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Effects of indirect sonication pretreatment and solvent extraction on the xanthone content and its antioxidant activities of freeze dried mangosteen (*Garcinia Mangostana* Linn.) pericarp powder extracts

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Abstract

Garcinia Mangostana Linn. (mangosteen) belongs to the Guttiferae family; about 2/3 of the fruit weight is pericarp which is a great source of polyphenols compounds and xanthones with strong antioxidant and biological activity, but thrown out as a waste from the fruit processing industries due to its unpleasant astringency taste. Owing to the above concern, the extraction and utilization of these bioactive compounds for functional products is increasing. In present study effect of indirect sonication and solvent extraction of freeze dried mangosteen pericarp powder (MPP) on total xanthones, α- mangostin, total polyphenol content and its antioxidant activities were studied. Indirect sonication showed significantly higher total xanthone, α-mangostin content and α- mangostin percentage of MPP, irrespective of the sonication power and time ($p<0.05$). Among the pretreated freeze dried MPP, low power 20 mins pretreatment showed higher α- mangostin content and α- mangostin percentage which was about 56.3 mg/g and 58.3%, respectively. Furthermore, indirect sonication pretreatment enhances the porosity of the MPP which can be clearly observed by the scanning electron microscope images with more loosen structure, whereas control showed more compact structure. This may enhance the release of total xanthones and α- mangostin during pretreatment. These results confirmed that indirect sonication with solvent extraction has synergistic effect on the release of total xanthones and α- mangosteen content of MPP.

Introduction

Mangosteen (*Garcinia mangostana* Linn.) is rich in bioactive compounds with various health enhancing functions like antioxidant, antinflammation and it is also used for the treatment of diarrhea, infected wound, abdominal pain, dysentery and chronic ulcer (Pedraza-Chaverri *et al.*, 2008; Suttirak and Manurakchinakorn, 2014). Inner fleshy part of the fruits are most widely used for the consumption and the outer pericarp is remained as the waste from most of the fruit processing industries and other small scale sectors of the fruit market (Palakawong *et al*., 2013). Outer pericarp is rich in various bioactive compounds including oligomeric pro-anthocyanins, anthocyanins, tannin, α- mangostin and xanthones (Palakawong *et al*., 2013; Janhom and Dharmasaroja, 2015). Recently xanthones and α-mangostin from pericarp of the mangosteen showed neuroprotective activity in the Parkinson's disease model (Jindarat, 2014; Janhom and Dharmasaroja, 2015). Furthermore, the pericarp extract was extensively used in production of herbal cosmetic and herbal medicines such as products for treatment of acne (Choi *et al*., 2014; Peerapattana *et al.*, 2015). Even though mangosteen pericarp powder is rich in bioactive compounds and xanthones, their extraction method influence the bioactive compounds activity from the mangosteen pericarp.

Extraction of bioactive compounds from mangosteen pericarp powder by conventional technologies showed various limitations such as thermal damage of bioactive ingredients, loss of xanthone contents and its antioxidant activities. In order to enhance the yield of xanthone and α- mangostin contents, various low temperature technologies were studied such as ultrasound assisted extraction (UAE) (Zou *et al*., 2014). Even though they showed higher extraction of xanthones, combination of the two processes may enhance the yield of the xanthone and α- mangostin. Some combination of extraction process such as ultrasound

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and vacuum distillation showed higher release of bioactive compounds in spearmint plants (Da Porto and Decorti, 2009). To the best of our knowledge, the combination of indirect ultrasound pretreatment and solvent extraction of xanthones and α- mangostin from freeze dried pericarp powder are not yet studied. The aim of current research is to evaluate the synergistic effect of the indirect sonication pretreatment and solvent extraction of freeze dried mangosteen pericarp powder on the amount of xanthone, α- mangostin, total polyphenol contents and its antioxidant activities.

Materials and Methods

Materials

Mature mangosteen fruits were collected from local farm in Hulu Langat, Selangor, Malaysia. Sigma-Aldrich was the supplier of 1, 1-Diphenyl-2-picryldydrazyl (DPPH), 2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) di ammonium salt (ABTS) and trolox standards. Folin–Cicalteau and 2, 4, 6-Tri (2-pyridyl)-1, 3, 5-triazine (TPTZ) were purchased from Merck. Absolute ethanol (99.99%), ethanol 95%, methanol (99.98%) and HPLC grade acetonitrile were provided by Fisher Scientific. All other chemicals used were of analytical grade.

Preparation of freeze dried mangosteen pericarp powder

Collected mangosteen fruits were cleaned, peeled and cut into small cubes and packed in labeled vacuum pouches at the University Putra Malaysia in Selangor, Malaysia. To prevent degradation they were stored under -18°C and dried using freeze drier at -35ºC (Labcono USA) until pericarp reached to moisture content less than 10%. Dried samples were ground to fine powder using commercial blender (Calverton, UK). The mangosteen pericarp powder (MPP) was sieved through stainless steel laboratory test sieve, aperture 500 µm, mesh No. 35, to obtain powder with particle size smaller than 500µm and stored under -18°C until further analysis.

Extraction of bioactive compounds

The freeze dried MPP was mixed with 95% ethanol at the ratio of 1:100 and pre-treated randomly following defined conditions that was set in the full factorial design with sonication power at 2 levels: low and medium (10 & 50W) ; sonication time at two levels: 10 & 20 mins by using a bath sonicator (Powersonic 420, Gyeonggi-do, S. Korea). After that, the sample mixture was subjected to solvent extraction for 1hr at room temperature using ethanol 95% and magnetic stirrer, then the mixture was centrifuged at 4500 rpm for 15 mins, filtered with Whatmann No.1 filter paper. The solvent in the samples were evaporated under vacuum using rotary evaporator. The sample without ultrasonic pretreatment was taken as control and stored at -18°C for further analysis.

Determination of total xanthones content

Preparation of standard solution

A stock solution 1000 ppm (1000 µg/mL) and standard solutions of α-mangostin standard was prepared by dissolving accurately 10mg of α-mangostin standard powder with purity 99.99% in 10 mL of HPLC grade methanol in a volumetric flask, and filtered through CNW Nylon 0.45µm filter (Zarena *et al*., 2012). Standard curve equation was obtained from the standard curve of α-mangostin plotted based on the concentration of α-mangostin versus area under the peak.

HPLC Instrumentation and Analytical Condition

HPLC analysis of the xanthones was performed through the method of Zarena *et al.* (2012) on a Shimadzu LC-10 (Japan), Eclipse XDB-C18 (4.6×150 mm, 5 µm particle size) reverse-phase column and UV-visible detector with a little modification (running mobile phase B for 25 mins instead of 20 mins). Injection volume of sample was 20 µL, and they were analyzed and detected through gradient mobile phase at 254 nm − 319 nm of wavelength. Orthophosphoric acid in deionized water (0.03% v/v) was used as mobile phase A. Solvent B was acetonitrile: methanol (75:25 v/v). Samples and standard solutions were injected at the flow rate 0.8mL/min under the initial condition of 0 mins 75% B, 25 mins 90% B, 30 mins 95% B, 35 mins 75% B, all under the constant temperature (40°C). A class of 10 A Software was utilized in data collection and data processing for instrument control. The results were reported as mg α-mangostin/g mangosteen pericarp powder according to the standard curve equation (of α-mangostin standard solutions (0 to 400 ppm).

Extraction yield

The extraction yield of the freeze dried MFP extracts were expressed in the percentage of dried MFP sample according to Equation 1.

$$Yield\ (\%) = \frac{W3 - W2}{W1}) \times 100 \qquad Equation\ (1)$$

W_1 is the weight of mangosteen fruit pericarp used for extraction, W_2 is weight of empty flask (g),

and W_3 is weight of flask with extract (g) after rotary evaporation and oven drying at 40°C.

Total polyphenolic contents (TPC)

The TPC of freeze dried MPP extracts were determined according to the modified method of Cheok *et al*. (2013). One mL of diluted crude extract from MPP was mixed with 5.0 mL of Folin–Cicalteau reagent (diluted 1:10 with distilled water) and shook for 3 mins. Later, 4.0 mL of sodium carbonate solution (7.5% w/v) was added to the mixture and mixed well, then incubated for 30 mins in the dark at ambient temperature until the solution turns into blue color. Finally their absorbance was measured at 765 nm. The TPC value was reported based on the mg of gallic acid equivalents (GAE) per g dry weight (DW) mangosteen fruit pericarp powder using the standard curve equation (y= 9.7053x+ 0.0399, R^2=0.9974) of the absorbance of Gallic acid (0.1 to 0.8 µg/mL) standard solutions.

DPPH radical scavenging activity

The DPPH radical scavenging activity of freeze dried MPP extracts were determined according to the modified method of Pan *et al*. (2011). Around 60 µl of diluted crude extract were mixed vigorously with 3 mL freshly prepared DPPH reagent (0.05 g/ mL methanol), allowed to stand for 20 mins in dark at room temperature. Then, their absorbance was measured at 517 nm by a UV-visible spectrophotometer. As a control, 60 µl of ethanol was mixed with DPPH reagent and the decrease in absorbance of the resulting solution was monitored at 517nm and reported as Abs control. After calculation their % inhibition based on equation (2):

$$\%\,Inhibition = \left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) \times 100 \qquad Equation\ (2)$$

Using equation from the standard curve (y= 26.918x+2.8237, R^2=0.986), based on the % inhibition of different concentration of Trolox standard solutions versus mM Trolox, results were corrected for dilution and expressed in mM Trolox equivalent per 100g dry weight sample. (mM Trolox/100g DW).

Ferric ion reducing antioxidant power (FRAP) assay

The FRAP value of freeze dried MPP extracts were measured by a modified method of Hossain *et al.* (2012). For FRAP reagent preparation 300 mM acetate buffer at pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM FeCl3 was mixed together at the ratio of 10:1:1, v/v/v and incubated in water bath 37°C. Then 100 µL of a standards solutions or sample extracts was mixed with 900 µL of FRAP reagent and their absorbance at 593 nm was read (UV-visible spectrophotometer; Thermo Scientific Co.) against a reagent blank at a pre-determined 40 mins time after mixing and incubation at 37°C. Using the equation from the FeSo4 standard curve (y= 1.1609x+ 0.0115, R^2=0.9953), the results were corrected for dilution and expressed in mM $FeSo_4$ equivalent per 100 g dry weight sample (mM $FeSo_4$/100 g DW) through Equation (3).

$$\left(mM\frac{FeSO4}{100g}\right) = C \times V \times df \times 100 / (M \times 1000) \qquad Equation\ (3)$$

Where, C = concentration of $FeSO_4$ established from calibration curve (mM), V = volume of extraction solvent (mL), df = dilution factor, M = weight of plant material in extraction, (×100 g to convert the ratio in mM/100 g; ÷1000 to convert the extract weight from mg to g).

ABTS radical scavenging activity

ABTS radical scavenging activity of freeze dried MPP extracts were done by the modified method of Suvarnakuta *et al.* (2011). The 20 µL of diluted extract as a sample, was mixed with 1 mL of diluted radical $ABTS^+$ and mixed for 10 second, stored at dark, absorbance reading was done exactly 1 mins after initial mixing. As and Ao refer to the absorbance of the sample and control, respectively. Percentage of inhibition was calculated by equation (4).

$$\%\ Inhibition = \left(\frac{Ac - As}{Ac}\right) \times 100 \qquad Equation\ (4)$$

Based on the equation from the standard curve (y= 70.81x -2.4364, R^2= 0.970) of the % inhibition of different concentrations of trolox standard solutions versus mM Trolox (0.2-1.2 mM in 95% ethanol), and correction for dilution, results were reported in mM trolox equivalent per 100 g dry weight (DW).

Scanning electron microscope (SEM)

In order to investigate the morphology of freeze dried MPP before and after indirect ultrasonic pretreatment the scanning electron microscope (JSM 6390 LV, Tokyo, Japan) was used at an accelerating voltage of 15KV and magnification between X 190-4500. Briefly, some mangosteen pericarp powders were placed on one surface of a double-faced adhesive tape mounted on SEM stubs and coated with a thin layer of gold by a vaccum-sputtering coater (Leica, EM SCD 500, Wetzlar, Germany) (Palakawong *et al*., 2013), then kept in oven 45ºC for 1 hr, before running the sample.

Statistical analysis

Statistical analyses were performed using Minitab, version 16. The results were expressed as mean ± SD of triplicate. A one-way ANOVA was performed by Turkey's, test at P< 0.05 to determine significant differences among the samples.

Results and Discussion

Effect of indirect sonication pretreatment and solvent extraction on the total xanthone and α-mangostin content of freeze dried MPP extracts

The effect of indirect sonication and solvent extraction on the total xanthone and α-mangostin content of the freeze dried MPP were shown in Fig. 1. Indirect sonication significantly increase the xanthone and α-mangostin content of the MPP, irrespective of the sonication pretreatments (P<0.05). Increase in the release of xanthones favors the α-mangostin percentage content in the treated samples. Ultrasound pretreatment sample with low power 10 mins sonication showed significantly higher xanthones, α-mangostin and α-mangostin percentage than control. This was most likely due to the sonication pretreatment that may have enhanced the cavitation of the cell compartments resulting in higher release of the xanthones. Similarly, Cheok *et al*. (2013) also studied the effect of direct and indirect sonication on anthocyanin content in the mangosteen hull. They found that release of bioactive compound was enhanced with ultrasound treatment due to the cavitation effect and increased penetration of the solvents. However, different indirect sonication pretreatments with variable power and time, does not significantly affects the release of total xanthones, and α-mangostin content (P>0.05) in this study. In overall, the total release of xanthone and α-mangostin contents were enhanced with the indirect sonication pretreatment of the freeze dried pericarp powder without much degradation.

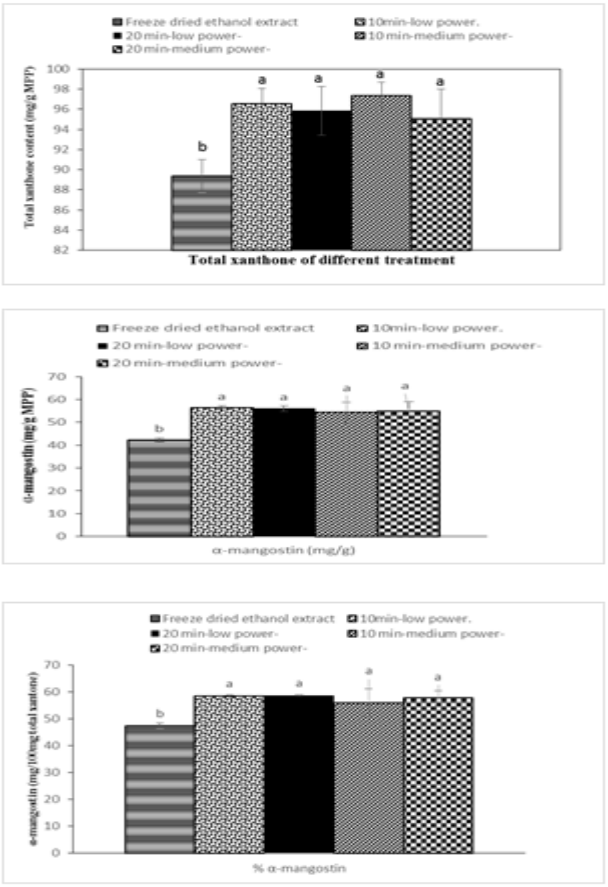

Figure 1. Effect of indirect sonication pretreatment and solvent extraction on the total xanthone and α-mangostin content of freeze dried mangosteen pericarp powder extracts.

Effect of indirect sonication pretreatment on the extraction yield and TPC of freeze dried MPP extracts

The effect of indirect sonication and solvent extraction on the extraction yield and TPC of the freeze dried MPP are shown in Fig. 2. Sonication pretreatment of freeze dried MPP extracts significantly decreased the extraction yield and TPC of the freeze dried MPP extracts (P<0.05), irrespective of treatments. However, different sonication pretreatments does not significantly affects the extraction yield and TPC of freeze dried MPP extracts (P>0.05). Among the sonication pretreatments, by increasing the sonication pretreatment time to 20 mins, the extraction yield decreases, irrespective of the power. This was most likely due to the degradation of bioactive compounds, other than xanthones and α-mangostin, through sonication pretreatment by increasing temperature, which may result in the lower yield percentage of the extraction of bioactive compounds. Similarly, ultrasound pretreatment of the air dried apple resulted in the lower extraction yield and TPC (Opalic *et al*., 2009). However, among the sonication pretreatments, at low power sonication, increasing sonication pretreatment time to 20 mins, results into increases the yield and TPC of the freeze dried pretreated MPP extracts. Further increase in the sonication power from low to medium reduces the TPC content of the freeze dried MPP extracts. It confirmed that increase in indirect sonication time and power may affects the yield and TPC content of the freeze dried MPP extracts other than xanthones and α-mangostin contents.

Effect of indirect sonication pretreatment on the DPPH, FRAP radical scavenging activity, and ABTS of freeze dried MPP extracts

The effect of indirect sonication and solvent

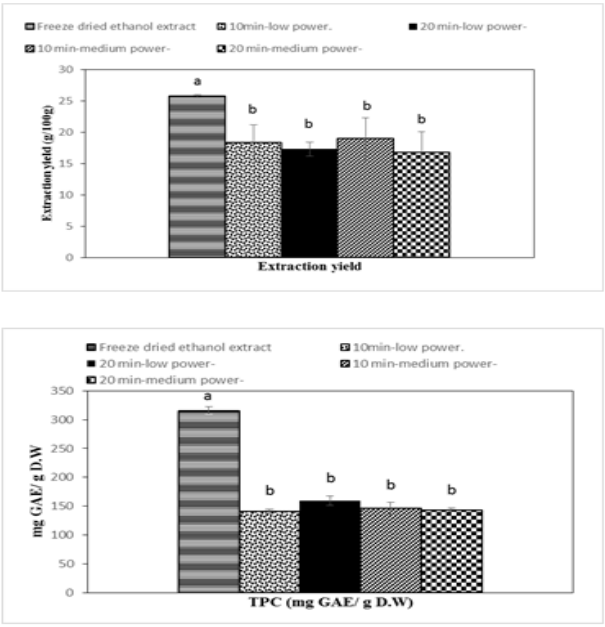

Figure 2. Effect of indirect sonication pretreatment and solvent extraction on the extraction yield and total polyphenol contents of freeze dried mangosteen pericarp powder extracts.

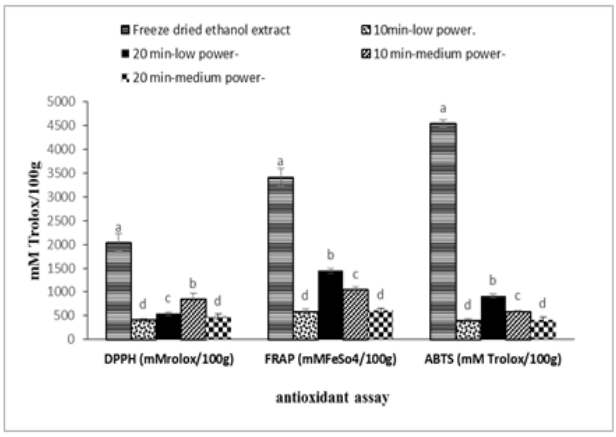

Figure 3. Effect of indirect sonication pretreatment and solvent extraction on the DPPH, ABTS and FRAP radical scavenging activity of freeze dried mangosteen pericarp powder extracts.

extraction on the DPPH, FRAP radical scavenging activity, and ABTS of the freeze dried MPP is shown in Fig. 3. Sonication pretreatment of freeze dried MPP extracts significantly decreases the DPPH, FRAP radical scavenging activity, and ABTS of the freeze dried MPP ($P<0.05$), irrespective of sonication power and times. In addition, different sonication pretreatments do not significantly affect these antioxidant properties of freeze dried MPP extracts ($P>0.05$). This was highly correlated with TPC content of the freeze dried MPP extracts. Among the sonication pretreatments, increase in sonication pretreatment time to 20 mins, increases the DPPH, FRAP radical scavenging activity, and ABTS in the

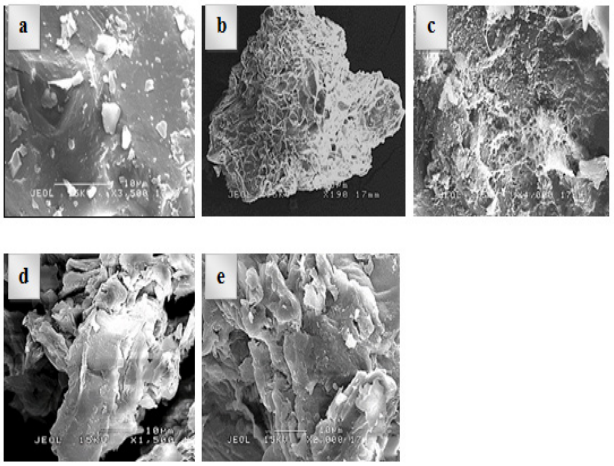

Figure 4. Scanning electron microscope image of freeze dried mangosteen pericarp powder extracts: a: Control (X 3500); b: low power-20 mins sonication (X 190); c: low power-20 mins sonication (X 4000); d: medium power-10 mins sonication (X 1500); e: medium power-10 mins sonication (X 2000).

low power treatment. In contrast, at medium power, increase in sonication time to 20 mins decreases the DPPH, FRAP and ABTS value, and it was most likely due to the degradation of bioactive compounds through sonication pretreatment by increasing temperature. Ultrasonic pretreatment lead to the loss of polyphenol which ultimately reduced DPPH, FRAP and ABTS value. Similarly, ultrasound treatment of the *Terminalia catappa* L. leaves resulted in the lower DPPH, FRAP radical scavenging activity, and ABTS with the extraction time more than 60 minutes, due to the degradation of the bioactive compounds (Annegowda *et al.*, 2010). These results confirm that increase indirect sonication time and power may affect the antioxidant properties of the freeze dried MPP extracts.

Effect of indirect sonication pretreatment on the microstructure of freeze dried MPP

The effect of indirect sonication and solvent extraction on the microstructure of the freeze dried MPP is shown in Fig. 4. Sonication pretreatment may loosen the freeze dried powder cell wall structure irrespective of the treatment, which may results in the enhanced penetration of the solvents into the cell and increase the amount of release of xanthones and α-mangostin into the freeze dried MPP extracts (Fig.1). Further, microstructure of the control without pretreatment shows more compact structure, and it may results in the lower degradation of the TPC by the sonication pretreatment. It confirms that sonication pretreatment of the freeze dried MPP enhances the release of xanthone while other polyphenol contents reduced. In general the microstructure during

sonication pretreatment gives valuable information for the food processing and enhanced extraction of the bioactive compounds.

Conclusion

In conclusion, indirect ultrasonic pretreatment has positive synergistic effect of the release of total xanthone and α-mangostin content of the freeze dried MPP. However, it has negative effect on TPC and its antioxidant activities. For the commercial purpose, the industries which focus on the higher extraction of xanthones and α-mangostin content, without much effect in the biological activities, indirect sonication pretreatment with medium sonication power of 10 mins followed by solvent extraction will be a suitable alternative.

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