

## RESEARCH ARTICLE

# *Psidium guajava* Linn Leaves Extracts: A Potential Digestive Enzyme Inhibitor in the Treatment of Obesity

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## ABSTRACT

Obesity is a major worldwide problem and is associated with metabolic disorders, including hyperglycemia and hyperlipidemia. Many researchers focus on herbal plants rich with polyphenols are potent inhibitors of digestive enzymes and could be used as antihyperlipidemic and antihyperglycemic and hence useful in obesity treatment. The present work deals with the determination of the inhibitory effect of *Psidium guajava* Linn (Guava) leaves extract on digestive enzymes. Our findings confirmed that guava leaf extracts inhibit two primary enzymes,  $\alpha$ -amylase and  $\alpha$ -glucosidase. Similarly, investigated pancreatic lipase inhibition potential. The leaf extracts of *P. guajava* demonstrated significant anti-obesity properties by inhibiting key enzymes associated with obesity development. The aqueous extract exhibited pancreatic lipase inhibition ranging from 19.25 to 38.51%, while the methanolic extract showed inhibition between 22.96 to 46.66%. Additionally, the aqueous extract displayed  $\alpha$ -amylase inhibition ranging from 43.10 to 62.06%, and the methanolic extract showed inhibition between 48.27 to 62.06%. Moreover, the aqueous extract demonstrated  $\alpha$ -glucosidase inhibition between 13.11 to 49.18%, and the methanolic extract displayed inhibition ranging from 29.50 to 60.65% for 200 to 1000  $\mu$ g/mL concentrations. The inhibitory activity observed was dose-dependent for all three enzymes.

**Keywords:** Obesity, Lipase,  $\alpha$ -Amylase, Extract, Orlistat.

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## INTRODUCTION

Obesity is a global problem primarily due to its widespread prevalence across countries and regions worldwide. It impacts individuals, communities, and societies in several ways. Obesity's global impact spans health, economic, social, and environmental realms.<sup>1</sup> It drives chronic diseases like diabetes and cardiovascular issues, straining healthcare systems. Socially, it fosters stigma and inequality. Environmentally, it fuels resource depletion. Addressing it demands holistic approaches, considering interconnected factors like food systems and cultural norms. Nowadays obesity does not remain a problem in any particular country or particular age group.<sup>2</sup> It has become the global emerging cause of many diseases that affect almost all organs of the human body from head to toe and hence needs to be attention on measures to control obesity and automatically related health problems. Diabetes, metabolic syndrome, strokes, and cancer are escalating globally and stand as significant contributors to morbidity rates.<sup>3</sup> Obesity occurs when the body accumulates excess

fat, typically stemming from a prolonged imbalance between energy intake and expenditure. This imbalance often arises from lifestyle shifts and poor dietary habits. The mainstays of obesity treatment involve dietary adjustments and regular physical activity. Additionally, individuals may opt for anti-obesity medications to curb appetite or hinder fat absorption. In severe cases of obesity, surgery may be recommended as a last resort.<sup>4</sup> Allopathic drugs have gained popularity in the treatment of obesity; however, their usage is now limited due to severe side effects. Orlistat is an anti-obesity drug used in the treatment of obesity which acts by reducing fat absorption by inhibiting intestinal pancreatic lipase enzyme, but still, it has many more side effects. In the quest to treat obesity, researchers have delved into molecular targets found in natural products, such as enzymes and receptors. These targets offer the potential for developing medications that work through enzymatic inhibition, leading to beneficial changes in metabolism. This approach presents a promising avenue for the safe and efficient development of anti-obesity drugs.

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Digestive enzymes are essential for carbohydrate metabolism.<sup>5</sup> Plant-derived inhibitors targeting these enzymes present a promising approach to assist in obesity treatment. Additionally, another digestive enzyme, lipase, which participates in lipid metabolism, is emerging as an intriguing target for inhibitors. Inhibiting lipase can lead to a reduction in triglyceride absorption, consequently lowering caloric intake and promoting weight loss.<sup>6</sup> Therefore, inhibitors of digestive enzymes that restrict the intestinal absorption of carbohydrates and fats during the initial stages could potentially serve as valuable adjuncts in obesity management. By taking consideration many researchers have evaluated the enzyme inhibition activity of numerous herbal plants. Building on this background, our present study conducted an *in-vitro* enzyme inhibition assay using aqueous and methanolic extracts of *Psidium guajava* Linn leaves. Among the all-bioactive compounds of medicinal plants, phenolic compounds have great value in developing new molecules as they possess strong antioxidant activity and other activities like anti-inflammatory<sup>7</sup> and can be used in obesity treatment.<sup>8,9</sup> Primarily, polyphenols play a key role in regulating nutrient availability by inhibiting digestive enzymes involved in breaking down lipids and starch. This modulation can impact conditions such as obesity and hyperglycemia.<sup>10</sup> *P. guajava* Linn leaves have been studied for their potential in obesity treatment due to their bioactive compounds. Research suggests they may help regulate lipid metabolism, improve insulin sensitivity, and inhibit adipogenesis, thereby potentially aiding in weight management. Additionally, they possess antioxidant properties that could mitigate oxidative stress associated with obesity.

## MATERIAL AND METHODS

### Materials

Orlistat, oil emulsion, acetone, alcohol, phenolphthalein indicator, phosphate buffer, dinitro salicylic acid, acarbose, sodium dihydrogen phosphate, disodium hydrogen phosphate, glucose reagent, p-nitrophenyl  $\beta$ -D-glucopyranoside, spectrophotometer, etc. In the present study porcine pancreatic lipase (SIGMA), porcine pancreatic  $\alpha$ -amylase (SIGMA), and  $\alpha$ -glucosidase enzymes were used and they were obtained from SRL Lab.

### Methods

#### *Plant collection and authentication*

Leaves of the plant were obtained from the region of Karad, Maharashtra, in July 2018. The authentication of the leaves was performed from a botanical survey of India, Pune. The voucher (BSI/WRC/IDN.CER./2018/H3/97) was deposited in the herbarium for further reference.

#### *Preparation of extracts*

- *Aqueous extract (AEPG)*<sup>11</sup>

About 100 gms of coarse powder is mixed with water and chloroform (9:1) and kept in a glass container for 7 days with intermittent occasional shaking of the container. After 7 days,

the mixture was filtered through a clean, sterile muslin cloth and filter paper. The filter was boiled to evaporate water. The brown color residue was obtained after evaporation, which is stored in an airtight container and labeled as aqueous extract of *P. guajava* (AEPG).

- *Methanolic extract*

About 100 gms of coarse powder of *P. guajava* L was defeated by petroleum ether (60–80°C) residue was dried at RT and then extracted with 200 mL of 95% methanol in a soxhlet extraction apparatus for a period of 72 hours. Then, the extract was collected and subjected to solvent evaporation. The dried green-colored extract was obtained, which is stored in an airtight container and labeled as methanolic extract of *P. guajava* (MEPG).

- *In-vitro lipase inhibition activity*<sup>12,13</sup>

The experiment involved testing various concentrations of extracts (200, 400, 800, 1000  $\mu$ g/mL). Each concentration of the extract (100  $\mu$ L) was combined with a 7 mL oil emulsion containing 2.5 mL deionized water, 3 mL olive oil, 1-mL of 200 mM tris HCl buffer (pH 7.2), and 0.5 mL Tween 80. This total emulsion was stirred for 15 minutes to ensure proper emulsification. Following this, 1-mL of lipase (150 mg) was added, and the incubation was performed at 37°C for 60 minutes, 1.5 mL of a mixture of acetone and 95% alcohol (1:1 ratio) was added to stop the reaction. This reaction was titrated with 0.02 M NaOH using phenolphthalein as an indicator, observing the color change from pink to colorless.

The percentage lipase inhibition activity was determined as follows

$$\text{Lipase inhibition} = [ ( A - B ) / A ] \times 100$$

Where

Activity of lipase in absence of extract,  
Activity of lipase in presence of extract.

- *In-vitro  $\alpha$ -Amylase inhibition activity*<sup>14</sup>

About 200  $\mu$ L of  $\alpha$ -amylase enzyme and 100  $\mu$ L of 2 mM phosphate buffer (pH 6.9) were combined with aqueous and methanolic extracts (100  $\mu$ L each) at concentrations of 200 to 1000  $\mu$ g/mL. The incubation of this mixture was performed for 20 minutes before the addition of starch solution. The identical protocol was used for the control samples, with the exception that 200  $\mu$ L of the enzyme was swapped out for the buffer. Both the control and test samples were treated with 500  $\mu$ L of dinitrosalicylic acid reagent after a 5-minute incubation period. After that, the samples were submerged for five minutes in a bath of boiling water. With a spectrophotometer set at 540 nm, absorbance was determined. The following equation was used to calculate % inhibition.

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{100/\text{Abs 540 (control)}} \times 100$$

- *$\alpha$ -Glucosidase inhibition*<sup>14,15</sup>

In a 96-well microplate, a 50  $\mu$ L sample solution was combined with 50  $\mu$ L glutathione, 50  $\mu$ L  $\alpha$ -glucosidase solution in phosphate buffer (pH 6.8), and 50  $\mu$ L pNPG (4-Nitrophenyl  $\beta$ -D-glucopyranoside). For 15 minutes, incubation was done

at 37°C. A blank was made in the same way, but without the enzyme ( $\alpha$ -glucosidase) solution. By adding 50  $\mu$ L of 0.2 M sodium carbonate, the process was halted. At 400 nm, the absorbances of the sample and the blank were measured.

$$\% \text{inhibition} = \frac{\text{control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

## RESULT AND DISCUSSION

Inhibiting the enzymes lipase,  $\alpha$ -amylase, and  $\alpha$ -glucosidase is crucial for controlling obesity, as it impacts the digestion and absorption of fats and carbohydrates. Compounds that inhibit these key digestive enzymes can help regulate nutrient absorption, metabolism, and energy balance. This regulation can potentially aid in the treatment of obesity.<sup>16</sup> So, in this research work, we have evaluated the anti-obesity activity of the *P. guajava* Linn leaves extract.

### Percentage Yield

The yield of AEPG was 6.2% and MEPG was 11.66 % was observed concerning the total quantity of *P. guajava* leaves powder used for extraction.

### In-vitro Pancreatic Lipase Inhibition Activity

By inhibiting lipase activity, the digestion and absorption of fats are reduced, leading to decreased calorie intake and potentially lower fat accumulation in the body. This can contribute to weight loss or weight management.<sup>17</sup>

In our experiments, orlistat exhibited significant pancreatic lipase inhibitory activity ranging from 26.66 to 48.88%. In contrast, the aqueous extract displayed inhibitory activity ranging from 19.25 to 38.51%, and the methanolic extract showed inhibition between 22.96 to 46.66%, corresponding to concentrations ranging from 200 to 1000  $\mu$ g/mL. IC<sub>50</sub> values of extract and orlistat were 1028.59  $\pm$  9.9 whereas 1308.90  $\pm$  7.44 and 1071.71  $\pm$  10.1  $\mu$ g/mL for aqueous and methanolic extracts of *P. guajava*, respectively (Table 1).

The results indicated that the percentage inhibition of lipase activity by both standard orlistat and *P. guajava* extracts was dependent on the dose. As the concentration increased, there was a corresponding increase in percent inhibition, as illustrated in Figure 1.

### In-vitro $\alpha$ -Amylase Inhibition Activity

An enzyme called  $\alpha$ -amylase is essential for dissolving complex carbohydrates, or starches, into simpler sugars like glucose. By inhibiting  $\alpha$ -amylase activity, glucose is released into the circulation gradually as a result of slowed carbohydrate digestion and absorption. This procedure helps control blood sugar levels, lowers the chance of developing insulin resistance, and minimizes the following accumulation of fat.<sup>18</sup>

Acarbose exhibited significant inhibitory potential, with inhibition percentages ranging from 41.37 to 84.48%. In contrast, the aqueous extract displayed inhibitory activity ranging from 43.10 to 62.06%, and the methanolic extract showed percent inhibition between 48.27 to 62.06%, corresponding to concentrations ranging from 200 to 1000  $\mu$ g/mL. IC<sub>50</sub> values of extract and acarbose were 151.04  $\pm$  1.44 whereas 575.00  $\pm$  0.83  $\mu$ g/mL and 417.89% for aqueous

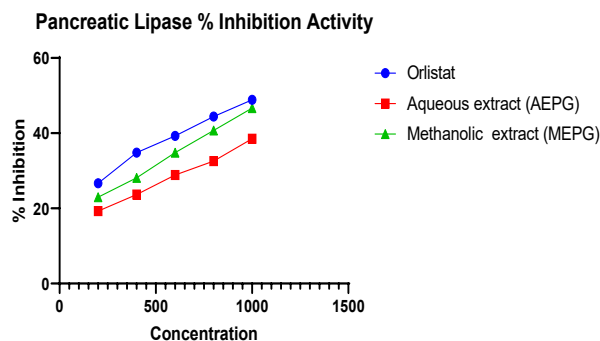


Figure 1: Pancreatic lipase inhibitory activity by aqueous and methanolic extracts with standard

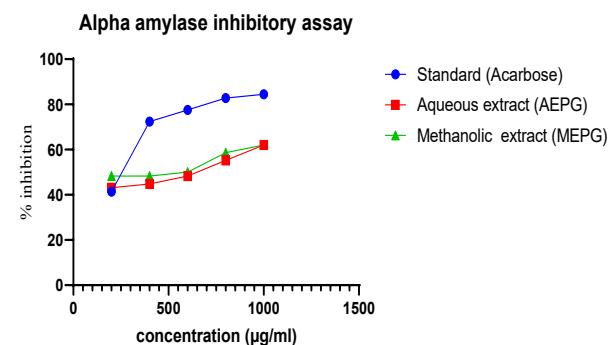


Figure 2:  $\alpha$ -Amylase inhibition by aqueous and methanolic extracts with standard

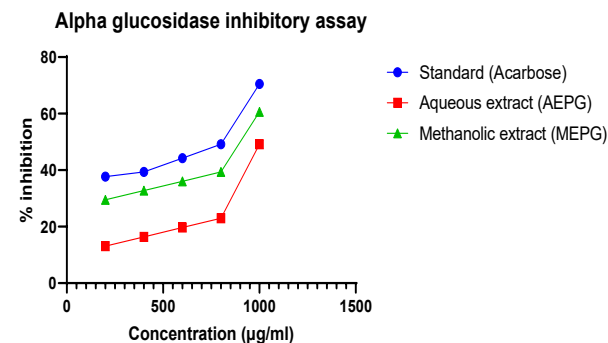


Figure 3:  $\alpha$ -Glucosidase inhibitory activity by aqueous and methanolic extracts with standard

methanolic extracts of *P. guajava*, respectively (Table 2).

The results indicated %inhibition of  $\alpha$ -amylase enzyme activity by both standard acarbose and *P. guajava* extracts was dependent on the dose. As the concentration increased, there was a corresponding increase in percent inhibition, as illustrated in Figure 2.

### In-vitro $\alpha$ -Glucosidase Inhibition Activity

This enzyme is located in the small intestine and further breaks down complex sugars (such as disaccharides) into absorbable monosaccharides (such as glucose). Inhibiting  $\alpha$ -glucosidase activity delays the absorption of glucose from carbohydrates, leading to a slower increase in serum sugar after meals.<sup>19, 20</sup>

**Table 1:** Pancreatic lipase inhibitory activity by aqueous and methanolic extracts

Conc.	% Inhibitory activity by Orlistat	IC <sub>50</sub>	% Inhibitory activity by AEPG	IC <sub>50</sub>	% Inhibitory activity by MEPG	IC <sub>50</sub>
200	26.66		19.25		22.96	
400	34.81	1028.59	23.70	1308.90	28.14	1071.71
600	39.25		28.88		34.81	
800	44.44		32.59		40.74	
1000	48.88		38.51		46.66	

Conc in µg/mL

**Table 2:** α-Amylase inhibition activity by aqueous and methanolic extracts

Conc. (µg/mL)	% Inhibitory activity standard Acarbose	IC <sub>50</sub>	% Inhibitory activity AEPG	IC <sub>50</sub>	% Inhibitory activity MEPG	IC <sub>50</sub>
200	41.37		43.10		48.27	
400	72.41	151.04	44.82	575.00	48.27	417.89
600	77.58		48.27		50.00	
800	82.75		55.17		58.62	
1000	84.48		62.06		62.06	

Conc in µg/ml

**Table 3:** α-Glucosidase inhibitory activity by aqueous and methanolic extracts

Conc.	% Inhibition (standard acarbose)	IC <sub>50</sub>	% Inhibitory activity by AEPG	IC <sub>50</sub>	% Inhibitory activity by MEPG	IC <sub>50</sub>
200	37.70		13.11		29.50	
400	39.34	660.54	16.39	1265.38	32.78	911.76
600	44.26		19.67		36.06	
800	49.18		22.95		39.34	
1000	70.49		49.18		60.65	

Conc in µg/mL

AEPG and MEPG also possess significant inhibitory activity on α-glucosidase activity. AEPG varies percent inhibition between 13.11 to 49.18% and MEPG shows percent inhibition between 29.50 to 60.65% for 200 to 1000 µg/mL conc (Table 3). So, from the result, it is clear that α-glucosidase inhibitory activity is dependent upon concentration. IC<sub>50</sub> values of AEPG and MEPG were 1265.38 ± 0.54 and 911.76 ± 0.84 µg/mL, respectively.

Figure 3 illustrates the inhibition percentage of α-glucosidase enzyme activity. Acarbose demonstrated robust α-glucosidase inhibitory activity, ranging from 37.70 to 70.49% for concentrations between 200 to 1000 µg/mL. IC<sub>50</sub> value was 660.54 ± 1.32 µg/mL.

### CONCLUSION

The leaf extracts of the *P. guajava* demonstrated excellent anti-obesity activity by inhibiting lipase, α-amylase and glucosidase enzymes, which are involved in the development of obesity. Aqueous extract displayed pancreatic lipase inhibitory activity ranging from 19.25 to 38.51%, and the methanolic extract showed inhibition between 22.96 to 46.66%. While the aqueous

extract displayed α-amylase inhibitory activity ranging from 43.10 to 62.06%, and the methanolic extract showed percent inhibition between 48.27 to 62.06%. Also, the aqueous extract displayed α-glucosidase inhibition between 13.11 to 49.18% and the methanolic extract showed inhibition between 29.50 to 60.65% for 200 to 1000 µg/mL concentration.

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