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ANTI-PANCREATIC LIPASE ACTIVITY OF LEAVES OF *OPERCULINA TURPETHUM*

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ABSTRACT

Hyperlipidemia is one of the greatest risk factors in the development of atherosclerosis and coronary heart disease (CHD). It is expected that there would be around 62 million patients with CHD by 2015 in India. Pancreatic lipase is a primary enzyme which hydrolyzes dietary fat molecules in digestive system converting triglyceride to monoglyceride and free fatty acids. Agents which inhibit pancreatic lipase play an important role in treatment of hyperlipidemia. Phytochemical studies and pancreatic lipase inhibitory activity was carried out in different extracts of leaves of *Operculina turpethum* at different concentrations. The inhibition of lipase by the different extracts were concentration dependent and ethanol showed an maximum inhibition of 85.24% at concentration of 100µg/ml. Phytochemical studies showed the presence of alkaloids, glycosides, phenolics, terpenoids, phytosterols and saponins in the different extracts. Ethyl acetate and ethanol extracts showed the presence of maximum phytoconstituents. The presence of these phytoconstituents may make this plant to be of value in treating hyperlipidemia.

INTRODUCTION

Hyperlipidemia is a metabolic disorder characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentration of total cholesterol, low density lipoprotein, very low density lipoprotein and triglycerides with a concomitant decrease in concentration of high density lipoprotein in blood circulation.[1, 2] It is a major cause of atherosclerosis and atherosclerosis associated conditions such as Coronary heart disease, Ischemic cerebrovascular disease and Peripheral vascular disease.[3, 4] According to WHO, high blood cholesterol level leads to approximately 56% of cardiac disease worldwide and about 4.4 million deaths each year.[5] Controlling the lipid absorption offers a possible way to prevent hyperlipidemia. The process of lipid absorption is divided into four steps. In the first step, fat and cholesterol form droplets by amphipathic molecules. In the second step, droplets are hydrolysed by pancreatic lipase into fatty acids and monoglycerides.[6] Then the hydrolysed products are solubilized by bile acids to form micelles which facilitates absorption.[7] Naturally, the enterohepatic circulation of bile acids interrupts to impact the micelle formation and subsequently reduce the absorption of fat and cholesterol. In the final step, the transportation of hydrolyzed products occurs.[8] Hence inhibition of pancreatic lipase plays an important role in the treatment of hyperlipidemia.

Currently available allopathic drugs used in treatment of hyperlipidemia are associated with number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. [9] Medicinal plants play a major role in treatment of hyperlipidemia. Literature survey suggests that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine.[10] A herbal treatment for hyperlipidemia is expected to have minimal side effects and is relatively cheap and locally available. [11]

The plant *Operculina turpethum* is an important herb used in Ayurvedic system of medicine since ages. Root, bark, stem and leaves of this herb have high medicinal value. [12] It is abundantly found in India, Australia, China, Srilanka and Pacific Islands. In India, it is found throughout the country up to an altitude of 900m and is occasionally grown as an ornamental garden plant.[13,14] It has traditional and folklore claims of usefulness in Ulcer, Diabetes, Hyperlipidemia, Cancer, Constipation and Liver disorders.[15] *Operculina turpethum* (L) Silva Manso belongs to the family Convolvulaceae. It is properly known as Indian jalap or transparent wood rose. It is large stout perennial climbers with winged stem, fleshy branched roots with funnel shaped flowers. Leaves are simple, alternate and scabrous texture with acuminate apex, smooth on both surfaces with sinuate and dentate margin. Stem is very long, twinning and much twisted, angled, and winged.

[16] The present study was aimed to investigate the pancreatic lipase inhibitory activity of various extracts of leaves of *Operculina turpethum*.

MATERIALS AND METHODS

Collection of plant materials

The plant material was collected from the local areas of Mekkarai, Tenkasi, Tirunelveli district, Tamilnadu and authenticated by V. Chelladurai, Research officer- Botany (Scientist-C), Tirunelveli. The plant material was authentified as *Operculina turpethum* of family Convolvulaceae. The leaves of the plant were separated, washed and then dried in shade. The dried leaves were coarsely powdered and used for further studies.

Preparation of extracts [17]

Continuous hot percolation method

The coarsely powdered leaf material of *Operculina turpethum* was extracted successively with Petroleum Ether, Ethyl Acetate and Ethanol using Soxhlet apparatus by continuous hot percolation method for 48 hours. The solvents were completely recovered from the collected extract under reduced pressure by distillation. The concentrated extracts were dried on a water bath and preserved in vacuum dessicator for further studies.

Cold maceration

The dried marc obtained from the ethanol extract was charged in an aspirator bottle and extracted with 800ml of water by cold maceration method for 7days. After decanting and filtering, nearly 80% of the solvent was removed by distillation over boiling water bath and the remaining under reduced pressure. The extract obtained was further dried in vaccum dessicator and this extract was used for further studies.

Preliminary phytochemical analysis [18, 19]

The various extracts were subjected to chemical test for detection of various phytoconstituents.

Pancreatic lipase inhibitory activity [20]

Extraction of lipase from chicken (*Gallus domesticus*) pancreas

Pancreas of freshly slaughtered chicken were collected from slaughter house, washed and placed in ice cold sucrose solution (0.01M). The pancreas were homogenized in 0.01M sucrose and centrifuged. The supernatant was separated and subjected to ammonium sulphate precipitation (50% saturation). The pellet obtained after centrifugation was dissolved in sucrose solution and again saturated to 50% ammonium sulphate saturation and centrifuged. The pellet obtained was dissolved in phosphate buffer and used as enzyme source.

Determination of chicken pancreatic lipase activity

Lipase inhibitory activity of different type of extracts was tested by mixing 25-100µg/ml of extract, 8ml of olive oil and 1ml of chicken pancreatic lipase followed by incubation for 60 minutes. The reaction was stopped by adding 1.5ml of a mixture containing acetone and 95% of ethanol (1:1). The liberated fatty acids were determined by titration against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as indicator. Blank was also performed without adding the extracts.

Percentage inhibition of lipase activity was calculated using the formula

Lipase inhibition= [A-B / A] X 100

A= Lipase activity of control

B=Activity of lipase when incubated with the extract.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Phytochemical analysis of the various extracts of leaves of *Operculina turpethum* revealed the presence of alkaloids, flavonoids, glycosides, phytosterols, phenols, saponins, terpenoids and tannins. A wide variety of secondary metabolites were present in Ethyl acetate and Ethanol extracts. These may be responsible for the folklore claims and traditional uses of *Operculina turpethum*. (Table.1)

Anti-lipase activity of various extracts of leaves of *O.turpethum*

The inhibitory activity of pancreatic lipase was studied using different concentrations of 25, 50, 75, 100 µg/ml for various extracts. From the study, it was found that hexane, ethyl acetate and aqueous extract showed 44.26%, 63.11% and 53.27% inhibition of lipase respectively. The maximum inhibition was observed in ethanol extract which showed an inhibition of 85.24%. (Table.2)

Table.1: Phytochemical screening of leaves of *Operculina turpethum*

S.No	Phytochemical Tests	Hexane extract	Ethyl acetate Extract	Ethanol extract	Aqueous extract
1	Alkaloids	-	-	+	-
2	Flavonoids	-	+	+	-
3	Glycosides	-	+	+	+
4	Phytosterols	+	+	+	-
5	Phenols	-	+	+	+
6	Saponins	-	+	+	+
7	Terpenoids	-	+	+	-
8	Tannins	-	+	+	+

+ indicates presence - indicates absence

Table.2: *In vitro* inhibition of chicken pancreatic lipase activity

S.no	Concentration (µg/ml)	Anti- lipase activity				% Inhibition			
		Hexane	Ethyl acetate	Ethanol	Aqueous	Hexane	Ethyl acetate	Ethanol	Aqueous
1	25	8.8±0.2	6.5±0.1	3.8±0.4	6.7±0.5	27.86	46.72	68.85	45.08
2	50	7.9±0.3	6.2±0.3	3.1±0.3	6.5±0.5	35.24	49.18	72.95	46.72
3	75	7.2±0.2	5.1±0.3	2.4±0.3	6.2±0.1	40.98	58.19	80.32	49.18
4	100	6.8±0.5	4.5±0.4	1.8±0.4	5.7±0.3	44.26	63.11	85.24	53.27

Values are expressed as Mean ± S.E.M

CONCLUSION

The study was carried out to find whether the leaves of the plant *Operculina turpethum* possess any anti-hyperlipidemic activity. The phytochemical studies showed that both ethyl acetate and ethanol contains several phytoconstituents such as alkaloids, flavonoids, glycosides, phytosterols and phenolics and saponins. The *in vitro* pancreatic lipase inhibitory activity also showed that all the extracts possess this activity. Ethanol extract showed maximum activity at all concentrations studied ie, 25, 50, 75, 100µg/ml. The highest concentration (100 µg/ ml) showed 85.24% inhibition of pancreatic lipase. The presence of phytoconstituents in the ethanol extract could be responsible for its lipase inhibitory activity. Further *in vivo* studies of the ethanol extracts will help in confirming the antihyperlipidemic activity of the plant. Hence we conclude from this study that the ethanol extract of *Operclina turpethum* inhibits the activity of pancreatic lipase which indicates its protective role in treating hyperlipidemia. This also correlates with the fact that, of all the extracts, ethanol extract contains several phytoconstituents.

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