC in a final volume of 0.25 ml. Lipase activity was expressed as moles of oleic acid released per ml reaction mixture per min.

PIPETTED (IN MILLILITERS) THE FOLLOWING REAGENTS INTO SUITABLE CUVETTES:

	Test	Blank
Reaction Mixture (Substrate Suspension)	0.1	0.1
Equilibrate to 37°C. Then add:		
Pancreatic Lipase (Enzyme Solution)	0.05	------
Distilled Water	------	0.02
Test 1 ACS (0.5 g/l)	0.1	
Test 2 ACS (1.0 g/l)	0.1	
Test 3 ACS (2.0 g/l)	0.1	
Test 4 MCA (0.5 g/l)	0.1	
Test 5 MCA (1.0 g/l)	0.1	
Test 6 MCA (2.0 g/l)	0.1	

Calculations:

Units/ml enzyme =

$$\frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})\,(df)}{(0.001)\,(0.05)}$$

df = Dilution factor; 0.001 = Change in absorbance per unit; 0.05 = Volume (in milliliter) of enzyme used units/ml enzyme; Units/mg protein = mg protein/ml enzyme

RESULTS AND DISCUSSION:

Extraction Yield:

Extracts	% yield (%w/w)
Aqueous Extract (ACS)	11.34
Methanolic Extract (MCS)	15.12

Phytochemical Analysis:

Plant Constituents	Extracts	
	ACS	MCS
Carbohydrates	+VE	+VE
Glycosides	-VE	-VE
Protein and Amino Acids	-VE	-VE
Alkaloids	+VE	+VE
Phenol	+VE	+VE
Flavonoids	+VE	+VE
Phytosterols	+VE	+VE
Fats and Fixed oils	-VE	-VE
Saponins	+VE	+VE
Tannins	+VE	-VE
Sugar	+VE	+VE

Inhibition of Lipase Activity (*In-vitro* Anti-Obesity Assay): The aqueous and methanolic extracts (ACS, MCS) of *Camellia sinensis* was further characterized by an enzymatic assay method involving inhibition of LPL. As shown in **Table 1, fig. 1**, the methanolic extract of *Camellia sinensis* inhibited the lipase activity using triolein emulsified with lecithin.

TABLE 1: EFFECT OF VARIOUS EXTRACTS ON PANCREATIC LIPASE ACTIVITY

Sample	Concentration	Percentage of control
Control	(0.0 g/l)	100±0.0
Test 1 ACS	(0.5 g/l)	09±1.0
Test 2 ACS	(1.0 g/l)	08±1.6
Test 3 ACS	(2.0 g/l)	10±1.8
Test 4 MCS	(0.5 g/l)	70±1.2
Test 5 MCS	(1.0 g/l)	74±1.4
Test 6 MCS	(2.0 g/l)	80±1.4

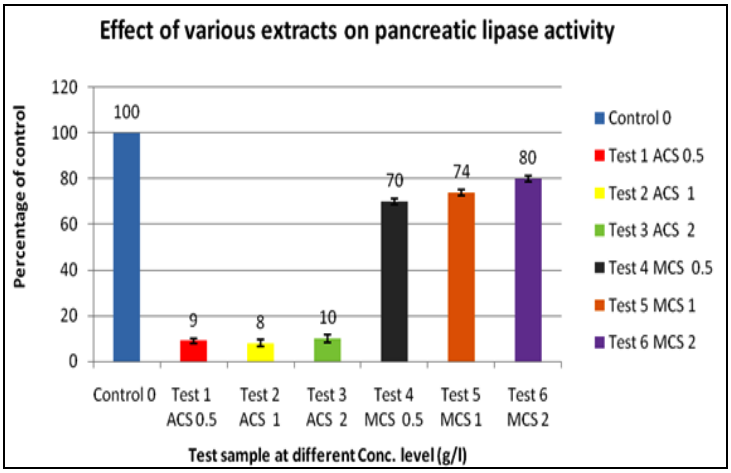

FIGURE 1: EFFECT OF VARIOUS EXTRACTS ON PANCREATIC LIPASE ACTIVITY

From the results of the lipase inhibition activity, it was observed that all the aqueous extracts of *Camellia sinensis* showed poor activity in comparison to methanolic extracts at various concentration levels. Among the methanolic extracts test sample 6 at the dose of 2g/l showed maximum inhibitory effect on lipase activity. Therefore, methanolic extract of *Camellia sinensis* at the dose of 2g/l can be considered as the best effective extract. However it was not more effective than orlistat at 0.076 µg/ml [21].

Conclusion: The result of the study reveals that methanolic extract of *Camellia sinensis* has a strong anti lipase activity at the tested dose level 2 g/l. The activity attributed may be due to the presence of different phytoconstituents like Carbohydrates, Alkaloids, Phenol, Flavonoids, Phytosterols and Saponins.

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