

**SCREENING OF *IN VITRO* PANCREATIC LIPASE INHIBITORY
EFFECT OF *SESAMUM TRIPHYLLUM* WELW. EX ASCH. CRUDE
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Coimbatore District, Tamil Nadu, India.DOI: <https://doi-doi.org/101555/ijarp.6433>**ABSTRACT**

The positive effects of using natural medicines include its affordability, ease of access, and lack of side effects. The increasing prevalence of obesity worldwide is related to a number of metabolic illnesses and the worsening of global health issues. Pancreatic lipase is a crucial enzyme involved in the absorption of triglycerides in the small intestine, therefore inhibiting the absorption of dietary fat is a sensible target for treating obesity. It has been proposed that intestinal lipase inhibitors serve as anti-obesity medicines. Novel lipase inhibitors with few side effects have recently been the focus of increased research in natural resources. According to this perspective, the current study details the screening of an herb, *Sesamum triphyllum*, for its ability to prevent obesity. Ethanol extracts of four different parts (leaf, stem, root, and seed) of *S. triphyllum* were prepared and studied for their anti-obesity activity. Among the four crude extracts of the species, two extracts had strong anti-lipase activity (above 76%). Another two extracts demonstrated an inhibiting impact on pancreatic lipase of over 70%. These findings imply that preparing herbal extracts from the investigated plant parts will result in the production of potent anti-obesity medications. More research was necessary to ascertain the molecular mechanism and clinical effectiveness of this natural anti-obesity herb.

KEY WORDS: *Sesamum triphyllum*, Extracts, Anti-obesity, Pancreatic Lipase, Inhibition.

INTRODUCTION

A chronic metabolic disease called obesity is brought on by an imbalance between energy intake and expenditure. Being overweight or obese is characterized by abnormal or excessive fat storage that poses a health risk (Roh & Jung, 2012). According to Must *et al.* (1999) and Poulos *et al.* (2010), obesity is frequently linked to various illnesses such osteoarthritis, diabetes, cancer, hypertension, and arteriosclerosis. Approximately 500 million people worldwide are obese, and the prevalence of obesity is rising exponentially (Sweeting *et al.*, 2015).

The molecular regulation of triglyceride synthesis and pharmaceutical strategies to lessen fat absorption and storage caused by phytochemicals have drawn the attention of numerous scientific communities, offering an exciting prospect for the development of novel anti-obesity agents (Bray & Tartaglia, 2000; Hino *et al.*, 2011). The activity of lipolytic enzymes involved in fatty acid metabolism in adipose tissue controls the availability of fatty acids and triglycerides in biological reactions (Vázquez-Vela *et al.*, 2008; Tanzi & Fare, 2009). A large number of promising targets for medications to treat obesity and other metabolic syndromes have been identified through the characterisation and identification of many genes involved in lipid metabolism (Shi & Burn, 2004; Reilly & Lee, 2008; Kos & Wilding, 2010).

The primary lipid-digesting enzyme, pancreatic lipase, is released by the pancreas and is essential for breaking down fifty to seventy percentage of fat into monoglycerides and free fatty acids that the enterocytes can absorb. Inhibition in the digestion and absorption of fat usually reduces its accumulation in the adipose tissue (Hanefeld & Sachse, 2002). Long chain saturated and polyunsaturated fatty acids as well as the lipolytic product β -monoglyceride are produced when pancreatic lipase separates fatty acids from α and α' sites of dietary triglycerides. One attractive targeted strategy for the invention of effective anti-obesity medications for the treatment of obesity is the inhibition of pancreatic lipase (Thomson *et al.*, 1997; Shizhen & Xiwen, 2003).

Traditional natural remedies for a variety of illnesses have been made from plants. In particular, biological activity have been identified for a number of oriental medicinal herbs. Finding strong lipase inhibitors from plant extracts is a screening method used in the quest for anti-obesity medications (Shizhen & Xiwen, 2003). Scientists are looking for safe and efficient natural bioactive chemicals because of the adverse effects of several anti-obesity medications (Abdul Rahman *et al.*, 2007). *Sesamum triphyllum* Welw. ex Asch. (also known as wild sesame) is an erect annual plant that grows to a height of one to eight feet and may have simple or bushy stems. According to Leffers (2003) and Oyen (2011), this species is

used as an aphrodisiac and to treat stomach and chest pain, epilepsy, malaria, and snakebite. Scientists are not evaluating this species for any biological activity. Hence, the current study was designed to assess *S. triphyllum*'s ability to prevent obesity.

MATERIALS AND METHODS

Collection and preparation of materials

The wild sesame plant was gathered from the Karamadai region in Coimbatore district. The specimen was confirmed by the Botanical Survey of India (BSI), Coimbatore, and the voucher specimen was kept in our lab.

The leaves, stems, roots, and seeds of the plant were detached, cleaned under running water, and then dried at 37°C in the shade condition. The dried materials were pounded into a coarse powder using a mortar and pestle to retain the active substances from disintegrating. Using a Soxhlet apparatus, 100 g of each powder were extracted independently for 48 hours using an ethanol solvent (Das *et al.*, 2010). The entire extract was collected and concentrated using a rotary evaporator for further investigation.

Pancreatic lipase inhibitory activity

A slightly modified version of the pancreatic lipase inhibition method proposed by Kim *et al.* (2007) and Roh & Jung (2012) was used to assess the anti-diabetic activity of *S. triphyllum* extracts. Different concentrations of test samples (100, 50, 25, 12, 6.25, 3.125, and 1.565 µg/ml) and orlistat (standard) dissolved in DMSO solvent (1 mg/ml) were prepared using the serial fold dilution method.

The enzyme buffer was prepared by combining 6 µl of porcine pancreatic lipase solution (1 mg/ml) in a buffer containing 10 mM MOPS (3-morpholinepropane-1-sulphonic acid) and 1 mM EDTA (ethylene diamine tetra acetic acid) (pH 6.8), with 164 µl of Tris buffer (100 mM Tris-HCl and 5 mM CaCl₂, pH 7.0). Following that, 20 µl of different concentrations of either the plant extracts or orlistat were blended with 170 µl of enzyme buffer on a 96-well quartz microplate, and the mixture was incubated for 15 minutes at 37 °C. After adding 10 µl of the substrate solution [10 mM p-nitrophenyl butyrate (p-NPB) in dimethyl formamide], the mixture was incubated for 15 minutes at 37 °C. The lipase activity was assessed by measuring the hydrolysis of p-NPB to p-nitrophenol. After the reaction was carried out, an ultraviolet/visible microplate spectrophotometer was used to detect the absorbance at 405 nm. The inhibition of lipase activity by the test samples was estimated using the formula shown below.

$$\text{Lipase inhibition (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

The IC₅₀ value was derived as the sample extract concentration required to inhibit 50% of pancreatic lipase inhibitory activity under test conditions.

Data analysis

The experiment was conducted in triplicate, and the results were expressed as mean \pm standard deviation (SD) using Microsoft Office 2013 Excel program. The data were analyzed statistically using SPSS software (version 20.0), with one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Pancreatic Lipase Enzyme inhibition activity

The inhibitory effect of different extracts against porcine pancreatic lipase was concentration-dependent (Table 1 and Figure 1). The higher concentrations of the samples (100 $\mu\text{g/ml}$) significantly inhibited the pancreatic lipase enzyme activity ($> 70\%$). Although the leaf and stem extracts provided the strongest protection against lipase activity at maximum concentration, they showed 78.90 ± 0.87 and $76.32 \pm 0.46\%$ inhibition, respectively. The leaf and stem samples inhibition values are nearly identical. A stronger pancreatic lipase inhibition was also seen in the root and seed samples (around 71–73%). The IC₅₀ values of leaf and stem was 30.18 ± 1.84 and 31.42 ± 2.54 $\mu\text{g/ml}$, respectively. On the other hand, the standard drug, had an inhibitory effect with an IC₅₀ value of 19.09 ± 1.16 $\mu\text{g/ml}$. The tested samples were found to possess pancreatic lipase activity with an IC₅₀ value in the following order: leaf \geq stem $>$ seed $>$ root (Figure 2). The IC₅₀ values of the tested plant samples and the orlistat were statistically different at the $p < 0.05$ level of significance.

Table 1. Effect of *S. triphyllum* extracts on pancreatic lipase inhibition.

Extracts	% of Inhibition						
	1.565 ($\mu\text{g/ml}$)	3.125 ($\mu\text{g/ml}$)	6.25 ($\mu\text{g/ml}$)	12.5 ($\mu\text{g/ml}$)	25 ($\mu\text{g/ml}$)	50 ($\mu\text{g/ml}$)	100 ($\mu\text{g/ml}$)
Leaf	14.99 ± 0.49	31.69 ± 1.64	40.44 ± 1.37	47.03 ± 1.41	60.18 ± 0.78	69.84 ± 0.84	78.90 ± 0.87
Stem	19.97 ± 1.49	29.73 ± 1.51	37.98 ± 2.04	44.70 ± 1.67	60.52 ± 1.26	69.52 ± 1.08	76.32 ± 0.46
Root	13.55 ± 1.45	21.55 ± 1.75	31.28 ± 1.00	39.68 ± 1.89	52.39 ± 1.16	61.04 ± 1.19	71.20 ± 0.39
Seed	16.29	21.23	34.16	41.53	55.12	63.17	72.95

	±1.25	±1.59	±1.28	±2.25	±1.25	±1.19	±1.09
Orlistat (Standard)	23.48 ±1.32	35.07 ±1.59	44.02 ±1.31	54.47 ±1.32	65.62 ±0.74	76.88 ±0.41	86.07 ±1.00

Values are mean of triplicate determination (n=3) ± standard deviation.

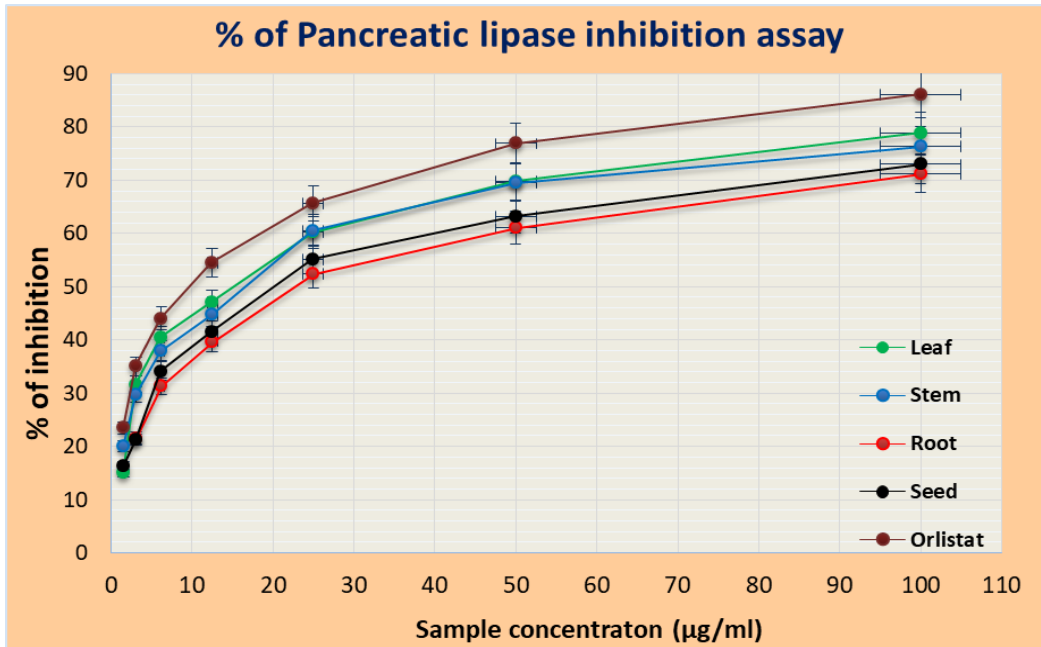


Figure 1. Line chart of inhibition % of *S. triphyllum* extracts on pancreatic lipase.

Values are mean of triplicate determination (n=3) ± standard deviation

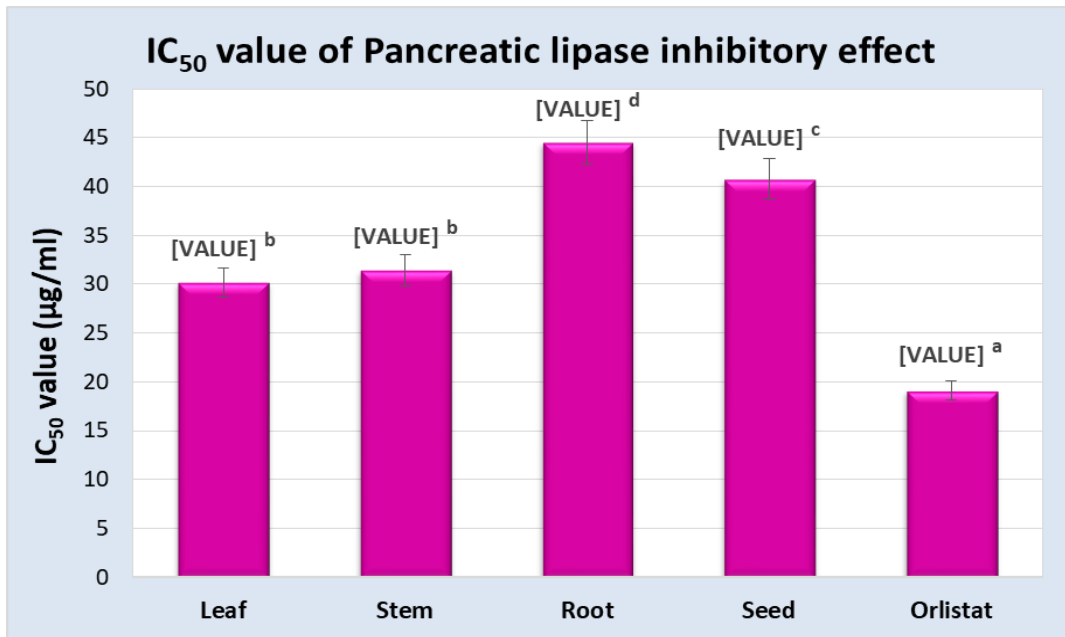


Figure 2. IC₅₀ value of pancreatic lipase inhibitory effect of *S. triphyllum* extracts.

Values are mean of triplicate determination (n=3) ± standard deviation.

Values are statistically significant at $p < 0.05$ where $a > b > c > d > e > f$.

Anti-obesity drugs are pharmaceuticals that affect one of the three main metabolic processes involved in regulating body weight: appetite, calorie intake, or metabolism. Anti-obesity medications may have greater side effects than favourable ones (Daneschvar *et al.*, 2016; Velazquez & Apovian, 2018). The enzyme pancreatic lipase has been utilized extensively to assess the potential effectiveness of natural products as anti-obesity medications (Sugiyama *et al.*, 2007).

As of right now, the only medication in Europe with clinical approval for managing obesity is orlistat. Long-term use of this medication in conjunction with an energy-restricted diet causes weight loss because it inhibits pancreatic lipase activity and reduces triglyceride absorption (Neovius *et al.*, 2008). Orlistat, when taken as prescribed by the physician, has been demonstrated to be safer and more effective than diet alone in reducing the risk of coronary artery disease and other obesity-associated comorbidities in addition to helping people lose weight. A variety of gastrointestinal discomforts, including a steathorrhsea, bloating, oily spotting, fecal urgency, and fecal incontinence, as well as hepatic complications, are the most frequently reported side effects of orlistat (Viner *et al.*, 2010).

The need to search for alternate therapies is clearly shown by the dismal history and disappointing track record of anti-obesity drugs (Daneschvar *et al.*, 2016; Velazquez & Apovian, 2018). Natural products especially medicinal plants in the form of pure components or extracts are increasingly accessible on the market as alternatives to traditional therapies and the problems they entail (Najmi *et al.*, 2022). Phytochemicals can demonstrate anti-adipogenic actions through a variety of biological processes, by inhibiting digestive enzymes (amylase and pancreatic lipase), controlling hunger, and reducing the formation of white adipose tissue (Fu *et al.*, 2016).

Thus, the goal of the current study was to find natural anti-obesity compounds from the wild. The current study discovered that, ethanolic leaf and stem extracts of *S. triphyllum* exhibited a promising inhibitory effect on the pancreatic lipase enzyme at their highest concentrations. The root and seed extracts were also found to have a strong anti-obesity effect. Consequently, accumulating fat and triglyceride content may be effectively inhibited by the *S. triphyllum* extracts.

CONCLUSION

Untreated obesity can lead to serious consequences. Plants have several natural compounds that work against obesity through a variety of mechanisms. The current study focused in particular on the natural agent *S. triphyllum*'s ability to prevent obesity. The *S. triphyllum*

ethanol extracts had the strongest pancreatic lipase enzyme inhibitory effect, it was proved by the current anti-obesity activity results. More research was necessary to ascertain the molecular mechanism and clinical effectiveness of the investigated anti-obesity agent *S. triphyllum*, and it may be a fascinating and captivating topic in future.

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