Effects of Extraction Solvents on Phytochemicals and Antibacterial Activities of *Garcinia mangostana* Pericarp

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ABSTRACT— ***In this paper, the effects of different solvents (hexane, ethyl acetate, acetone, ethanol, and methanol) on the content of phytochemicals including total polyphenols, flavonoids and anthocyanin, as well as antibacterial activities of extracts from Garcinia mangostana pericarp were investigated. The results showed that the contents of total polyphenols, flavonoids, and anthocyanin were significantly affected by extraction solvents, and resulting in variation of antibacterial activities of Garcinia mangostana pericarp. The methanol extract exhibited high antibacterial activities because it possessed the highest total polyphenols content. The acetone and ethanol extracts had the highest content of flavonoids and anthocyanin respectively and also exhibited higher antibacterial activities, while other extracts had both lower the content of active compounds and bioactivities. These results indicated that selective extraction from Garcinia mangostana pericarp, by an appropriate solvent, is important for obtaining fractions with high antibacterial activity, which will be useful for the developing and application of mangosteen pericarp as a new local source of bioactive compounds in foods and medicine industries.***

Keywords—*Garcinia mangostana* pericarp, Extraction solvents, Phytochemicals, Antibacterial activity

1. INTRODUCTION

In recent years, food spoilage and food poisoning are one of the most important issues facing the food industry (Sokmen *et al*., 2004), and there has been a dramatic increase throughout the world in the number of reported cases of foodborne illness (Mead *et al*., 1999; Shan *et al*., 2007), even in developed countries. Many attempts, such as use of synthetic chemicals, have been made to control microbial growth and to reduce the incidence of food poisoning and spoilage with antimicrobial chemicals. Recently, however, consumers have grown concerned about the side effects of synthetic antimicrobial chemicals and want safer materials for preventing and controlling pathogenic microorganisms in foods (Cakir *et al*., 2005). Thus, development of safe and natural antibacterial compounds for resistant organisms is becoming critically important (Michael & Stuart, 2007).

Plants can be an excellent source of natural antimicrobials and can be effectively in reducing the dependence on antibiotics, minimize the chances of antibiotic resistance in food borne pathogenic microorganisms as well as help in controlling cross-contaminations by food-borne pathogens. The plants or their extracts can also be used as natural colorants of foodstuffs, and they are believed to be safe, and non-toxic to humans (Burt 2004; Rymbai *et al*., 2011). Of late, many compounds, especially of plant origin, have been reported to exhibit rich antioxidant and antimicrobial properties (Tiwari *et al*., 2009; Delgado-Adámez *et al*., 2012).

The mangosteen (*Garcinia mangostana* L.), belonging to the family Guttiferae, is a tropical evergreen tree widely distributed in India, Myanmar, Malaysia, Philippines, Sri Lanka, and Thailand (Pedraza-Chaverri *et al*., 2008). The mangosteen-fruit and its products are now widely available and are highly popular because of their perceived role in enhancing human health. The pericarp of mangosteen fruit has also been used as a medicinal agent by Southeast Asians for centuries in the treatment of abdominal pain, dysentery, suppuration, infected wound, leucorrhoea, chronic ulcer, cholera and fever (Suksamrarn *et al*., 2006; Obolskiy *et al*., 2009). It contains abundant bioactive substances such as mangostin, tannin, xanthone, flavone and phenolic compounds and so on (Mahabusarakam & Wiriyachitra 1987; Zadernowski *et al*., 2009; Wittenauer *et al*., 2012), which can make it possess a wide range of biological activities, such as antioxidant activity (Jung *et al*., 2006; Yu *et al*., 2007), antibacterial activity (Sakagami *et al*., 2005; Chomnawang *et al*., 2005), anti-inflammatory activity (Chen *et al*., 2008), antitumor activity (Yu *et al*., 2009), cytotoxic activities (Suksamrarn *et al*., 2006) and so on (Pedraza-Chaverri *et al*., 2008; Cui *et al*., 2010). However, the content of bioactive substances is affected by genetic, cultural practices and climatic factors during the plant growth cycle, but the extraction

yield is influenced by extraction methods (Aspé & Fernández 2011; Cheok *et al*., 2013) and extraction solvents (Alothman *et al*., 2009; Cheok *et al*., 2012) during extraction due to differences in the structure of these compounds and their physicochemical properties. So depending on the solvent used for extracting bioactive compounds, extracts obtained from the same plant may vary widely with respect to their concentration and activities (Alothman *et al*., 2009; Cheok *et al*., 2012). To the best of our knowledge, data on the pericarp of *Garcinia mangostana* in this respect are still scarce. In order to assess the effect of solvent system on the content and bioactivities of bioactive substances from mangosteen pericarp, we compared the content of total phenolics, flavonoids, and anthocyanin, as well as antibacterial activities of mangosteen pericarp under five extracting solvents. The expected results will be useful for the developing and application of mangosteen pericarp as a new local source of bioactive compounds for economic and health utilization.

2. MATERIALS AND METHODS

2.1 Plant materials

The *Garcinia mangostana* fruit at the commercially mature stage was bought in the summer of 2013 from a local supermarket. The plant material was identified by Prof. Qing-Ping Hu, College of Life Sciences, Shanxi Normal University. A voucher specimen was deposited in the College of Life Sciences, Shanxi Normal University, Linfen City, China. They were cleaned with distilled water and then peeled manually. Subsequently, the fruit pericarp was lyophilized and then stored in polyethylene bags at 4 ^{o}C until analysis.

2.2 Microbial strains and culture

Three Gram-positive strains were *Staphylococcus aureus* ATCC 25923, *Staphylococcus albus* ATCC 8799, and *Bacillus subtilis* ATCC 6051. Three Gram-negative bacteria were *Salmonella typhimurium* ATCC 19430, *Shigella dysenteriae* CMCC (B) 51252 and *Escherichia coli* ATCC 25922. The strains were provided by the College of Life Science, Shanxi Normal University, and cultured at 37 °C on NA or NB mediums.

2.3 Preparation of extracts

The dried fruit pericarps were finely ground with a micro plant grinding machine (FZ102; Tianjin Taisite Instruments, Tianjin, China). Ground samples (500 g) were blended with 5 L solvent and shaken with a laboratory rotary shaker at 150 rpm for 4 h at 30 °C, and then the homogenates were centrifuged for 20 min at 4 ^{o}C and 5 000 g in a centrifuge (Eppendorf 5417R, Germany). After centrifugation, the supernatants were pooled, and vacuum-evaporated to dryness at 40 ^{o}C. Extracts were obtained using different solvents with increasing polarity: hexane, ethyl acetate, acetone, ethanol, and methanol. All extracts were stored at -4 ^{o}C until analysis was performed.

2.4 Determination of total phenolic content (TPC)

Total phenolic content was determined as described by Rebey et al. (2012) with slight modifications. An aliquot (0.1 mL) of diluted extracts, 2.8 mL of deionized water and 0.1 mL of 1.0M Folin-Ciocalteu reagent were mixed and stirred. After 8 min, 2 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured at 765 nm wavelength after incubation for 2 h at room temperature. Gallic acid was used for calibration of the standard curve and total phenolic content was expressed as milligram gallic acid equivalent per gram dried weight (mg GAE/g DW). All extracts were tested in triplicates.

2.5 Determination of total flavonoid content (TFC)

The level of total flavonoid was measured as described by Rebey et al. (2012) with some modifications. Briefly, an aliquot (1.0 mL) of diluted extracts and 0.3 mL of 5% $NaNO_2$ solution were mixed for 6 min. Then 0.3 mL 10% $Al(NO_3)_3$ was added and incubated for 6 min. Next, 4 mL of 4% NaOH was added. The final volume was adjusted to 10 mL with distilled water and mixed thoroughly. After 15 min, absorbance of the mixture was determined at 510 nm against the same mixture. Rutin was used for calibration of the standard curve and the content of flavonoids was expressed as milligram rutin equivalent per gram dried weight (mg RE/g DW). All samples were tested in three replications.

2.6 Determination of the total anthocyanin content (TAC)

The TAC was determined as described by Hu and Xu (2011). Absorbance of appropriately diluted extracts at 535 nm was immediately measured to detect anthocyanin. Anthocyanin levels were expressed as milligrams of cyanidin 3-glucoside equivalents (CGE) per 100 g of DW, using the reported molar extinction coefficient of 25 965 M^{-1} cm^{-1} and a molecular weight of 449.2 g/mol.

2.7 Antibacterial activities

2.7.1 Agar disc diffusion assay

The agar disc diffusion method described by Rammanee and Hongpattarakere (2011) with some modifications was used to determine the antibacterial activity of the samples. Briefly, a suspension (0.1 mL of 2×10^6 CFU/mL) of each bacterium was spread on the solid medium plates. Whatman no.1 sterile filter paper discs (6 mm diameter) were impregnated with the extracts sterilized by filtration through 0.22 μm Millipore filters and then placed on the inoculated plates; these plates were incubated at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition (ZOI) against the tested bacteria. Each assay was performed in triplicate.

2.7.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The MIC and MBC were determined according to the method described by Diao et al. (2013) with minor modifications. Two fold serial dilutions of samples were prepared in sterile NB medium. To each tube 0.1 mL of the exponentially growing bacterial cells was added to give a cell concentration of approximately 2×10^6 CFU/mL. The tubes were incubated at 37 °C for 24 h and then examined for evidence of the growth. The MIC was determined as the lowest concentration of the extracts that demonstrated no visible growth. The MBC was determined as follows. After the determination of the MIC, 100-fold dilutions with NB from each tube showing no turbidity were incubated at 37 °C for 48 h. The MBC was the lowest concentration of the extracts that showed no visible growth in the drug-free cultivation.

2.7.3 Kill-time analysis

The kill-time curve assay method was used to study the bactericidal effects of the essential oil according to the technique described by Joray et al. (2011). The cultivation with the essential oil was done the same as the above MIC assay and controls containing only DMSO were simultaneously run. At selected time intervals, samples from the test culture were taken, serially diluted in sterile water, and plated in Plate Count Agar (PCA) medium. All plates were then incubated for 24 h at 37 °C, and CFU were counted.

2.8 Statistical analysis

All results are expressed as mean ± SD (n=3). One-way analysis of variance (ANOVA) and Duncan's test were performed with significant level being considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Contents of total polyphenols, flavonoids and anthocyanin

The levels of total polyphenols, flavonoids and anthocyanin in different extracts from *Garcinia mangostana* pericarp were given in Table 1. Results showed that total phenolic contents of different extracts varied considerably and ranged from 8.43 to 85.65 mg GAE/g DW, respectively, for hexane and methanol. With respect to total phenolic content, solvents used in the present study could be classified in the following decreasing order: methanol > acetone > ethanol > ethyl acetate > hexane. The extraction of flavonoids was also influenced significantly by extracting solvent ($P < 0.05$), and its contents varied from 3.42 to 21.45 mg RE/g DW, respectively for hexane and acetone. With regard to flavonoid content, solvents could be sequenced in the following decreasing order: acetone > ethanol > methanol > ethyl acetate > hexane. However, no difference in both TPC and TFC was found between acetone and ethanol extracts. For anthocyanin, there was a significant difference among different extracts. The TAC of ethanol extracts was the highest (4.68 mg CGE/g DW), followed by methanol, acetone, and ethyl acetate extracts, the lowest for hexane extract (0.12 mg CGE/g DW). These results were basically consistent with previous studies (Cheok *et al*., 2012; Rebey *et al*., 2012; Turkmen *et al*., 2006). However, unlike the present results, Cheok et al. (2013) reported that the TPC of 245.78 mg CGE/g DW from mangosteen was obtained in ethanol, and TAC of 2.92 mg CGE/g DW was achieved using methanol aqueous solvent using ultrasonic treatments, which may result from different extraction methods.

Table 1: The contents of total polyphenols, flavonoids, and anthocyanin of different solvent extracts

	TPC (mg GAE/g, DW)	TFC (mg RE/g, DW)	TAC (mg CGE/g, DW)
Methanol	85.65±8.32 a	14.22±0.82 b	2.35±0.12 b
Ethanol	62.22±6.65 b	19.74±1.55 a	4.68±0.52 a
Acetone	68.38±4.52 b	21.45±1.35 a	0.78±0.06 c
Ethyl acetate	16.95±0.75 c	8.36±5.41 c	0.26±0.05 d
Hexane	8.43±0.52 d	3.42±1.45 d	0.12±0.04 e

Values are represented as mean ± standard deviation of triplicates; Different letters within a column indicate statistically significant differences between the means at $P < 0.05$ for the solvents.

Generally speaking, differences in the content of phytochemicals from different solvent extracts may be come from differences in the polarity of solvents. However, there was not a one-to-one relationship between the polarity of solvent and the content of phytochemicals; for instance, the TPC and TFC in their extracts was no difference, though there was obvious different in the polarity between acetone and methanol, which implied that the effect of solvents on extraction was not solely dependent on the polarity, but also possibly being influenced by dielectric constant, chemical structure of organic solvents (Cheok *et al*., 2012) as well as chemical properties of plant phytochemicals (Jayaprakasha *et al*., 2003), and on the other hand also indicated complexity of chemical constituents of polyphenols and flavonoids from *Garcinia mangostana* pericarp.

3.2 ZOI, MIC and MBC of different extracts

The effect of the different extracts from *Garcinia mangostana* pericarp on growth of different bacteria is presented in Table 2, and the MIC and MBC of different extracts is shown in Table 2. Different extracts inhibited growth to variable extents, depending on the bacterium in question. Among these extracts, methanol extract was found to be the most effective against each bacteria with a larger ZOI values (13.4-19.8 mm) and with both the lower MIC (62.5-125 µg/mL) and MBC (62.5-250 µg/mL) compared with other extracts, followed by ethanol and acetone (no difference), ethyl acetate extracts, the lowest for hexane extract, and inhibitory effects of hexane extract on tested Gram-negative bacteria were not found. Moreover, the MIC and MBC values of the hexane extract for tested Gram-negative bacteria strains have not yet been gained at the highest concentration of extract in this study. Sundaram et al. (1983) found that bacteria *S. aureus*, *P. aeruginosa*, *S. typhimurium* and *B. subtilis* were highly susceptible to xanthones, whereas *Proteus* sp., *Klebsiella* sp. and *E. coli* were only moderately susceptible to them. Some studies reported that extracts of *Garcinia mangostana* pericarp inhibited the growth of *S. aureus* (Voravuthikunchai & Kitpipit 2005), *opionibacterium acnes* and *Staphylococcus epidermidis* (Chomnawang *et al*., 2005), which is further supported by our findings. In addition, we also found that Gram-positive bacteria were more sensitive than the Gram-negative ones to each of extracts from *Garcinia mangostana* pericarp. Similar trend for inhibition of bacterial growth have been observed in earlier studies and other plant extracts (Diao *et al*., 2013; Adámez *et al*., 2012), which could be ascribed to the differences between their cell wall compositions. The Gram-positive bacteria contain an outer peptidoglycone layer, which is an ineffective permeability barrier (Diao *et al*., 2013; Gao *et al*., 1999).

Table 2: Antibacterial activities of different extracts from *Garcinia mangostana* pericarp

	Zone of inhibition (mm)					
	S. aureus	*S. albus*	*B. subtilis*	*S. typhimurium*	*S. dysenteriae*	*E. coli*
Methanol	18.7±1.0 aA	18.0±0.5aA	19.8±0.5 aA	17.2±1.2 aA	13.4±0.8 aB	14.8±0.6 aB
Ethanol	16.1±0.5 bB	16.2±0.8 bB	19.1±0.9 aA	14.8±0.5 bB	10.6±0.4 bC	14.5±0.4 aB
Acetone	15.4±0.8 bB	15.8±0.6 bB	18.5±0.4 aA	14.1±0.7 bB	10.2±0.7 bC	13.8±0.2 aB
Ethyl acetate	11.2±0.9 cA	11.3±0.7 cA	12.4±0.8 bA	11.5±0.7 cA	8.6±0.5 cB	8.3±0.5 bB
Hexane	9.2±0.9 cA	10.5±1.0 cA	9.0±0.5 cA	NI [a]	NI	NI

Values are represented as mean ± standard deviation of triplicates; Different small letters within the same column indicate statistically significant differences between the means of different solvent extracts against the same tested strains at P < 0.05; Different upper case letters within the same row indicate statistically significant differences between the means of the same extract against different tested strains at P < 0.05.

[a] *Not inhibition.*

Table 3: The MIC and MBC values (µg/mL) of different extracts from *Garcinia mangostana* pericarp

	Methanol		Ethanol		Acetone		Ethyl acetate		Hexane	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	62.5	62.5	62.5	62.5	62.5	125	250	500	500	500
S. albus	62.5	62.5	125	125	125	125	250	500	250	500
B. subtilis	62.5	62.5	62.5	62.5	62.5	62.5	250	500	500	500
S. typhimurium	62.5	125	125	250	125	250	250	500	ND	ND
S. dysenteriae	125	250	125	250	125	250	500	1000	ND	ND
E. coli	125	250	125	250	125	250	500	500	ND	ND

To further investigate their relationship, the correlation among the content of different phytochemicals and bioactivities of different extracts was established, and correlation coefficients (*R*) are shown in Table 4. In this study, the antibacterial activities were highly correlated to the content of total polyphenols (Table 4, $R \geq 0.8745$), and the flavonoids and anthocyanin were moderately correlated to the bioactivities of extracts ($0.8990 \geq R \geq 0.4832$), indicating total polyphenols, flavonoids and anthocyanin are the main constituents contributing to the antibacterial activities of extracts from *Garcinia mangostana* pericarp, which was supported by previous reports studied on other plants (Delgado-Adámez *et al*., 2012; Alothman *et al*., 2009; Rebey *et al*., 2012). On the basis of these results, it is possible to conclude that *Garcinia mangostana* pericarp is a kind of food resources with some high healthy functions, but total polyphenols, flavonoids and anthocyanin of extracts were significantly affected by the extracting solvents, which results in variation of the antibacterial activities.

Table 4: Correlation analysis among phytochemicals and antibacterial activities of different extracts

	Antibacterial activity					
	S. aureus	*S. albus*	*B. subtilis*	*S. typhimurium*	*S. dysenteriae*	*E. coli*
Polyphenols	0.9877**	0.9841**	0.9699**	0.9250*	0.8745*	0.8950*
Flavonoids	0.7734	0.7687	0.8990*	0.7800	0.6542	0.6815
Anthocyanin	0.6667	0.6597	0.6956	0.6173	0.5320	0.4832

Values are correlation coefficient R. [a] *Significantly different:* ** *P < 0.01,* * *P < 0.05*

3.3 Kill-time analysis

Based on the sensitivity of the test food-borne pathogens, methanol extracts had the best antibacterial effect against both tested bacteria. Therefore, one Gram-positive (*S. aureus*) and one Gram-negative (*S. typhimurium*) bacteria was selected as the model organisms for further study the effect of the methanol extracts from *Garcinia mangostana* pericarp on the viable counts of tested bacterial pathogen in the present study. As observed in Figure 1, compared to the control, susceptible *S. aureus* treated with the methanol extracts at the MIC value showed a slow decrease in the number of viable cells over the first 5 h period of the test, and the number of viable cells decreased by 13.33% from 6.0 to 5.2 $\log_{10}$ at the cultivation time of 5 h. Subsequently, an obvious decrease in cell numbers was detected and decreased to 3.5 $\log_{10}$. Unlike the changing trend of the number of viable cells at 1×MIC, in treatments at 2×MIC, the number of viable cells decreased obviously from the first hour after cultivation and decreased by 97.5% to 0.15 $\log_{10}$ over 20 h of incubation. Similarly, the effect of the methanol extracts on the viable counts of *S. typhimurium* was similar to the result on *S. aureus.* Somewhat differently, the extent of change was not in keeping with the former. These results showed that the treatment time and concentration of essential oil had great influences on antibacterial effects.

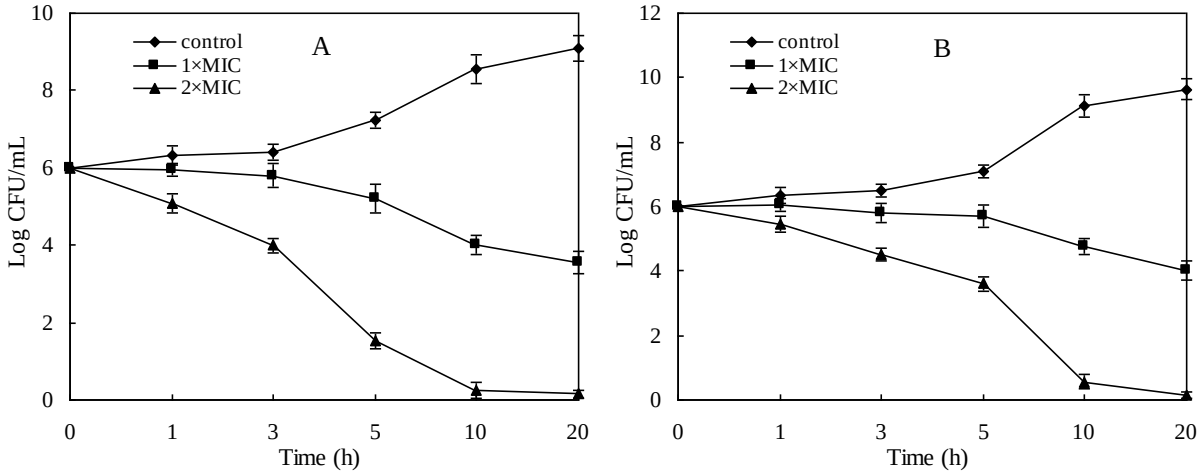

Figure 1: Effect of the methanol extracts on the viability of the tested *S. typhimurium* (A) *and S. aureus* (B)

4. CONCLUSION

The extracting solvents significantly affected total polyphenols, flavonoids, anthocyanin content as well as DNA damage protective effect, antioxidant and antibacterial activities of *Garcinia mangostana* pericarp. In our study, methanol, acetone, and ethanol extracts exhibited the better protective effect. The methanol extract from *Garcinia mangostana* pericarp possessed the highest content of total polyphenols and the strongest antioxidant activity, as well as the relatively higher antibacterial activity. The acetone and ethanol extracts had the highest content of flavonoids and

anthocyanin respectively and also exhibited higher antioxidant and antibacterial activities, while other extracts had both lower the content of active compounds and bioactivities. These results indicated that selective extraction from natural sources, by an appropriate solvent, is important for obtaining fractions with high antioxidant and high antibacterial activity.

5. ACKNOWLEDGEMENT

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