

PANCREATIC LIPASE AND α -AMYLASE INHIBITORY POTENTIAL OF MANGOSTEEN (*GARCINIA MANGOSTANA* LINN.) PERICARP EXTRACT

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ABSTRACT

Background: Obesity is a disorder of lipid metabolism and the enzyme involved in this process could be selectively targeted to develop anti obesity drugs. Inhibition of digestive enzymes is one of the most widely studies mechanisms used to determine the hypolipidemic and hypoglycemic agent of natural products for anti-obesity agent screening. **Aims:** To evaluate the inhibitory potential of *G. mangostana* extract, xanthone and α -mangosteen compound toward pancreatic lipase and α -amylase enzyme as once of anti-obesity mechanism. **Material and Methods:** The IC₅₀ value of the mangosteen pericarp extract, xanthone, and α -mangosteen toward pancreatic lipase and α -amylase were determined *in vitro* compared to orlistat and acarbose as standard drugs. **Results:** Mangosteen pericarp extract contains phenol, terpenoid, saponin, flavonoid and tannin. Mangosteen pericarp extract is a more active compound in inhibiting the PPL activity compared to α -mangosteen, and xanthone. Mangosteen pericarp extract shows the higher activity compared to xanthone but still lower activity compared to α -mangosteen. However, its activity is still lower than standard drugs. **Conclusions:** Our *in vitro*, confirmed that the mangosteen pericarp extract has the phytochemical bioactive content that possesses anti-obesity potential through pancreatic lipase and α -amylase inhibitory activity.

INTRODUCTION

Obesity is a complex metabolic disorder, which affects the normal functions of the whole body. Obesity has become a worldwide public health threat, since it is involved in various serious diseases, including type 2 diabetes, hypertension, coronary heart diseases, apoplexy, osteoarthritis and cancers.^[1] Preventing the epidemic of obesity become one of the greatest public health challenges in the first half 21st century.^[2] The number of studies to prevent and treat obesity continues to rise.^[3]

There are various ways for effective therapy in obesity, such as suppression on food intake, stimulation o energy expenditure, lipase inhibition, regulation on lipid metabolism, and inhibition of adipocyte differentiation.^[4] Pancreatic lipase and α -amylase that synthesized and secreted by the pancreas plays a significant roles in the principal lipolytic and glycosidic chain hydrolysis for efficient digestion of triglycerides, starch and glycogen. Therefore, compounds that exhibited pancreatic and α -amylase inhibitory activities would reduce energy absorption and intake and have potential use for obesity treatment.^[5,6] Orlistat is a commercial drug that commonly used as anti-obesity medications by inhibiting pancreatic lipase activity. It has adverse effects including abdominal pain, bloating, flatulence, oily stools, diarrhea, and decreasing in fat soluble vitamins absorption.^[7] Acarbose have been potent reversible inhibitor of α -amylase and α -glucosidase. However, undesirable side effects limit its use.^[8,9]

Many plants and their active chemical compounds have demonstrated activity in the treatment of obesity that have

fewer side effect and less toxicity compare to synthetic drugs.^[10-14] Mangosteen (*Garcinia mangostana* Linn.) is a tropical tree from India, Myanmar, Malaysia, Philippines, Sri-Lanka, and Thailand. The fruit hull of Mangosteen has been used for hundreds of years around the world, mostly in Southeast Asia, as a medicine for a great variety of medical conditions such as treatment of skin infections and wounds, amoebic dysentery, etc.^[15] Previous studies have shown that the various parts extract contain varieties of secondary metabolites such as prenylated and oxygenated xanthones. The major bioactive secondary metabolites of *G. mangostana* are xanthone derivatives. Xanthones were reported to have a great variety of pharmacological activities including antioxidant, antifungal, anti-bacteria, cytotoxic, anti-inflammatory, antihistamine, anti-HIV, and other activities.^[15] α -Mangosteen, the dominant xanthone found from the fruit hulls of *G. mangostana* L., has been demonstrated by pharmacological studies to possess antioxidant, antibacterial, anti-inflammatory, antitumor and renoprotective activities.^[16-21] Therefore, the aim of this study is to evaluate the inhibitory potential of *G. mangostana* extract, xanthone and α -mangosteen compound toward pancreatic lipase and α -amylase enzyme as once of anti-obesity mechanism.

MATERIAL AND METHOD

Study Design: This *in vitro* study was carried out in Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia in collaboration with

School of Pharmacy, Bandung Institute of Technology. The laboratory experiment has been performed for 4 months.

Extraction Procedure and Sample Preparation: *G. mangostana* L. pericarp were collected from Indonesian farms in Cicantayan, Sukabumi, Bandung, West Java. The plants were identified by staff herbarium, Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The mangosteen pericarps were dried, ground, and successively extracted by using reflux method in 50% ethanol and water. The extract obtained from the reflux Method was then freeze drying.^[22]

Qualitative Phytochemical Screening Assay: The mangosteen pericarp extract was tested by phytochemical assay using modified Farnsworth method including phenol, steroid/triterpenoid, saponin, tannin, terpenoid, flavonoid and alkaloid as listed below.

Phenol Identification: The sample was added into the dropping plate. FeCl₃ dissolved in 1% water or ethanol was added into the sample. The formation of green/ red/ purple/ dark blue was an indication of the presence of phenol.^[23,24]

Steroid/ Triterpenoid Identification: Sample was put on the dropping plate. Asetat anhirat acid then added into the sample until it is soaked. After 10-15 minutes, one drop of absolute sulfate acid (H₂SO₄) was added into the sample. Green/blue coloration was an indication of steroid while red/ orange sediment was an indication of triterpenoid.^[23,24]

Saponin Identification: Sample was shaken with water in the test tube. Saponin content was indicated by persistence of froth on the surface of the mixed liquid.^[23,24]

Tannin Identification: The sample was soaked with HCl liquid in a test tube then heated on water bath for 30 minutes. The positive reaction was shown by red/ orange color formation on the surface of amil alcohol liquid layer or surface layer.^[23,24]

Terpenoid Identification: The sample was added into the dropping plate, and then the vanillin and H₂SO₄ liquid were added into the sample. The formation of purple color was an indication of terpenoid.^[23,24]

Flavonoid Identification: The sample was put into the test tube then Mg/Zn and HCl 2N were added. The sample then incubated for 5-10 minutes and the amil alcohol was added into the filtrate. The positive reaction was shown by the formation of red color.^[23,24]

Alkaloid Identification: 10% liquid ammonia was added into the sample then the sample was extracted with chloroform until 2 layers were formed. The bottom layer was then placed into the first new tube. HCl 2N was added into the first new tube. The upper layer was placed into the second new tube then the dragendorf solution was added into the second new tube. The positive reaction was shown by the formation of yellow or dark red sediment.^[23,24]

Porcine Pancreatic Lipase (PPL) Inhibition Assay Report: The inhibitory activity of the mangosteen pericarp extract on PPL was observed compared to the xanthone and alpha mangosteen as pure compound and orlistate as the standard of anti obesity drugs as positive control. The assay was performed based on Liu, et al (2013)

method.^[25] 20 µL of sample in various concentrations (125; 62,5; 31,25; 15,63; 7,81; and 3,91 µg/mL) was added into the sample well and 20 µL of aquades was served as negative control. 20 µL of PPL was added into each well, except for blank well. 100 µL of potassium phosphate buffer (0.1 mM; pH = 7.2) was added into each well. After 15 minutes of incubation in 37°C incubator, 20 µL of *p*-nitrophenyl butyrate was added into each well as substrate then incubated for 5 minutes in 37°C incubator. The plate then placed in cold temperature (-20°C) for 2 minutes to stop the reaction. The absorbance was measured in 405 nm of wavelength. The percentage of inhibition was measured according to equation 1.

$$\% \text{ of Lipase Inhibitor Activity} = \frac{C-S}{C} \times 100\% \dots \dots \dots \text{equation 1}$$

Description: C = absorbance of negative control,

S = absorbance of sample

-Amylase Inhibition Assay: The inhibitory activity of mangosteen pericarp extract on alpha amylase activity was observed compared to the xanthone and -mangosteen as pure compound and acarbose as the standard anti obesity drugs as positive control. The modified method from Etondi, *et al* and Sudha, *et al* was used to evaluate -amylase inhibitory activity.^[26,27] 30 µL of sample in various concentration (176.47; 88.24; 44.12; 22.06; 11.03; and 5.51 µg/mL) were added into the sample well and 30 µL of aquades was served as negative control. Furthermore, 20 µL of alpha-amylase was added into each well except for blank well. After 10 minutes of incubation in 37°C incubator, 20 µL of starch solution was added into each well as substrate except for negative control well then incubated for 15 minutes in 37°C incubator. Enzymatic reaction was terminated by the addition of 100 mL of acidic iodine solution in each well. The absorbance was read in 620 nm of wavelength. The percentage of inhibition was measured according to equation 2.

$$\% \text{ of Amylase Inhibitor Activity} = \frac{C-S}{C} \times 100\% \dots \dots \dots \text{Equation 2}$$

Description: C = absorbance of negative control

S = absorbance of sample

Statistical Analysis: Data were presented as means ± standard deviation. Statistical comparisons were performed using One Way ANOVA statistics methods and Tukey's HSD Post Hoc Test by SPSS software (version 20.0. *p*-values <0.05) were considered as statistically significant.

RESULTS

Qualitative Phytochemical Screening Assay: The qualitative pythochemical screening assay carried out on mangosteen pericarp assay revealed a high content of phenol and flavonoid and low content of terpenoid, saponin, and tannin. The steroid, triterpenoid, and alkaloid were absent in the sample (Table 1).

Porcine Pancreatic Lipase (PPL) Inhibitory Activity: Mangosteen pericarp extract, -mangosteen, and xanthone have the PPL inhibitory activity in

concentrations dependent manner (Table 2; Fig. 1). Based on the IC₅₀ value, Mangosteenpericarp extract was more active in inhibiting the PPL activity compared to -mangosteen, and xanthone. However, its activity was still lower than orlistat as standard drugs (Table 3). Pancreatic lipase inhibitory effect is one of the most important factors for determining anti-obesity agent potential since decreasing the lipid absorption is important for the anti-obesity mechanism.^[28]

-Amylase Inhibitory Activity: Pancreatic -amylase is a key enzyme in the digestive system and catalyses the initial step in starch hydrolysis.^[27] Mangosteen pericarp extract, xanthone, and -mangosteen exhibited the -amylase inhibitory activity in a concentration dependent manner as well as acarbose (Table 3; Fig.2).

Nevertheless, its activity on inhibiting -amylase was lower than acarbose. Mangosteen pericarp extract showed higher activity than xanthone but it showed lower activity compared to -mangosteen (Table 5).

Table 1: Phytochemical screening of mangosteen pericarp extract

Phytochemical content assay	mangosteen pericarp extract
Phenol	+++
Steroid/ triterpenoid	-/-
Terpenoid	++
Saponin	++
Flavonoid	+++
Tannin	++
Alkaloid	-

++++: very high content , +++: high content, ++: moderate content, +: less content, -:undetected

Table 2. The PPL inhibitory activity of orlistat (1); -mangosteen (2); xanthone (3); and mangosteen pericarp extract (4) in various concentrations.

Concentrations (µg/mL)	Samples			
	orlistat	-mangosteen	xanthone	mangosteen pericarp extract
125	93.84±0.07 fD	71.35±0.86 fA	80.60±0.65 fB	89.10±0.70 fC
62.5	79.85±0.13 eD	62.13±0.60 eA	67.36±1.15 eB	70.90±0.10 eC
31.25	68.52±0.10 dD	48.32±0.46 dA	58.92±0.42 dB	56.85±0.56 dC
15.63	57.89±0.57 cD	44.05±0.61 cA	45.16±0.68 cB	50.13±0.19 cC
7.81	44.32±0.50 bD	41.34±1.44 bA	41.28±0.61 bB	45.00±1.20 bC
3.91	37.22±0.44 aD	38.18±1.66 aA	32.40±0.16 aB	36.19±2.28 aC

*The data are presented as means ±standard deviation. The different small letters at the same column (among concentrations) and capital letters at the same row (among anti sample) are significant at p<0.05 (Tukey Post Hoc test).

Table 3: The IC₅₀ of PPL inhibition activity of mangosteen pericarp extract, -mangosteen, xanthone, and orlistat

Samples	IC ₅₀ value (µg/mL)
orlistat	9.32±1.11 a
-mangosteen	47.32±2.87 d
xanthone	36.67±0.42 c
mangosteen pericarp extract	26.50±1.15 b

*The data are presented as mean ± standard deviation. Different letters in the same column (among samples) are significant at p < 0.05 (Tukey's HSD post hoc test)

Table 4. The -amylase inhibitory activity of acarbose (1); -mangosteen (2); xanthone (3); and mangosteen pericarp extract (4) in various concentrations.

Concentrations (µg/mL)	Samples			
	acarbose	-mangosteen	xanthone	mangosteen pericarp extract
176.47	91.19±0.28 fD	72.94±0.84 fC	29.48±0.57 fA	70.33±0.88 fB
88.24	78.65±0.57 eD	64.31±0.65 eC	25.95±0.29 eA	53.88±0.84 eB
44.12	64.50±1.20 dD	57.79±0.38 dC	22.20±1.06 dA	45.81±0.81 dB
22.06	53.44±0.49 cD	54.75±0.37 cC	18.75±0.70 cA	35.76±0.49 cB
11.03	47.30±0.68 bD	43.70±0.57 bC	16.76±0.49 bA	29.23±2.76 bB
5.51	39.54±0.60 aD	41.34±0.37 aC	15.08±1.13 aA	25.95±0.21 aB

*The data are presented as means ±standard deviation. The different small letters at the same column (among concentrations) and capital letters at the same row (among anti sample) are significant at p < 0.05 (Tukey Post Hoc test).

Table 5. The IC₅₀ of -amylase inhibition activity of mangosteen pericarp extract, -mangosteen, xanthone, and acarbose

Samples	IC ₅₀ value
acarbose	14.33±1.24 a
-mangosteen	29.67±1.98 b
xanthone	517.85±8.52 d
mangosteen pericarp extract	105.36±2.73 c

*The data are presented as mean ± standard deviation. different letters in the same column (among samples) are significant at p < 0.05 (Tukey's HSD post hoc test)

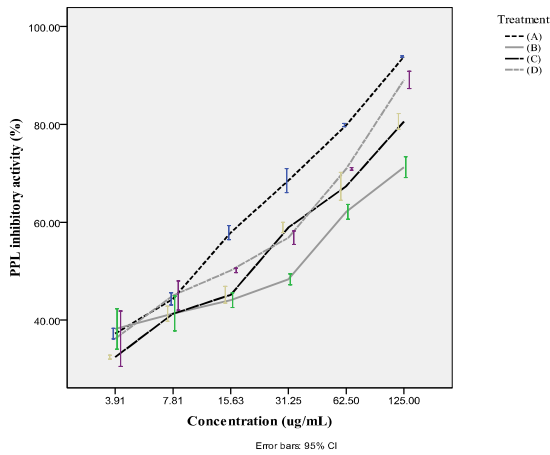


Fig 1. The PPL inhibitory activity of Orlistat (A); Mangosteen (B); Xanthone (C); and Mangosteen pericarp extract (D) in various concentrations.

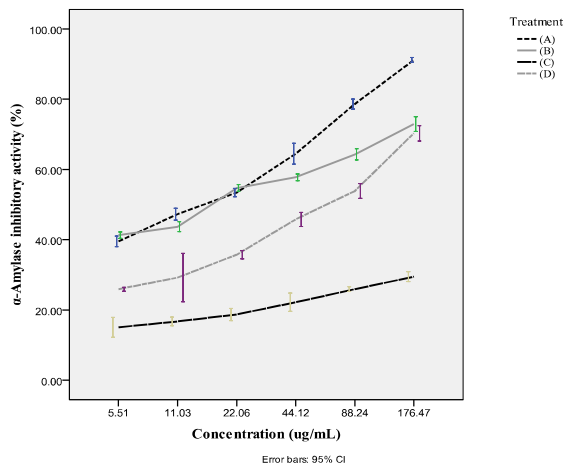


Fig 2. The alpha-Amylase inhibitory activity of Acarbose (A); Mangosteen (B); Xanthone (C); and Mangosteen pericarp extract (D) in various concentration..

DISCUSSION

Developing anti obesity drugs that are efficacious and have minimal side effects become a pressing need.^[29] The development of nutrient digestion and absorption inhibitors become one of the most important strategy in the obesity treatment.^[30] Inhibition of digestive enzymes is one of the most widely studies mechanisms used to determine the hypolipidemic and hypoglycemic agent of natural products for anti-obesity treatment development.^[30] In this *in vitro* study, the porcine pancreatic lipase and alpha-amylase inhibitory activity of mangosteen pericarp extract, mangosteen, and xanthone compared to orlistat and acarbose were evaluated.

Dietary fats are important components in human nutrition as an energy supply, thermal regulators, membrane constituents, and some play an important role in body function as essential fatty acids and fat-soluble vitamins which absorbed up to 95% of ingested fat by human intestine.^[31,32] Pancreatic lipase is the key enzymes in lipid absorption that hydrolyses triacylglycerols in the gastrointestinal tract.^[33] Inhibition of this enzyme becomes important in obesity treatment. Based on this study, orlistat has become the most active compound in inhibiting porcine pancreatic lipase. Orlistat is a hydrogenated derivatives of lipstain derived from *Streptomyces toxicicini* that has the

inhibitory activity toward gastric, pancreatic and carboxyl ester lipase and has been proved to be effective for the human obesity treatment.^[34-36] Mangosteen pericarp extract also showed higher pancreatic inhibitory activity than mangosteen and xanthone, although its activity is still lower than orlistat. The lipase inhibitory activity of mangosteen was also confirmed by Lin, *et al* (2012) study that observed the crude extract of mangosteen pericarp having the most significant inhibitory effects on pancreatic lipase activity ($IC_{50} = 0.75\text{mg/mL}$) *in vitro* among other samples tested in the study.^[28] The presence of flavonoids, tannins, and saponin in mangosteen pericarp extract could be responsible for its pancreatic lipase inhibitory activity although exact mode of action is still unclear.^[37] Inhibition of alpha-glucosidase and alpha-amylase enzymes involved in carbohydrate digestion can significantly decrease the postprandial blood glucose.^[38] In this study, acarbose showed the highest inhibitory activity on alpha-amylase. Acarbose-a microbial pseudo-tetrasaccharide is an inhibitor to both of alpha-amylase and alpha-glucosidase that widely used clinically as an oral hypoglycaemic agent.^[39,40]

Mangosteen, mangosteen and xanthone showed the activity in inhibiting alpha-amylase respectively. Only few studies were performed to analyse the alpha-amylase inhibitory potential of mangosteen pericarp extract. Based on Loo, *et al* (2007), alpha-amylase inhibitory activity was detected in methanol soluble part of precipitate obtained upon evaporation of acetone ($IC_{50} = 5.4 \mu\text{g/mL}$) and methanol solution portion of powder obtained from drying water soluble extract after removal of acetone that binds to XAD2 resin ($IC_{50} = 10.1 \mu\text{g/mL}$). alpha-amylase inhibitor activity was not detected in diethyl ether soluble part of precipitate obtained upon evaporation of acetone and was found to constitute xanthone found in mangosteen pericarp besides mangosteen.^[42] Interestingly, mangosteen was detected more active in inhibiting alpha-amylase compared to mangosteen pericarp extract in this study. Other than pericarp extract, Manaharan T, *et al* (2012) found that mangosteen rind possesses an alpha-amylase inhibitory activity ($IC_{50} = 41.7 \pm 3.2 \text{ mg/mL}$).^[43] Phenol, flavonoid, tannin, and saponin that found in this mangosteen pericarp extract were confirmed exerted hypoglycemic activity by alpha-amylase inhibitory activity.^[44]

Mangosteen pericarp has been used in in both of Chinese and Ayurvedic medicinal. The yellow exudate from mangosteen peel contain xanthone as the major class of compounds including mangosteen, mangostin, mangostin, garcinone-c and garcinone-d along with mangostinone, tanins, and flavonoid called epicatechin.^[45] Studies on the effects of mangosteen pericarp extracts on obesity also confirmed that the extract could reduce cholesterol level in rats.^[46,47] Mangosteen also showed anti-adipogenic activity through suppressing proliferator-activated receptor gamma (PPAR) expression and FAS activity.^[48] Our *in vitro*, confirmed that the mangosteen pericarp extract has an anti-obesity potential through pancreatic lipase and alpha-amylase inhibitory activity. This inhibitory activity may due to their active phytochemical content including phenol, terpenoid, saponin, flavonoid, tannin and xanthone.

CONCLUSION

Mangosteen pericarp extract contained phytochemical bioactive content that possesses the anti-obesity potential through pancreatic lipase and α -amylase inhibitory activity. The *in vivo* assay will be pursued to confirm the anti-obesity mechanism of mangosteen pericarp extract.

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Conflict of interest

All contributing authors declare no conflicts of interest.

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