

# Anti-obesity (Pancreatic lipase inhibitory) activity of *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae)

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## ABSTRACT

**Introduction:** Obesity has increased at an alarming rate and is now a worldwide health problem. *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae) is a foliose lichen and grow luxuriantly in tropical Himalayas, central India and higher altitudes of southern India. In this study, we report Anti-obesity activity, in terms of pancreatic lipase inhibitory activity, of methanol extract of *E. cirrhatum* for the first time. **Methods:** The powdered lichen material was extracted with methanol in soxhlet apparatus. The extract was tested for secondary metabolites by standard phytochemical tests and thin layer chromatography (TLC). Lipase enzyme was obtained from the chicken pancreas. Lipase inhibitory activity of different concentrations of methanol extract was determined in terms of inhibition of lipase activity using olive oil as substrate. **Results:** The extract was found to inhibit activity of chicken pancreatic lipase and the effect was found to be concentration dependent. Phytochemical analysis revealed the presence of alkaloids, saponins, tannins and terpenoids. TLC revealed Atranorin, Salazinic acid and Protolichesterinic acid. **Conclusion:** The result of lipase inhibitory activity of the lichen in this study is promising. The inhibitory activity may be attributed to the presence of secondary metabolites. In suitable form, the lichen could find its application as anti-obesity agent. Further studies on isolation of active principles from the extract and their enzyme inhibitory activity are under investigation.

**Keywords:** *Everniastrum cirrhatum* (Fr.) Hale, Anti-obesity activity, Pancreatic lipase, Lipase inhibitory activity, *Gallus domesticus*, Olive oil

## INTRODUCTION

Lichens are symbiotic organisms composed of a fungus (mycobiont) and an alga (photobiont). They produce characteristic secondary metabolites, lichen substances, which seldom occur in other organisms.<sup>[1]</sup> *Everniastrum cirrhatum* (Fr.) Hale (Parmeliaceae) is a foliose lichen in which phycobiont is a chlorolichen alga. It usually grows on the barks of trees in temperate regions.<sup>[2,3]</sup> It grows luxuriantly in tropical Himalayas, central India and higher altitudes of

southern India. It is characterized by linear, lacinate lobes which are grey in color. Thallus is tapering apically, 2-6mm wide and 10cm long. Thallus is loosely attached to the substratum and pendulous in nature. Apothecia are laminal, margins are inflexed, cilia are black in color, and spores are large.<sup>[4]</sup> In Ayurveda, it is mentioned as astringent, resolvent, laxative, carminative and aphrodisiac. It is also useful in bleeding piles, leprosy and excessive salivation. It is used as spices in Madhya Pradesh. Whole boiled material used as vegetable in Nepal and north Sikkim.<sup>[5,6]</sup> *E. cirrhatum* is traditionally used as antiseptic, used to heal wound and bronchitis in Sikkim.<sup>[7]</sup> *E. cirrhatum* has been used as material for sacrificial fire by Gaddi tribe of Kangra valley. It is as a spice and flavoring agent for meat and vegetables by Bhaiga, Bhil, Bhilala, Gond, Korka, Muria of Madhya Pradesh. It is used as vegetables in Lepchas and Nepalese of Sakyong valley, North Sikkim. In Uttaranchal, Uttar Pradesh and Sikkim, it is commercially sold as spice.<sup>[8]</sup> The mycobiont (lichen forming fungus) of *E. cirrhatum* was shown to cause mycelial growth inhibition of hot pepper

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anthracnose pathogen, *Colletortichum acutatum*.<sup>[1]</sup> Ethanol extract of *E. cirrhatum* has shown antimycobacterial properties against *Mycobacterium tuberculosis* H37Rv and H37Ra strains with minimum inhibitory concentration of 500µg/ml.<sup>[3]</sup> In a study, it was reported that the whole thallus of *E. cirrhatum* yielded a red brown dye.<sup>[9]</sup> Although it is used as traditional medicine in Indian subcontinent, literature surveys reveals that many of the bioactivities including Anti-obesity activity of this lichen has not yet been documented. In this study, we report for the first time the anti-obesity activity (in terms of lipase inhibitory activity) of methanol extract of *E. cirrhatum* from Bhadra wildlife sanctuary, Karnataka, India.

## MATERIALS AND METHODS

### Collection and identification of lichen

The lichen *E. cirrhatum*, growing on barks of trees, was collected from the Bhadra wildlife sanctuary, Karnataka, India, during August 2010. The lichen specimen was identified by morphological, anatomical, chemical tests.<sup>[10]</sup> The voucher specimen of the lichen (Voucher no. KU00703) was deposited in the University herbaria, Department of PG Studies and Research in Botany, Shankaraghatta-577451, Karnataka, India for future reference.

### Detection of secondary metabolites by thin layer chromatography (TLC)

The shade dried powdered lichen material was extracted with methanol, spotted on the silica plate and developed with solvent A (180 ml toluene: 60 ml 1-4, dioxine: 8 ml acetic acid) to detect secondary metabolites using standard protocols.<sup>[11,12]</sup>

### Preparation of extract using methanol

The lichen was dried at room temperature under shade. After drying, the lichen material was ground to fine powder and extracted by soxhlet apparatus using methanol as solvent. The extract was filtered using Whatman filter paper no. 1 and concentrated at 40°C under reduced pressure. The condensed methanol extract was stored at 4°C until use.<sup>[13]</sup>

### Phytochemical analysis of methanol extract

The extract obtained after solvent evaporation was subjected to standard tests for detection of alkaloids (Dragendorff's reagent and Mayer's reagent), tannins (ferric chloride test), saponins (frothing test and hemolysis test), glycosides (Salkowski test and Keller-Kilian test), sterols (Burchard test), flavonoids (Shinoda test) and terpenoids (Salkowski test).<sup>[14,15]</sup>

## Anti-lipase activity of methanol extract of *E. cirrhatum*

### Extraction of lipase from Chicken (*Gallus domesticus*) pancreas:

Pancreas of freshly slaughtered chicken were collected, washed and placed in ice cold sucrose solution (0.01M). The pancreas was homogenized in 0.01M sucrose, centrifuged, 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.<sup>[16]</sup>

### Determination of Chicken Pancreatic Lipase activity:

The activity of lipase was determined by incubating an emulsion containing 8ml of olive oil, 0.4ml of phosphate buffer and 1ml of chicken pancreatic lipase for an hour in rotary shaker, followed by stopping the reaction by addition of 1.5ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator.<sup>[17,18]</sup>

### Lipase Inhibitory activity of methanol extract of *E. cirrhatum*:

Lipase inhibitory activity of different concentrations of methanol extract was tested by mixing 100µl of each concentration of methanol extract, 8ml of oil emulsion and 1ml of chicken pancreatic lipase followed by incubation of 60 minutes. The reaction was stopped by adding 1.5 ml of a mixture solution containing acetone and 95% ethanol (1:1). The liberated fatty acids were determined by titrating the solution against 0.02M NaOH (standardized by 0.01M oxalic acid) using phenolphthalein as an indicator.<sup>[17-19]</sup> Percentage inhibition of lipase activity was calculated using the formula:

Lipase inhibition =  $A - B/A \times 100$ , where A is lipase activity, B is activity of lipase when incubated with the extract.

### Statistical Analysis

All data were expressed as mean  $\pm$  SD of the number of experiments (n =6). Past software version 1.92 was used.

## RESULTS AND DISCUSSION

### Secondary metabolites detected in *E. cirrhatum*

Preliminary phytochemical analysis of methanol extract of *E. cirrhatum* was determined by chemical tests. Phytoconstituents

namely alkaloids, saponins, tannins and terpenoids were detected in the extract (Table-1). TLC in solvent A showed the presence of Atranorin, Salazinic acid and Protolichesterinic acid in the lichen material.

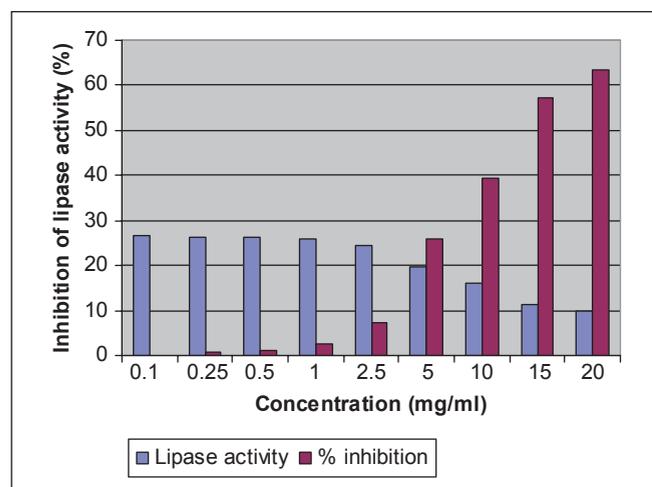
### Lipase inhibitory activity of methanol extract of *E. cirrhatum*

Inhibitory activity on chicken pancreatic lipase of different concentrations of methanol extract of *E. cirrhatum* was determined using olive oil as the substrate. The activity of pancreatic lipase was checked. It was found that the activity of lipase was affected when incubated with the methanol extract. The inhibitory activity was found to be dose dependent i.e., higher inhibition of enzyme was observed on increasing the concentration of extract. A marked inhibition of enzyme activity was observed with extract concentration 5mg/ml and higher (Figure 1).

**Table 1: Phytoconstituents detected in methanol extract of *E. cirrhatum***

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	+
	Mayer's test	+
Saponins	Frothing test	+
	Hemolysis test	+
Flavonoids	Shinoda test	-
Glycosides	Salkowski test	-
	Keller-Kiliani test	-
Tannins	Ferric chloride test	+
Sterols	Burchard test	-
Terpenoids	Salkowski test	+

'+' Present; '-' Absent



**Figure 1: Pancreatic lipase inhibitory activity of methanol extract of *E. cirrhatum***

Obesity has increased at an alarming rate and is now a worldwide health problem. It is widely accepted that obesity results from disequilibrium between energy intake and expenditure. Obesity is found to be strong risk factor for type 2 diabetes. Dietary lipids represent the major source of unwanted calories; therefore, lipid metabolism is a vital and subtle balance that maintains energy homeostasis. Once this balance is lost, obesity or hyperlipidemia develops, followed by a series of severe diseases, including atherosclerosis, hypertension, diabetes, and dysfunction of certain organs. Obviously, drug control of lipid metabolism offers a possible way to prevent or treat these diseases. The identification and characterization of several enzymes involved in lipid metabolism have yielded a rich pool of potential targets for drugs to treat obesity and other metabolic disorders.<sup>[20-24]</sup> Pancreatic lipase, the main lipid-digesting enzyme, removes fatty acids from the  $\alpha$  and  $\alpha'$  position of dietary triglycerides, which yields the lipolytic product  $\beta$ -monoglyceride and long-chain saturated and polyunsaturated fatty acids. Inhibition of pancreatic lipase is an attractive targeted approach for the treatment of obesity.<sup>[22]</sup> Naturally occurring compounds present an exciting opportunity for the discovery of newer anti-obesity agents. A number of studies have been carried on Anti-lipase activity of natural products. In a study, 95% ethanol extract of *Taraxacum officinale* inhibited porcine pancreatic lipase activity by 86.3% at a concentration of 250 $\mu$ g/ml.<sup>[25]</sup> The seed extract of *Vitis vinifera* was found to cause 80% inhibition of porcine pancreatic lipase at concentration of 1 mg/ml.<sup>[26]</sup> An extract of *Nomame herba* was found to possess inhibitory activity against porcine pancreatic lipase in a dose-dependent manner.<sup>[27]</sup> In our study also, a marked inhibition of chicken pancreatic lipase by the extract was observed which is consistent with earlier reports on lipase inhibition by natural products.

## CONCLUSION

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. A marked inhibition of activity of pancreatic lipase by methanol extract of *E. cirrhatum* was observed in this study. The inhibitory role could be attributed to the presence of various secondary metabolites in the extract. The extract may be used as anti-obesity agent in suitable form. Further studies on isolation of active principles from the extract and their inhibitory efficacy against lipases are under investigation.

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