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Assay-guided Fractionation Study of α-Amylase Inhibitors from *Garcinia mangostana* Pericarp

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α-Amylase inhibitor (α-AI) activity of *Garcinia mangostana,* commonly known as mangosteen, pericarp extracts was studied by assay guided fractionations from lipophilic to hydrophilic using combined solvent extraction and Amberlite XAD2 adsorption chromatography. Neither the lipophilic, xanthone containing fraction, nor the highly polar fraction, which has no affinity on Amberlite XAD2, showed any α-AI. The fraction that shows very high inhibitory activity contains primarily polyphenols and can be adsorbed on Amberlite XAD2. The IC50 of 5.4 μg/mL of this fraction is comparable to that of acarbose, a prescribed α-AI used in the control of type II diabetes, at 5.2 μg/mL. Total phenolic content (TPC) of each fraction was measured and the TPC has no correlation with the α-AI activity. The lipophilic fraction contains mainly xanthones as revealed by HPLC-MS analysis. Colorimetric analysis coupled with UV–vis and IR spectroscopic analysis demonstrated that the fractions with high α-AI activity are primarily oligomeric proanthocyanidins (OPCs) with little gallate moiety. There is also evidence to show that the α-AI by these OPCs is not purely by nonspecific protein complexation. Both tannic acid and *G. mangostana* OPCs precipitate BSA equally well but *G. mangostana* OPCs are 56 times more effective in inhibiting α-amylase.

INTRODUCTION

It has been suggested that insulin resistance is a manifestation of the body's inability to effectively metabolize a high glycaemic load (GL) diet (*1*). A high GL diet causes excessive stress on the β cells to secrete insulin at levels higher than they were originally designed for, leading to hyperinsulinemia and, subsequently, insulin resistance. Introducing α-AI into the diet can reduce the rate of postprandial glycaemia increase and thus relieve stress on β cells. Therefore, it is not surprising that acarbose, an α-amylase and α-glucosidase inhibitor, had been found to be effective in treating and possibly preventing noninsulin-dependent diabetes (*2*). However acarbose is a prescribed drug and cannot be added to foods or used as a supplement. Plants are a rich source of various antinutrients (*3*), including α-AI, which are not prescribed drugs. Therefore they are ideal candidates for novel α-AI to be used in functional foods.

Garcinia mangostana, commonly known as mangosteen, is a tropical fruit belonging to the Guttiferae family (*4*). It is widely cultivated in Thailand, Malaysia, and Indonesia. Worldwide production of *G. mangostana* is about 150000 tons per annum (*5*). The pericarp of the fruit is about two thirds of the whole fruit by weight, bright red in colour, and usually not eaten. It had been used for treating diarrhoea, wounds, and skin infection in traditional medicine (*6*). The pericarp is rich in polyphenols and contains 7–15% tannins (*3*). The polyphenols in the pericarp of *G. mangostana* and various other fruits could have evolved as a defensive mechanism against herbivores. The high tannin content not only makes the pericarp unpalatable but also inhibits various digestive enzymes (*7*). Thus it is common for inedible plant parts, such as the seeds, to contain high levels of polyphenols in the form of tannins to prevent it from being eaten (*8*). Therefore, the *G. mangostana* pericarp may also contain antinutrients such as α-AI as a defense mechanism.

There had been no known studies on the α-AI activity of phenolic compounds in *G. mangostana* pericarp. Utilization of the pericarp polyphenols is economically feasible as the pericarp is a waste material. In addition there is also an increased interest in low GL foods (*9*). Therefore, the purpose of this study is to determine if the α-AI activity of polyphenolic compounds from *G. mangostana* pericarp is comparable to other polyphenols and known α-AI using in vitro assays. The chemical properties of the polyphenolic compounds would also be determined so as to understand the structure and characteristics of these compounds.

MATERIALS AND METHODS

Instruments. FT-IR spectrum was acquired with Perkin-Elmer Spectrum One (Boston, MA). UV–vis spectroscopy and absorbance readings were carried out on a BioTek Synergy HT multidetection microplate reader (Winooski, VT) or on Mini 1240 Shimadzu (Kyoto, Japan) UV–vis spectrophotometer. LC/MS chromatograms, and spectrums were acquired using Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, CA) equipped with TSP 4000 HPLC system, which includes UV6000LP PDA detector, P4000 quaternary pump, and AS3000 autosampler. The heated capillary and spray voltage were

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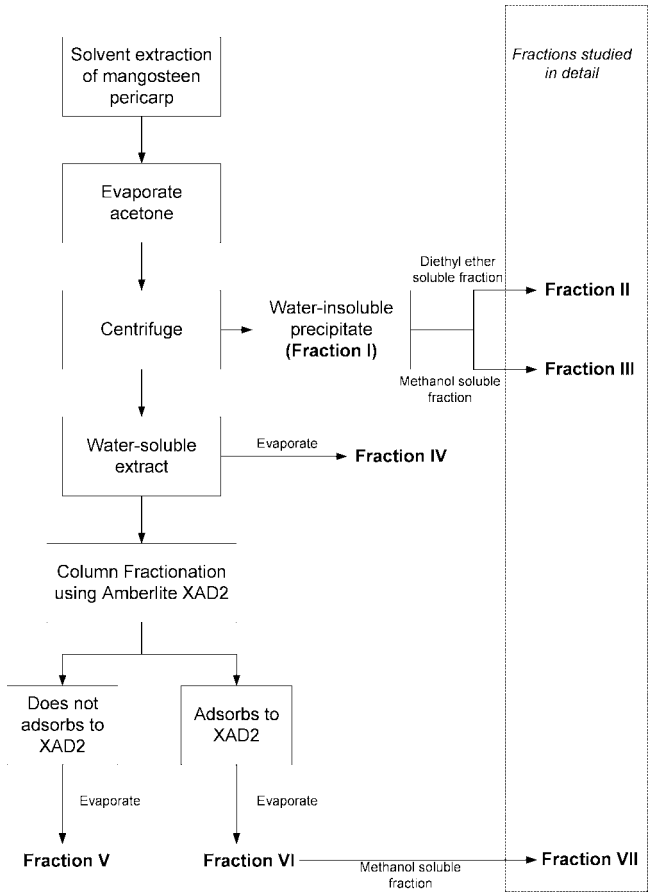
Scheme 1. Solvent fractionation of G. mangostana pericarps



maintained at 250 °C and 4.5 kV, respectively. Nitrogen is operated at 80 psi for sheath gas flow rate and 20 psi for auxiliary gas flow rate. The full scan mass spectra from *m/z* 50–2000 were acquired both in positive and negative ion mode with a scan speed of one scan per second. Chromatographic separations were accomplished on a Shim-Pack VP-ODS column (250 mm × 4.6 mm i.d.) (Shimadzu, Kyoto, Japan) with the following elution condition: 1.0 mL/min; room temperature (25 °C); solvent A, 0.1% formic acid in water; solvent B, 0.1% formic acid in methanol, starting from 30 to 60% B in 10 min, from 60 to 100% B in 40 min, from 100 to 60% B in 5 min, and from 60 to 30% B in 5 min, and lastly, reconditioning of the column for 20 min. Absorbance was recorded from 320nm.

Materials. Ripe *G. mangostana* from Thailand were purchased from local fruit stores and stored frozen in a −20 °C freezer for further use. All solvents used were of analytical reagent grade unless otherwise specified. Folin Ciocalteu reagent and KIO_3 were purchased from Merck (Darmstadt, Germany). Amberlite XAD-2, 4-dimethylamino-cinnamaldehyde, methyl gallate, starch from potatoes, amylase from hog pancreas with 46.3 U/mg, (+)- Catechin, bovine serum albumin, and tannic acid were purchased from Sigma chemical company (St Louis, MO). We purchased 4-hydroxybenzhydrazide 98% from Alfa Aesar (Ward Hill, MA). Gallic acid was purchased from Acros Organics (Geel, Belgium). Acarbose was obtained by dissolving a Glucobay tablet from Bayers containing 50 mg acarbose in phosphate buffer (pH 6.9, 50 mM, containing 6.7 mM saline), at the desired concentration. After standing for 15 min, the mixture was then centrifuged to remove the insoluble filler.

Extraction of α-AI. The extraction and fractionation steps are summarized in scheme **1**. The *G. mangostana* were thawed,and the pericarps were removed manually. The extraction method used was adapted from methods used in the extraction of tannins in cocoa (*10*). The first extraction was carried out by blending fruit hulls (250 g) with water (295 mL) in a Warring blender. After which, 5 mL acetic acid and 700 mL acetone was added to the slurry. Extraction was continued by stirring on an orbital shaker for 3 h. The slurry was then filtered using a suction pump and the residue was collected. Residues collected from two separate extractions were pooled together and further extracted with 1 L of acetone: water: acetic acid (70:29.5:0.5). The slurry was again stirred using an orbital shaker for 3 h. The filtration and extraction steps were repeated twice with less solvent (500 mL). All the filtrate was pooled and acetone was removed by evaporation at 40° under reduced pressure using a rotary evaporator. The extract was then centrifuged at 3000*g* to remove the insoluble solids that had precipitated upon removal of acetone. The sediments in the centrifuge tubes were carefully washed with water to remove the trace amount of the water-soluble extract and then dissolved in diethyl ether. The dissolved sediments were pooled together and evaporated at 30 °C under reduced pressure to dryness. The powder collected was labelled as fraction I.

The water-soluble extract was pooled together and a small sample of it was evaporated to dryness at 60 °C in vacuo. The powder collected was named as fraction IV. The remaining water-soluble extract was fractionated using XAD2 resin.

Column Fractionation of Water Soluble Extract. Fractionation by adsorption to XAD2 resin was carried out with slight modifications to instructions provided by the manufacturer. Resins were packed and equilibrated with water acidified with HCl to pH 2.0. The water-soluble extract was adjusted to pH 2.0 with HCl before loading into the column. Thirty milligrams of gallic acid equivalent (GAE), which is the binding capacity of the resin for *G. mangostana* pericarp extract, was loaded per mL of swollen XAD2 resin. Resin (500 g) was used during each separation. The column was then washed with 2 bed volume (B.V.) of acidified (pH 2.0) water. All flow rates were kept constant at 2 B.V. per hour. A sample of the extract not bounded to the column was collected and evaporated to dryness at 60 °C under reduced pressure. The powder collected was named fraction V. Desorption was carried out by washing the column with 2 B.V. of methanol followed by 2 B.V. of acetone. The flow was kept at a constant 2 B.V. per hour. The solvent was removed by evaporating to dryness at 40 °C under reduced pressure. The powder collected was named fraction VI.

Solvent Fractionation. Fraction I and VI were further solvent fractionated. The sample (1.0 g) was accurately weighed,and hexane (5.0 mL) was added. The mixture was allowed to stand for 15 min with occasional shaking before centrifuging at 3000*g*. The hexane and residue was separated and the above process repeated twice more. The hexane-extracted solid was obtained by evaporating hexane to dryness at 40 °C under reduced pressure. This extraction process was repeated on the hexane-insoluble residue using diethyl ether followed by ethyl acetate, methanol and DMSO in running order. For fraction I, only the diethyl ether and methanol fraction yield significant quantities for further characterisation and were termed fraction II and III respectively. For fraction VI, only the methanol fraction yield sufficient quantities and it was termed fraction VII.

Determination of Phenolic Contents. Total phenolic content was measured using a modified Folin-Ciocalteu assay as reported previously (*11*). Briefly, a 10-fold diluted Folin Ciocalteu solution (100 μL), sample (20 L) or standard solution of gallic acid was added together, followed by sodium carbonate solution (7%, w/v, 80 L). The solution was incubated for 30 min at 37° and absorbance read at 765 nm. Phenolic content was expressed as gram gallic acid equivalents (GAE).

Oligomeric proanthocyanidins (OPCs) was measured using 4-dimethylamino-cinnamaldhyde (DMCA) assay as reported by Shahidi and Naczk (*12*), using catechin as standard. Hydrolysable tannins was determined by reacting with KIO_3 using a modified method (*13*). Sample or standard (2.0 mL), methyl gallate, dissolved in water was added to methanol (2.0 mL) and 5% KIO_3 (100 μL). Absorbance was read at 525 nm after incubating in a 30 °C water bath for 30 min, the time was found to be optimal for color development in the standards. Anthocyanins were determined using a pH-differential spectrophotometry method as described by a method from the Institute of Nutraceutical Advancement (INA) method 116.00 (*14*).

Bovine Serum Albumin Precipitation Assay. BSA precipitation for the determination of tannins was carried as described by Hagerman (*15*, *16*). BSA was dissolved in acetic acid buffer (pH 4.9, 0.20M, containing 0.17 M NaCl). Sample or tannic acid standard (100 μL) dissolved in methanol was added to BSA (200 μL), were mixed thoroughly for 15 min before centrifuging for 5 min. The supernatant

Table 1. α-Amylase Inhibition Activity of Different Fractions of *G. Mangostana* Pericarp Extracts[a]

fraction	description	IC50 (μg/mL)	total phenolic content (g GAE/g extract)	tannic acid equivalent (g TAE/g extract)	yield (% FW)
I	precipitate obtained upon evaporation of acetone.	36.9	0.200 ± 0.004	–	2.90
II	diethyl ether soluble part of fraction I	nd	0.314 ± 0.001	–	2.11
III	methanol soluble part of fraction I	5.4	0.613 ± 0.010	1.03 ± 0.06	0.46
IV	powder obtained from drying of water soluble extract after removal of acetone	44.8	0.305 ± 0.008	–	2.27
V	portion fraction of IV that does not bind to Amberlite XAD2 resin	391.6	0.104 ± 0.008	–	0.95
VI	portion of fraction of IV that binds to XAD2 resin	27.5	0.533 ± 0.073	–	1.32
VII	methanol soluble portion of VI	10.1	0.601 ± 0.071	1.22 ± 0.06	1.05
tannic acid	known polyphenolic inhibitor of α-amylase	305.7	0.612 ± 0.080	–	–
acarbose	known carbohydrate-based inhibitor of α-amylase	5.2	–	–	–

[a] IC50 refers to the concentration of inhibitor required to produce a 50% inhibition of the initial rate of reaction, as determined by interpolation from **Figure 1a** and **b**. GAE is the total phenolic content of the extract expressed as g gallic acid. TAE is the tannin content expressed as g tannin acid. GAE and TAE are average of triplicates ± S.D.; nd, not detected, a dash implies that the assay was not carried out.

was discarded and the pellet washed with deionized water. The pellet was dissolved in SDS (400 μL, 1% w/v containing 5% (v/v) triethanolamine). Ferric chloride (100 μL of 0.01 M in 0.01 M hydrochloric acid solution) was added to the dissolved pellets and the mixture was allowed to stand at room temperature for 15 min for the formation of the ferric–phenolate complex. The absorbance readings were acquired using a microplate reader at 510 nm with 200 μL ferric-phenolate complex in each well of a 96-well plate. The results were expressed as tannic acid equivalent (TAE). The TAE refers to the amount of tannic acid (g) required to produce an equivalent amount of BSA precipitation as caused by 1 g of sample.

Amylase Inhibition Assay. A modified version of the assay introduced by Lever for the determination of reducing sugar was used (*17*). A 1% starch solution was prepared by suspending starch from potatoes in phosphate buffer (pH 6.9, 50 mM, containing 6.7 mM NaCl), and gelatinized at 100 °C for 10 min on a hotplate. Any evaporation losses were corrected by replacing the lost with water. α-Amylase solution was prepared by dissolving α-amylase from hog pancreas (35 mg) in calcium chloride (10 mL, 20 mM) topped up to 100 mL with phosphate buffer. A stock solution (5% (w/v) in 0.5 M HCl) of *p*-hydroxybenzoic acid hydrazide (PAHBAH) was prepared and stored at 4 °C. The stock solution was diluted five times with NaOH (0.5 M) to give the working PAHBAH reagent which was prepared daily. Control assays contained phosphate buffer (900 μL) and α-amylase solution (100 μL) and the reaction was started by the addition of gelatinised starch solution (500 μL). To monitor the production of reducing termini, sample (50 μL) was transferred out from each assay at 30 s intervals into PAHBAH reagent (1.0 mL). The reaction was monitored for 3.5 min. After heating for 10 min in a boiling water bath, the absorbance was acquired at 410 nm with a microplate reader. The initial rate of reaction was determined by using linear regression on Microsoft Excel software package. Inhibition assays contained phosphate buffer (400 μL), α-amylase solution (100 μL) and inhibitor (500 μL) dissolved in buffer at the desired concentration and with pH adjusted to 6.9. The reaction was started by the addition of gelatinized starch solution (500 μL) after the inhibitor was incubated together with α-amylase for 15 min. Production of reducing termini was monitored using the same method as that of the control assay. Blanks for control and inhibition assays was prepared by replacing α-amylase with phosphate buffer, so as to determine the amount of reducing termini present at 0 min. The percent inhibition was determined by comparing the initial rate of the inhibited assay to that of a control assay conducted on the same day.

RESULTS

Fractionation of Pericarp Extract and Screening of α-Amylase Inhibition Activity. Extracting *G. mangostana* pericarp with acetone–water-acetic acid (70:29.5:0.5) gave clear dark red solution. Subsequent removal of the acetone resulted in precipitation of a dark brown solid as fraction **I** (**Scheme 1**). Defatting treatment of fraction **I** removed yellowish–green substances, possibly carotenoids or chlorophyll, which yielded too little for α-amylase inhibition studies. The remaining fraction **I** was separated into diethyl ether soluble (fraction **II**) and methanol soluble (Fraction **III**) fractions. Fraction **II** was bright yellow with no measurable α-AI activity. In sharp contrast, fraction **III** has very high inhibition activity with IC50 of 5.4 μg/mL. This value is comparable with that of acarbose, a benchmark compound for α-AI. The water soluble portion Fraction **IV**, shows considerable inhibitory activity (IC50 44.8 μg/mL). Fraction **IV** was treated with Amberlite XAD2 resin and separated into those that have no affinity to amberlite (fraction **V**) and the fraction that binds Amberlite XAD2 (Fraction **VI**). Fraction V was shown to contain mainly anthocyanins (data not shown) and has rather low inhibition activity (IC50 of 391.6 μg/mL), whereas the α-AI of fraction **VI** has been enriched (IC50, 27.5 μg/mL). The IC50 was further improved to 10.1 μg/mL when fraction **VI** was extracted with methanol and yielding a methanol soluble fraction **VII** (**Table 1**). No inhibition was detected for (±) catechin and gallic acid for levels up to 1000 μg/mL. Tannic acid, a commercially available hydrolysable tannin, was found to be a weak α-AI with an IC50 of 305.7 μg/mL. The red pigment, anthocyanins does not exhibit significant α-AI activity. Typical inhibition assay plots are shown in **Figure 1**. The dose–inhibition relationship is linear and the IC50 was obtained by interpolation of points closest to 50% inhibition.

Total Phenolics Content and α-Amylase Inhibition Activity. The total phenolic contents (TPC) of the fractions and the results are tabulated in **Table 1**. Apparently, there is no relationship between TPC and α-AI activity. For example, fraction **II** has rather high TPC but there is no measurable inhibition activity. Fraction **VI** has about 5 times higher TPC than fraction **V** but has 14 times more α-AI. Tannic acid has similar TPC to fraction **VII** but its α-AI IC50 is thirty fold higher than that of fraction **VII**. Therefore, the specific chemical structure profiles of the fractions are more important factors than the TPC for determining α-AI activity, indicating there is specific interactions between the enzymes and the polyphenolic compounds, instead of none-specific binding as commonly believed.

Protein Precipitation Capacity. The ability of the fractions containing high α-AI activity (**III** and **VII**) to precipitate protein was measured and expressed as TAE. Both fraction **III** and **VII** precipitated BSA and their TAE is tabulated in **Table 1**. Fraction **III** has TAE of 1.03 ± 0.06 TAE while fraction **VII** has TAE of 1.22 ± 0.06. TAE values of above 1.0 imply that the samples were either able to precipitate BSA more efficiently than tannic acid or able to chelate more Fe (III) ions as compared to tannic acid. Catechin and gallic acid were not found to cause any

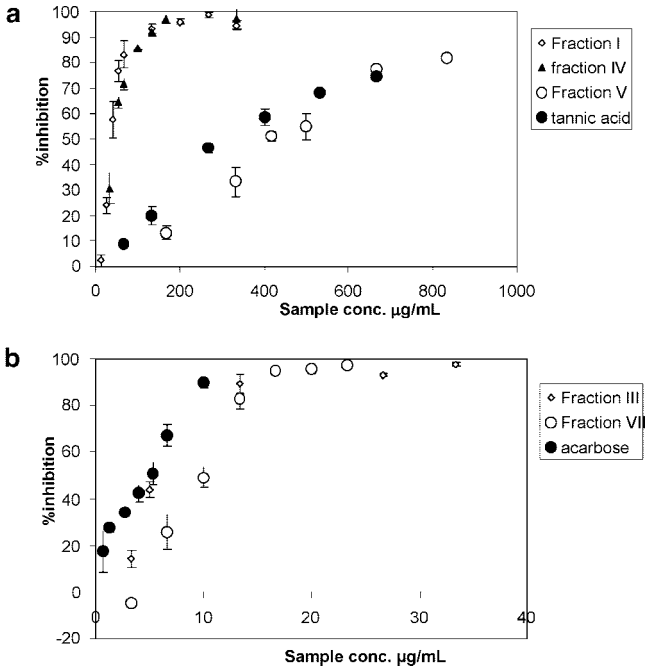


Figure 1. (**a**) Level of inhibition at different inhibitor concentration for weak inhibitors; (**b**) Level of inhibition at different inhibitor concentration for strong inhibitors. Average of triplicates ± SD.

precipitation of BSA, which had also been reported in literature (*16*). Therefore, fraction **III** and **VII** are polyphenols that can precipitate proteins.

OPC Contents. While the BSA precipitation assay confirmed that both fraction **III** and **VII** are tannins, the DMACA assay was carried out to determine if they are OPCs. The DMACA reagent reacts with the terminal unit of OPC or catechin at position C-8 of the A ring to produce a green chromophore. Catechin was used as the standard and fraction **III** and **VII** reacted positively with DMACA and therefore consist of flavonoids or OPCs. They contain 0.321 ± 0.009 and 0.424 ± 0.008 mmol terminal units/g respectively. Fraction II does not react with DMACA which confirm that it is not a flavonoid. Tannic acid, a gallotannin, was used as a negative control also does not react to DMACA.

Hydrolysable Tannin Contents. The oxidation of gallate esters by KIO_3 produces a red intermediate (λ_{max} 525nm) before further oxidation to a yellow product (*13*). The red intermediate is used as a test for hydrolysable tannins which contain large number of galloyl esters moiety. Methyl gallate was used as the standard and fraction II, III, and VII does not contain any gallate esters. Tannic acid, the positive control, reacted strongly with KIO_3.

Spectroscopic Properties. The UV–vis spectra of fraction II, III, and VII are shown in **Figure 2**. Fraction II showed maximum absorption at 317 and 352 nm which is similar to that of mangostin (318 and 351 nm) (*18*), the main xanthone in *G. mangostana*. Fraction III and VII has λ_{max} at 275nm, which is highly characteristic of flavonoids such as catechin (*19*) which are structural components of OPCs. However the composition of fraction III and VII appears to be slightly different. Fraction III shows some absorption at 370 nm, which could be contributed by small amounts of xanthones present in them. Fraction VII on the other hand has a weak absorption band at 450nm, possibly due to residual anthocyanins not removed during sorption to XAD2.

From the IR spectra (**Figure 3**), it can be seen that the transmittance bands from 1700 to 900 cm^{-1} for fraction **III**

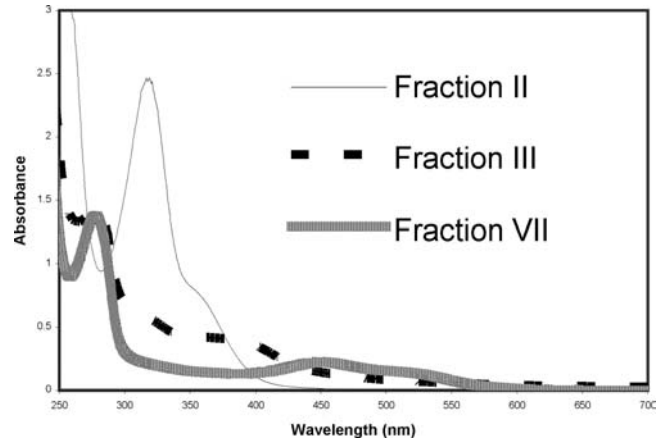


Figure 2. UV–vis spectrum of Fraction II, III, and VII at concentration of 100 ppm. Solvent, CH_3OH; room temperature.

and **VII** are almost identical. This further suggests that they are either structurally similar or perhaps identical. Fraction **II** on the other hand clearly has a different structure compared to the other two fractions. FT-IR had been used as a technique for differentiating OPCs and hydrolysable tannins (*20*). OPCs were found to have strong absorptions at around 1280 cm^{-1} due to their ethereal C-O stretching vibrations, whereas none could be observed in hydrolysable tannins. In agreement with the chemical assays, fraction **III** and **VII** are OPCs as they exhibit strong absorption in that region whereas tannic acid, a hydrolysable tannin, does not. In addition, as these two fractions have previously tested positive for flavonoids but does not contain the C=O stretching vibrations of flavones at 1670–1625 cm^{-1} (*21*), it can be concluded that they are either flavans, flavonoids lacking the carbonyl functional group (*22*), or polymers of flavans, i.e. OPCs. The C-ring structure of xanthones is similar to the C-ring of flavonoids so they are expected to have the IR absorption bands for the ethereal C–O and the flavone C=O stretching vibrations. The structural identification of the OPCs in mangosteen pericarps is further accomplished using MALDI-TOF mass spectroscopy, ^{13}C NMR, LC-MS, and thiolysis reactions. the results are reported in a separate paper (*23*). Our results revealed that there are little monomers like epicatechin or catechin in the pericarp. As expected, fraction **II** showed strong absorption bands in these two regions as well. LC-MS studies showed that fraction II contains mainly α-mangostin and other xanthones, which have also been reported by other groups (*24*).

DISCUSSION

α-AI were detected in fraction **III** and **VII** but none in fraction **II**. From LC-MS analysis, fraction **II** was found to constitute of xanthones of which mangostins are predominant. Fraction **III** and **VII** are found to be OPCs as they reacted positively to DMACA and had FT-IR spectrum similar to that of OPCs. We have further characterized the structural information of the OPCs *G. mangostana* and the results shown that they are mainly B-type oligomers (degree of polymerization ranging from 2 to 10) of epicatechin with low content of gallates and monomers. A plausible reason why OPCs could be found among the water insoluble extract (fraction **I**) as well as the water soluble extract (fraction **IV**) is that they might have complexed with the xanthones and precipitated together with them.

While the KIO_3 and DMACA assays are not strictly quantitative, we used them as assay to differentiate between OPC and

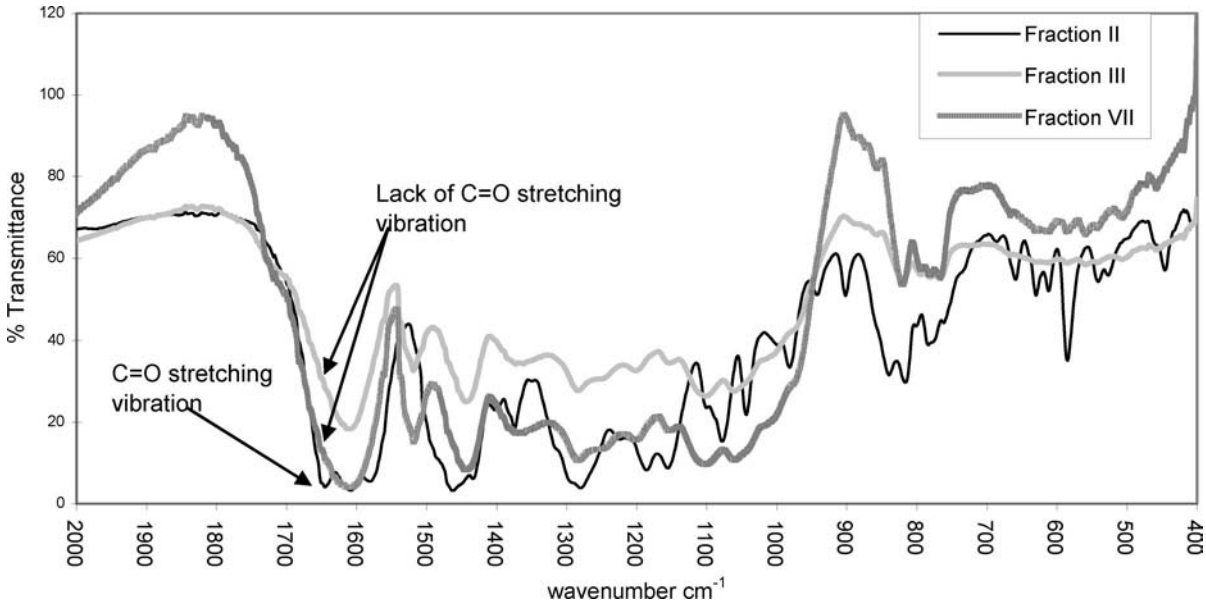


Figure 3. FT-IR transmittance spectrum of Fraction II, III, and VII pelletized with KBr.

hydrolysable tannins. KIO_3 reacts with gallate esters and the number of gallate esters on hydrolysable tannins can vary. DMACA reagent reacts with both monomers and polymeric flavonoids. Therefore the amount of OPCs in a sample could be over-estimated if monomers are present.

The α-AI activity has been shown to vary over several folds among tannins, indicating that not all tannins are effective α-AI. In our study, fraction **III** from *G. mangostana* pericarp are 56 times more effective α-AI than tannic acid and is as effective as acarbose. To the best of our knowledge, this is the first study on the α-AI activity of components in *G. mangostana* pericarp. The mechanism of inhibition is likely due to nonspecific interaction with α-amylase as OPCs can bind to proteins. Thus it is possible that *G. mangostana* OPCs would also bind to any available proteins within the small intestine and not specifically to α-amylase. While tannins used to be considered as interfering compounds in screening of bioactive natural products, this conventional wisdom needs to be re-examined (*25*) as our work indicates that tannins can act as potent α-AI. Further research is warranted to isolate the individual compounds from the OPC mixtures and study the specificity and potency of the enzyme inhibitions to establish a structure and activity relationship.

Another issue that affects the use of OPCs as α-AI is whether they are able to reach the small intestines intact. While such acid catalysed cleavage of the interflavan bond had been reported in vitro (*26*), in vivo studies on human and rats found that tetrameric or higher OPCs reach the small intestine unchanged. This had been attributed to the buffering effect that food provides, thus preventing the acid-catalysed hydrolysis (*27*). In the small intestines, it was found that phosphatidylcholine, a surfactant in bile, was able to prevent OPCs from inhibiting digestive enzymes (*28*). It appears that the metabolic fate of OPCs is complex and future studies should concentrate on their in vivo α-amylase inhibition activity.

The last but most important factor affecting the use of *G. mangostana* OPCs as α-AI is the alleged toxicity of tannins. Tannins are multidentate ligands and are therefore able to chelate metal ions. Thus they have been associated with decreasing bioavailability of iron (*29*) and calcium. Complexes of tannin and dietary proteins were also less digestible (*30*). Excessive consumption of betel nuts and sorghum, both of which are rich sources of tannins, had been associated with increased incidence of oesophageal cancers (*31*). There is also evidence that tannins might be hepatotoxic as feeding of tannins causes liver enlargement in rabbits (*32*). On the other hand, consumption of tannins has also been found to provide various health benefits. As reviewed by Prior and Gu (*33*), OPCs are cardioprotective, anticarcinogenic, and they promote urinary tract health. A recent study has shown that *G. mangostana* peels shows significant cytotoxicity against Caco-2 and peripheral blood mononuclear cells (*34*). However the cytotoxicity was carried out using extracts prior to any fractionation. More studies are needed to determine if the cytotoxic components are due to OPCs, anthocyanins or xanthones. To conclude, it was found that the secondary metabolites of *G. mangostana* pericarp is predominantly xanthones followed by OPCs. While xanthones does not have any α-AI activity, OPCs were found to be highly potent α-AI and are 56 times more effective than tannic acid. Their inhibitory activity of 5.4 μg/mL is comparable to that of acarbose at 5.2 μg/mL. However, more studies are required to determine the in vivo efficacy and possible toxicity. The structural and inhibiting activity relationship of individual OPCs can only be measured after successful separation of the oligomeric OPCs, a non-trivial task due to the complexity of the mixtures and the air-sensitivity of the OPCs.

ABBREVIATIONS USED

α-AI, alpha amylase inhibitor; TPC, total phenolic content; OPC, oligomeric proanthocyanidins; BSA, bovine serum albumin; FT-IR, Fourier transform infrared spectroscopy; nd, not detected.

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