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Original Research Article

Evaluation of anti-lipase activity of crude plant extract

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Abstract

Obesity is a chronic health problem, known to be risk factor for the development of metabolic disorders, type 2 diabetes, systemic hypertension, cardiovascular disease, dyslipidemia and atherosclerosis due to imbalance in energy uptake and expenditure. In this study, we screened twenty crude plant extracts from five plants to test their anti-lipase activity using porcine pancreatic lipase assay (PPL; triacylglyerol lipase, EC3.1.1.3). Qualitative phytochemical screening of all plant extracts is positive for presence of phenolic compounds might contribute to the lipase inhibition activity. Among five plants and 20 plant extract, ethanolic extracts of *Cassia auriculata* (L.) Roxb and *Momordica charantia* L at 1mg/ml concentration showed high percentage 91.1±2.7% and 92±32% lipase inhibition using 2, 4-dinitrophenylbutyrate as a substrate in porcine pancreatic lipase assay with antioxidant activity less comparable to ascorbic acid.
Key Words: Anti-lipase; lipase inhibition; crude plant extract; anti-oxidant.

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INTRODUCTION

Obesity is a serious health problem worldwide resulting in several pathophysiological disorders such as type 2 diabetes, hyperlipidemia, cardiovascular disease, congenstive heart diseases and cancer. These are considered to be major risk factor associated with obesity, and has been reported due to increased intake of foods. It is chronic metabolic disorder caused by an imbalance between energy intake and expenditure. Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health[1-16]. Only two drugs are currently approved for long term obesity treatment, even though both of them have undesirable side effects. They either regulate food intake by acting on neural circuit (sibutramine[TM]) or reduce nutrient absorption from gut (orlistat[TM])[7]. Pancreatic lipase, the main lipid digesting enzyme that removes fatty acids from the α and α' positions of dietary triglycerides, which yield the lipolytic product β-monoglyceride and long chain saturated and polyunsaturated fatty acids. Inhibition of pancreatic lipase is an attractive targeted approach for potent anti-obesity agent for obesity treatment[11-12]. One of the screening strategies used in the discovery of anti-obesity drugs is to search for potent lipase inhibitors from plant extracts. Plants have been used as traditional natural medicines for healing many diseases. In particular, various oriental medicinal plants are reported to have biological activity[13-16]. In this study, we screened *Syzigium cumini* (L.) Skeels, *Cassia auriculata* (L.) Roxb., *Momordica charantia* L., *Tridax procumbans* (L.) L., *Physalis anguilata* L. crude extracts from natural sources as potential anti-obesity agents by monitoring their anti-lipase activity.

MATERIALS AND METHODS

Chemicals: All the chemicals were procured from HiMedia Mumbai, India

Collection of plant materials: The *Syzigium cumini* (L.) Skeels, *Cassia auriculata* (L.) Roxb., *Momordica charantia* L., *Tridax procumbans* (L.) L., *Physalis anguilata* L.was collected from area of idoli Tq. Sengaon dist. Hingoli, Maharashtra. All plant species were identified and authenticated by Dr. Tukaram D Kamble, voucher specimen deposited at **BAMU** herbarium

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Preparation of natural crude plant extracts: The plant materials were air dried and grounded into fine powder. The powdered material (10 gm) was extracted with 100 ml various solvent in the order of ethanol, methanol, 70% ethanol, and distilled water by maceration method[16]. All the above extracts were dissolved at a final concentration of 1 % DMSO solution that did not affect enzyme activity, for further processing likewise phytochemical screening, antioxidant activity and anti-lipase activity.

Phytochemical qualitative screening: The following are some examples of chemicals constituent of biological process alkaloids mayer's test[18], flavonoids alkaline reagent test[19], phenolic compound ferric chloride test[20], lead acetate test[18] and alkaline reagent test[18] were determined qualitatively.

Antioxidant activity: The % radical scavenging activity (RSA) activity of different extract was determined using DPPH assay with small modification[17]. The decrease of the absorption at 517 nm of the DPPH solution after addition of the antioxidant (plant extract) was measured in a cuvette containing 3 ml 0.1mM methanolic DPPH solution was mixed with 1 ml of plant extract having concentration of 1mg/ml. Blank containing 0.1mM methanolic DPPH solution without extract and vortex thoroughly, the set up was left at dark at room temperature. The absorption was monitored after 20min. ascorbic acid (AA) is used as reference. The ability to scavenge DPPH radical was calculated by the following equation. % of DPPH radical scavenging activity (% RSA) = $Abs_{control}$ - Abs_{sample} × 100

$Abs_{control}$

$Abs_{control}$ is the absorbance of DPPH radical + ethanol; Abs_{sample} is the absorbance of DPPH radical + plant extract. Measurements were performed in the triplicate. Absorbance values were corrected for radical decay using solutions.

Lipase inhibition assay: The method used for measuring the pancreatic lipase activity was modified from the previously described method[11-13]. Porcine pancreatic lipase (PPL, type II) activity was measured using *p*-nitrophenyl butyrate (*p*-NPB) as a substrate. PPL stock solutions (1 mg/ml) were prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at −20 °C. To determine the lipase inhibitory activity, the extracts (final concentration 1 mg/ml) were pre-incubated with PPL for 1 h in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before assaying the PPL activity. The reaction was then started by adding 0.1 µl NPB as a substrate, all in a final volume of 100 µl. After incubation at 30 °C for 5 min, the amount of *p*-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer. The activity of the negative control was also examined with and without an inhibitor. The inhibitory activity (I) was calculated according to the following formula: Inhibitory activity (I %) = 100 − ((B − b) / (A − a) × 100) Where A is the activity without inhibitor; a is the negative control without inhibitor; B is the activity with inhibitor; and b is the negative control with inhibitor. DMSO was used as negative control and its activity was also examined.

RESULTS AND DISCUSSION

Dietary fat is absorbed by the intestine when it has been subjected to the action of pancreatic lipase. Pancreatic lipase is a key enzyme in dietary triacylglycerols absorption, hydrolyzing triacylglycerols to monoacylglycerols and fatty acids. Therefore inhibition of pancreatic lipase is suggested to be an effective therapy in the regulation of obesity[1-8]. Currently available anti-obesity medications attack the body fat dilemma in three different ways. They can stimulate metabolism, suppress appetite, affect serotonin, or they can impede digestion of fat[4]. Few substances interact directly with the lipases as orlistat. It is a derivative of the naturally occurring lipase inhibitor from *Strptomyces toxytricini*[3]. The potential of natural products for treating obesity is under exploration. This may be an excellent alternative strategy for developing future effective, safe anti-obesity drugs[1]. Plants showing anti-obesity potential mainly belong to the family Leguminaceae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae and Araliaceae[2]. There have been reports that naturally occurring polyphenols can inhibit pancreatic lipase and thereby influence fat digestion and affect energy intake[10]. Previous research also reported that flavonoids and alkaloids be able to inhibit pancreatic lipase[5]. Phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of newer therapeutics. As part of the continuing search for biologically active anti-obesity agents from natural herbal resources, various plants have been screened for their anti-lipase activity[13]. We screened some plants of the above mentioned families and some from other namely *Syzigium cumini* (L.) *Skeels*, *Cassia auriculata* (L.) Roxb., *Momordica charantia* L., *Tridax procumbans* (L.) L., *Physalis anguilata* L.

Table 1: Plant names and parts used

Sr. No.	Common name	Botanical name	Family	Part used
1	Jambhul	*Syzigium cumini* (L.) Skeels	Myrtaceae	Roots
2	Aura/ Kashid	*Cassia auriculata* (L.) Roxb.	Caesalpiniaceae	Leaf
3	Karali/ Karela	*Momordica charantia L.*	Cucurbitaceae	Seeds
4	Kodsan/Jakhamjodi	*Tridax procumbans* (L.) L	Asteraceae	Leaf
5	Matkifod/ Popati	*Physalis anguilata* L.	Solanaceae	Flowers

Table 3.1: Botanical names, families and parts used

Table 2: Phytochemical screening

Extracts		Flavonoids		Phenolic compounds			Alkaloids
		Shinods' test	Flavonoids alkaline reagent test	$FeCl_3$ test	Lead acetate test	alkaline reagent test	Mayer's test
Syzigium cumini (L.) Skeels	Ethanol	-	+	+	+	+	+
	70% ethanol	-	+	+	-	+	-
	Methanol	-	+	+	+	+	+
	Distilled water	-	+	+	+	-	-
Cassia auriculata (L.) Roxb.	Ethanol	-	+	+	+	-	+
	70% ethanol	-	+	+	-	+	+
	Methanol	-	+	+	-	+	+
	Distilled water	-	+	+	+	-	+
Momordica charantia L.	Ethanol	-	-	+	-	+	-
	70% ethanol	-	+	+	-	+	-
	Methanol	-	-	+	+	+	-
	Distilled water	-	-	-	+	-	-
Tridax procumbans (L.) L	Ethanol	-	+	+	-	+	+
	70% ethanol	-	+	+	+	-	+
	Methanol	-	+	+	+	+	+
	Distilled water	-	+	+	+	-	+
Physalis anguilata L.	Ethanol	-	-	+	-	-	-
	70% ethanol	-	-	+	-	+	+
	Methanol	-	+	+	-	+	+
	Distilled water	-	+	+	-	-	+

Table 3.2: phytochemical screening of plant extracts (+ = presence, - = absence)

Phytochemical qualitative analysis shown in table 3.2, all the extracts are rich in the phenolic compounds as almost every extract showing positive results of the test performed and shows presence of flavonoids in some extracts hence this may contribute to the activities as obtained through the investigation of present work

Table 3: Anti-oxidant activity

Extracts	*Syzigium cumini* (L.) Skeels	*Momordica charantia L.*	*Cassia auriculata* (L.) Roxb	*Tridax procumbans* (L.) L	*Physalis anguilata* L.
Ethanol	62.7±2.8	61.6±2.5	64.3±2.2	68.7±1.9	75.2±2.1
70% ethanol	70.2±3.2	59.9±2.6	68.2±1.4	61.8±2.3	82±3.3
Methanol	70.2±1.9	69.4±1.9	58.0±1.5	63.3±2.4	74.1±3.1
Distilled water	65.3±1.6	71.1±1.6	69.3±2.1	72.5±2.6	71±2.6
Ascorbic acid	86.53±1.1	86.53±1.1	86.53±1.1	86.53±1.1	86.53±1.1

Table 3.3: Percent (%) radical scavenging activity of plant extracts (values are mean ± S.D.) Antioxidant activity of the test extracts and ascorbic acid was performed using DPPH radical scavenging assay. The standard (Ascorbic acid) and all extracts are compared at the same concentration 1mg/ml. Anti-oxidant activity in table 3.3 indicates that ascorbic acid is very potent antioxidant in comparison with all extracts. Only 70% ethanolic extract of *Physalis anguilata* L shows 82±3.3 % radical scavenging activity lesser than ascorbic acid.

Table 4: Anti-lipase activity

Extracts	*Syzigium cumini* (L.) Skeels	*Momordica charantia L.*	*Cassia auriculata* (L.) Roxb	*Tridax procumbans* (L.) L	*Physalis anguilata* L.
Ethanol	69.15±2.6	72±2.2	91.1±2.7	89±2.4	57±1.8
70% ethanol	59.96±1.8	92±3.2	33.5±1.6	73±2.3	66±1.9
Methanol	69.51±1.9	62.2±1.7	53.6±2	36±1.4	35±1.3
Distilled water	84±2.4	89.83±2.6	31.8±1.9	46±1.7	43±1.6

Table 3.3: Lipase inhibition (values are mean ± S.D.) The results of pancreatic lipase inhibition by various plant extracts at the concentration of 1mg/ml have been summarized in table 3.3, where lipase inhibition is expressed in percentage. Evaluating the results, 13 extracts out of 20 can be regarded as poor lipase inhibitors (less than 70%)[16]. Only 4 extracts shows moderate anti-lipase activity greater than 70% inhibition. The highest lipase inhibition showed by 70% ethanolic extract of *Momordica charantia L.* is 92±3.2% and ethanolic extract of *Cassia auriculata* (L.) Roxb is 91.1±2.7%. These are considered to be potent ant-lipase agent for antiobesity target. Followed by distilled water extracts of *Momordica charantia L.* is 89.83±2.6% ethanol extract of *Tridax procumbans* (L.) L is 89±2.4% and distilled water extract of *Syzigium cumini* (L.) Skeels is 84±2.4 %. The result suggest these plants *Momordica charantia L.* and *Cassia auriculata* (L.) materials will prove a good source of effective crude drug for the treatment of obesity caused by a high fat diet. However, further biological investigations are needed, using animal models, to verify the inhibitory activities under in vivo conditions. These two plants will be examined for bioactive phytochemical compounds to further define the nature of their lipid lowering activity[16]. Further detailed analysis of individual constituent for the bioactive compound in its pure form through separation techniques is required in order to prove a therapeutic agent for obesity treatment.

CONCLUSION

In the study, we screened crude plant extracts anti-lipase activity from five plants. Two plant extracts were excellent in lipase inhibition. As the earlier report suggest this inhibition may be due to the phytochemicals present in extracts, suggesting their use as crude anti-obesity target. Our result showing a correlation between alkaloids, flavonoids, phenolic compound and the lipase inhibition which provides the strong support that these phytochemical compounds are key agents for pancreatic lipase.

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