Research Article

Comparison of Antioxidant and Antimicrobial Activities of Unripe and Ripe Fruit Extracts of *Momordica cochinchinensis* Spreng (Gac fruit)

Sirikhwan Tinrat*

*Department of Biotechnology, Faculty of Applied science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand.

***Corresponding author's E-mail:** sktinrat@gmail.com

Accepted on: 16-06-2014; **Finalized on:** 31-08-2014.

ABSTRACT

Three parts (peel, pulp and aril) of unripe/ripe *Momordica cochinchinensis* Spreng (Gac fruit) extracts with different solvents (methanol, acetone and hexane) were investigated antioxidant and antimicrobial activities. The highest total phenolics content in unripe fruit was the flesh acetone extract (41.6 ± 0.240 mg GAE/100g FW) and the aril methanolic extract in unripe fruit was significantly showed the best of the total flavonoids content which was 73.7 ± 0.00 mg RE/ 100g FW ($P < 0.05$). The antimicrobial activities of the extracts were determined against six pathogens with agar disc diffusion and broth macro dilution methods. The unripe flesh extract with acetone was the most active against the tested microorganisms especially *E. coli* ATCC 25922 with higher inhibition zones at all concentration (1, 10 and 100 mg/ml) and lower minimal bactericidal activities (MBC of 100 mg/ml) than the other extracts and the ripe peel methanolic extract was *S. aureus* ATCC 1216. By the MIC levels of both unripe and ripe fruit extracts ranged from 50.00 - 100 mg/ml. Based on solvents, methanol and acetone were suitable solvents for extraction when considering the result of high antioxidant activities and broad-spectrum antibacterial activities. These findings indicate the potential use of unripe and ripe fruit extracts could be promising source of antimicrobial and antioxidant agents for pharmaceutical and food industries.

Keywords: Antioxidant activity, Antimicrobial activity, *Momordica cochinchinensis* Spreng (Gac fruit), Unripe and ripe fruit.

INTRODUCTION

Momordica cochinchinensis Spreng is commonly called "Gac fruit" which is an edible plant using as herbal medicine. It is grown in many Asian countries, including China, Cambodia, Japan, Thailand, Laos and Malaysia.[1,2] Gac fruits are large, densely aculaeate, and green, turning to dark orange or red when ripe. Unripe fruits were used for cooking while ripe fruit were used to pharmaceutical and food product application. Because ripe fruit as amount beta carotene and lycopene higher than any other fruit and vegetable of all kinds[2-5] and most studies have focused on ripe gac fruit.[2-5] By considering the fact found that unripe gac fruit is very popular to use as food by scald and boil. It was eaten with chili sauce or taken sour curry (Gaeng Liang) and sour soup (Gaeng Som) in Thailand.[2] To provide the study about gac fruit has increase comprehensive. Therefore, the aims of this study were to: (1) evaluate and compare the total antioxidant capacity by four antioxidant activities method, including total phenolics and flavonoids content, DPPH radical scavenging activity and ferric ion reducing antioxidant power assay; (2) study and compare the antibacterial activities against six human pathogenic strains by agar disc diffusion assay and broth macro dilution method (MIC and MBC) from each part of unripe and ripe gac fruit extracts (peel, pulp and aril) in different solvents such as methanol, acetone, hexane as well as and gac juice (water) in order to guideline for the use of antioxidant and antibacterial agents or reducing the risk of disease by food in the future.

MATERIALS AND METHODS

Sample Preparation and Extraction

The unripe and ripe of gac fruits were collected from Nakhonpathom Province, in the central region of Thailand, on the 1st and 7th date after harvesting (unripe; green color and fully ripe; red color). The gac fruits were thoroughly cleaned with distilled water, and soaked with 70% alcohol for 15 min. The different parts of gac fruits including peel, flesh and aril were separated and soaked in methanol, hexane and acetone in ratio of 1:2 for 7 days, respectively. The mixtures were filtered through a filter paper (Whatman No. 1) and centrifuged at 8,000 rpm for 15 min. Then, the filtrates were subsequently concentrated under vacuum on a rotary evaporator. For gac juice (flesh and aril) was blended with distilled water and lyophilized in a freeze-dryer after boiling for 60 min. The concentrated extracts were stored at -20°C under dark condition until further analysis. The final weight of the crude extracts was weighted and calculated for the percentage yield.

Total phenolic content

Total phenolics content (TPC) of extracts were determined by Folin Ciocalteu method as described by Materska *et al.* (2005) [6] with some modifications. Briefly, 100 µl of each sample extract in all solutions was mixed with 750 µl of fresh Folin-Ciocalteu reagent (previously diluted 10 fold with distilled water) and allow standing at room temperature for 5 min. After that, 750 µl of 6% (w/v) sodium carbonate (Na_2CO_3) was added to the mixtures and allowed to completely react for 90 min at

the room temperature in the dark condition. Finally, the absorbance was read spectrophotometrically at 725 nm. The phenolic content was expressed in terms of mg of gallic acid equivalent per gram of fresh weight (mg GAE/100g FW) of the plant material through the standard calibration curve of the gallic acid (0.10-0.25 mg/ml).

Total flavonoid content

Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric assay according to method described by Marinova *et al.* (2005).[7] This method is based on a complex flavonoid-aluminium formation. Briefly, 200 µl of sample extracts were mixed with 2.3 ml 30% of methanol with aluminium trichloride ($AlCl_3$). The mixtures were added 100 µl of 0.5 M $NaNO_2$ and 100 µl 0.3 M $AlCl_3$, respectively. Next, the sample solution was thoroughly mixed with vortex and kept in the dark for 5 min. Finally, the absorbance was taken against a blank that without the $AlCl_3$ in the same solution at 506 nm using UV-spectrophotometer. Total flavonoid content was expressed in terms of mg of rutin equivalents (RE) per gram fresh weight (mg RE/100g FW) of the plant materials through the standard calibration curve of rutin (0.02-0.10 mg/ml).

Determination of free radical scavenging using DPPH method

Free radical scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in sample extracts were evaluated which based on the method proposed by Akowuah *et al*. (2005).[8] Briefly, 900 µl of 0.1 mM DPPH in methanolic solution was added into 100 µl of sample in methanol with vigorous shaking. After incubation in the dark for 15 min, the control was prepared by without sample (mixing 900 µl of DPPH with 100 µL methanol). The reduction of the DPPH radical was measured at 517 nm. Percentage of DPPH scavenging activity was calculated as %inhibition of DPPH used to evaluate the antioxidant activity of compounds. %Inhibition of free radical by DPPH was calculated in following way: % inhibition of DPPH = $[Abs_{control} – Abs_{sample} / Abs_{control}] \times 100$

Determination of ferric reducing/ antioxidant power assay (FRAP)

The ferric reducing antioxidant power (FRAP) assay was provided for measuring the reducing ability of the plant sample. FRAP assay was performed according to the method of Benzie and Strain (1996)[9] with minor modification and ascorbic acid was used as the standard. FRAP reagent was prepared from acetate buffer (1.6 g of sodium acetate and 8 ml of acetic acid make up to 500 ml; pH 3.6). The freshly prepared FRAP reagent was warmed to 37°C in oven prior to use. A total of 300 µl of plant extracts was mixed with 2.7 ml of the FRAP reagent. The absorbance was measured at 596 nm by using spectrophotometer after 30 min. Standard curve of ascorbic acid (0.01-0.05 mg/ml) was prepared using the similar procedure. The results were expressed as mg of ascorbic acid equivalents (AAE) per gram fresh weight (mg AAE/100g FW) of the plant materials.

Antimicrobial activities

Microorganisms and Culture condition

In-vitro antimicrobial activities of all sample extracts at different concentrations were determined by agar disc diffusion method and minimum inhibitory and bactericidal concentration (MIC and MBC) assay against six pathogenic strains including Gram-positive bacteria (*Staphylococcus aureus* ATCC 1216, *Bacillus cereus* DMST 5040) and Gram-Negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311) were obtained from the laboratory of the Department of Biotechnology, King Mongkut's University of Technology North Bangkok, Thailand. All tested strains were maintained on brain heart infusion (BHI, Difco) agar medium at 37°C.

Disc diffusion method

The antimicrobial activity of the plant extracts was carried out by agar disc diffusion method [10] against six indicator pathogenic strains. Discs were used in assay agar plates. Briefly, overnight bacterial cultures of tested strains were adjusted the OD_{600} to 0.2 (10^8-10^9 cfu/ml) by spectrophotometer. Then, the suspensions of tested strains were swabbed onto the surface of BHI plates. After about 10-15 min, sterile paper discs were placed on the surface of these plates. Next, 10 µl of different concentration of plant extracts was applied on sterilized paper discs and all plates were incubated at 37°C for 24 h. Finally, antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). Antibiotic discs of Ampicillin (Amp, 10 µg/disc) and Streptomycin (S, 10 µg/disc) were used as positive controls.

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values of plant extracts were determined for the bacterial strains sensitive to the sample extracts in the broth macro-dilution method. The gac fruit extracts were first dissolved in 10% DMSO and then diluted to the highest concentration (100 mg/ml) to be tested, and then serially two-fold dilutions were made in a concentration range from 100 to 0.195 mg/ml with BHI in tested tubes. Then, 0.5 ml of a standard inoculum of the pathogenic strains was added to each concentration of gac fruit extracts. Contents of each tube were vortexed for 20 sec and then incubated at 37°C for 24 h. similar tests were performed simultaneously for growth control (BHI + inoculums) and sterility control (BHI + test sample). The tube with least concentration of extract without growth after 24 h of incubation was recorded as the minimum inhibitory concentration (MIC).

Determination of the Minimum Bactericidal Concentration (MBC)

After MIC determination, all tested tubes showing complete absence of tested strains growth were identified. One loop full of each tested tube was transferred on a BHI agar (Difco) plate. After overnight incubation at 37°C, the lowest concentration of crude extract with complete absence of growth on the agar plates was considered as the Minimum Bactericidal Concentration (MBC).

Statistics analysis

Results obtained were reported as mean ± standard deviation (SD) of triplicate measurements. Statistical analyses (ANOVA) were performed with the statistical program MS Excel (Microsoft Office 2010 Professional) to Analyze whether there was significant difference between each extract.

RESULTS AND DISCUSSION

The extractive yield of the unripe/ripe peel, flesh and aril extracts of *M. cochinchinensis* Spreng with different solvents are presented in Table 1. The maximum yield was obtained for the ripe peel acetone extract (28.58%) followed by unripe flesh methanolic extract (23.87%) while unripe fruit extracts with hexane (0%) yielded least. Consequently, different parts of unripe gac fruits with hexane fraction are not able to analyze the antioxidant and antimicrobial activities. Methanol and acetone fraction was the best suitable solvents for extraction of unripe and ripe gac fruits, respectively. The differences between the yields of extraction might be attributed to the availability of different extractable components,[11,12] polarity of solvents and ripeness of the fruit.

Table 1: The yield, total phenolic and flavonoid content and antioxidant activities of gac fruit extracts

Solutions	Plant	Part	%Extract yield (w/w)	TPC (mg GAE/g FW;%)	TFC (mg RE/g FW;%)	Antioxidant activities	
						DPPH assay (mg AAE/g FW; %)	FRAP assay (mg AAE/g FW; %)
Methanol	Unripe	Peel	12.85±0.10	20.30±0.10	15.10±0.30	5.12±0.21	0.69±0.00
		Flesh	23.87±0.53	4.70±0.00	11.70±0.00	8.59±0.08	0.06±0.00
		Aril	7.86±0.17	35.10±0.10	73.70±0.00	11.26±0.51	0.74±0.00
	Ripe	Peel	4.73±0.84	13.90±0.10	28.40±0.10	8.20±0.14	0.14±0.00
		Flesh	7.54±0.17	23.30±0.10	51.80±0.41	8.99±0.07	0.21±0.00
		Aril	9.11±0.19	33.50±0.10	10.80±0.00	7.83±0.14	0.37±0.00
Acetone	Unripe	Peel	22.48±0.44	11.00±0.40	17.90±0.30	7.25±0.14	0.02±0.00
		Flesh	20.99±0.21	41.60±0.24	12.20±0.24	8.94±0.24	0.02±0.00
		Aril	17.32±0.23	16.60±0.34	18.90±0.16	8.55±0.51	0.03±0.00
	Ripe	Peel	28.58±0.48	4.60±0.00	7.90±0.14	9.03±0.10	0.04±0.00
		Flesh	23.67±0.44	8.70±0.10	6.40±0.30	8.84±0.34	0.03±0.00
		Aril	18.45±0.61	23.50±0.10	23.20±0.00	7.83±0.07	0.09±0.00
Hexane	Unripe	Peel	ND	ND	ND	ND	ND
		Flesh	ND	ND	ND	ND	ND
		Aril	ND	ND	ND	ND	ND
	Ripe	Peel	1.74±0.09	3.50±0.10	22.20±0.00	9.01±0.24	0.060±0.00
		Flesh	7.47±0.28	3.60±0.00	19.30±0.13	8.70±0.00	0.08±0.00
		Aril	1.59±0.03	4.00±0.10	29.90±0.70	5.99±0.27	0.07±0.00
Gac juice			14.41±0.51	32.50±0.10	50.90±0.70	7.97±0.17	0.21±0.00

ND = Not determined

Total phenolic content and total flavonoid content

Phenolic compounds are widely distributed in many plants[12] which have antioxidant activities and free radical-scavenging abilities, multiple beneficial effects on human health.[13] The total phenolics content (TPC) of gac fruit by using the diluted Folin-Ciocalteu reagent were examined and presented in Table 1. The TPC was determined in comparison with standard gallic acid (mg GAE/100g FW). The results showed that the different parts of the gac fruits varied in three solvents and had a range of TPC from 3.50±0.10 to 41.60±0.24 mg GAE/100g FW. The aril methanolic extract of unripe fruit showed the highest TPC (35.10±0.10 mg GAE/100 gFW) which was significantly different from the other part of ripe fruits ($P<0.05$) (Figure 1). The TPC of gac juice had significantly higher than ripe fruit extracts with acetone and hexane ($P < 0.05$). Most of the TPC was significantly presented the gac fruits in all solvents as: aril>flesh>peel. The highest TPC

values were mostly obtained for the peel fraction of ripe fruit followed by flesh whereas aril had the lowest TPC. Furthermore, the result also indicated that the unripe fruit had significantly higher phenolics content than ripe fruit ($P < 0.05$) that may provide good sources of antioxidants (except hexane fraction). However, the radicals scavenging activity is not only due to the phenolic content, but it was also depended on other various antioxidant compounds.[14] Thus, TPC does not incorporate necessarily to all the antioxidants that may present in the extracts. Therefore, sometimes there is a vague correlation between TPC and antioxidant activity of several plant species.[15] It may be due to the physiological changes that accompany ripening that brings about changes in pigments, which may have caused an increase in the total phenol content.[16,17]

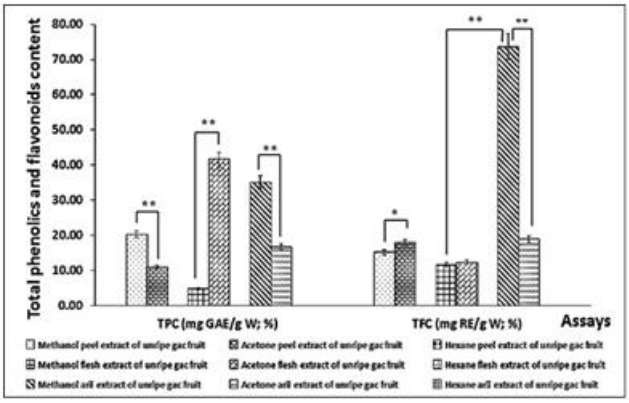

Figure 1: Total phenolics and flavonoids content of different parts of unripe Gac fruit extract in different solvents (* = $P < 0.05$; ** = $P < 0.01$; one way ANOVA and paired T-test; non appear column = not determined)

Other than that, the concentration of flavonoids in fruits of the species *M. cochinchinensis* (Spreng) was determined by Spectrophotometric method with aluminum chloride. The content of flavonoids (TFC) was expressed in terms of rutin equivalent. All extracts had a range of TPC from 6.40±0.30 and 73.70±0.00 mg RE/100g FW as shown in Table 1. The unripe aril and ripe flesh methanolic extract showed the highest capacity of (73.70±0.00 and 51.80±0.41 mg RE/100 g FW, respectively) which were significantly different ($P < 0.05$) in comparison with the other part extracts (Figure 1 and 2). The total flavonoids content of gac juice had significantly higher than extracts of ripe fruit with acetone and hexane fractions ($P < 0.05$). The total antioxidant activities of the parts of gac fruit were presented as: aril>peel>flesh. And most of the unripe fruits were showed significantly higher total phenolics content than ripe fruits ($P<0.05$) (Table 1). According to the study of Kubola and Siriamornpun (2011)[2] reported that the contents of total phenolic in peel and pulp of gac fruit decreased during the fruit development stage (immature > ripe fruit). Based on solvent extraction, most of the TPC was significantly presented the solvent for all part of ripe gac fruits extracts as: methanol>acetone>hexane ($P<0.01$) (Figure 2) while for unripe fruit extracts depended on each part of fruits. And most of the TFC from all parts of unripe/ripe gac fruit extract varied the solvent extraction (Figure 1 and 2).

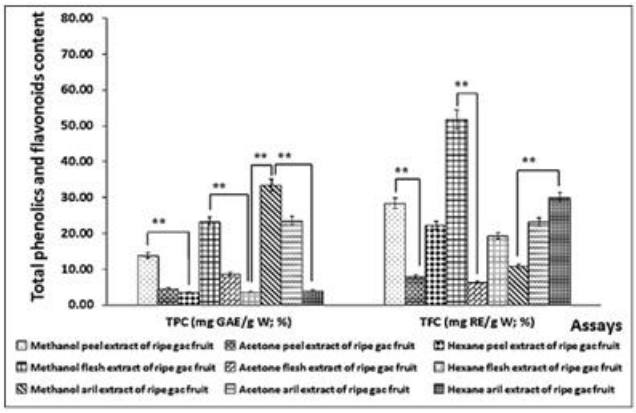

Figure 2: Total phenolics and flavonoids content of different parts of ripe Gac fruit extract in three solvent (** = $P < 0.01$; one way ANOVA and paired T-test)

Antioxidant activities

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant components. Decrease in absorbance of DPPH˙ radical is caused by a reaction between antioxidant molecules and the radical, which result in the scavenging of radical by hydrogen donation, and then occurred discoloration from purple to yellow.[18] The DPPH radical scavenging activity of gac fruit extracts were depended on parts of plants and polarity of solvents. The result clearly showed that the gac fruit extracts had DPPH radical scavenging activity range from 5.120±0.205 to 11.256±0.512 mgAAE/100 gFW (Table 1). The unripe aril extract with methanol (11.26±0.51%) showed the highest activity, followed by ripe peel extract with acetone (9.03±0.10%) and hexane (9.01±0.24%) whereas unripe peel extract with methanol showed the lowest activity (5.12±0.21%). The varied radical scavenging activity of the extracts depended on the amount of total phenolic in each fraction.[19] These findings support the data previously reported in a study which the antioxidant activity was dependent on the actual composition of the peel, pulp and aril fractions.

FRAP assay

Different studies have indicated that the electron donation capacity and reflecting the reducing power, of bioactive compounds is associated with antioxidant activity.[20] The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The ferric reducing/antioxidant power (FRAP assay) is widely used in the evaluation of the antioxidant component in dietary polyphenols.[9,21,22] Antioxidant activity increased proportionally to the polyphenol content. In this study, antioxidant activity of gac fruit was calculated with a linear equation based on a standard curve using ascorbic acid and shown in Table 2. All parts of gac fruit extracts examined reduced ferric ion to

different solvents. The aril methanolic extracts of unripe and ripe gac fruit had the highest reducing capacity (0.741±0.001 and 0.372±0.00 mgAAE/100 gFW, respectively). The reducing power of gac fruit extracts are mostly presented in the order of aril > peel > flesh. The aril ripe extract with methanol gave high FRAP values. These may be due to their higher TPC.

Antimicrobial activity

In the present study, the antimicrobial activities of unripe and ripe gac fruit including peel, flesh and aril extracts in different solvents were determined against microorganisms on the basis of agar disc diffusion and broth macro dilution assays. Their potencies were quantitatively assessed with the presence or absence of inhibition zones (mm) and MIC and MBC values. Gac fruit extracts were tested the antibacterial activity on six pathogenic strains and presented in Table 2-3. The inhibition zone around the disc impregnated with plant extracts was quantitatively determined the antibacterial activity. The result showed that the antibacterial activities of plant extracts were increased with increasing concentration of crude extracts. Most of the extracts showed inhibitory activity against both Gram negative and Gram positive bacteria, but *B. cereus* DMST 5040 and *S. typhimurium* ATCC 13311 appeared the resistance to all plant extracts. Among the three solvent extracts with different parts of unripe fruits, peel methanolic extract and flesh acetone extract showed the highest inhibitory activity of 10.330±0.260 mm (100 mg/ml) inhibition zone against *P. aureginosa* ATCC 27853 and *E. coli* ATCC 25922, respectively, and followed by aril methanolic extract that showed 10.170±0.260 mm (100 mg/ml) inhibition zone against *P. aureginosa* ATCC 27853 (Table 2).

Table 2: The antimicrobial activity of unripe gac fruit extracts by agar disc diffusion method

Bacterial strains	Clear zone of unripe gac fruit extracts (mm±SD)					
	Methanol			Acetone		
	1 mg/ml	10 mg/ml	100 mg/ml	1 mg/ml	10 mg/ml	100 mg/ml
Peel						
B. cereus DMST 5040	R	R	R	R	R	R
S. aureus ATCC 1216	R	R	R	R	R	R
E. coli ATCC 25922	R	R	R	R	7.83 ± 0.26	9.42 ± 0.20
P. aeruginosa ATCC 27853	7.58 ± 0.20	8.83 ± 0.26	10.33 ± 0.26	R	6.83 ± 0.26	8.25 ± 0.27
S. typhimurium ATCC 13311	R	R	R	R	R	R
K. pneumoniae	R	R	R	R	R	R
Flesh						
B. cereus DMST 5040	R	R	R	R	R	R
S. aureus ATCC 1216	R	R	R	R	6.75 ± 0.27	8.17 ± 0.27
E. coli ATCC 25922	R	R	R	7.67 ± 0.26	8.92 ± 0.20	10.33 ± 0.26
P. aeruginosa ATCC 27853	R	R	R	R	R	R
S. typhimurium ATCC 13311	R	R	R	R	R	R
K. pneumoniae	R	R	R	7.33 ± 0.26	8.42 ± 0.38	10.17 ± 0.26
Aril						
B. cereus DMST 5040	R	R	R	R	R	R
S. aureus ATCC 1216	R	7.42 ± 0.20	8.25 ± 0.27	6.58 ± 0.20	7.92 ± 0.20	9.08 ± 0.20
E. coli ATCC 25922	R	R	R	R	R	7.33 ± 0.26
P. aeruginosa ATCC 27853	7.33 ± 0.26	8.75 ± 0.27	10.17 ± 0.26	6.67 ± 0.26	7.58 ± 0.20	8.58 ± 0.20
S. typhimurium ATCC 13311	R	R	R	R	R	R
K. pneumoniae	7.25 ± 0.27	8.25 ± 0.27	8.25 ± 0.27	R	7.75 ± 0.27	8.75 ± 0.27

R = Resistance

Among the three solvent extracts with different parts of ripe gac fruits, peel methanolic extract showed the highest potential activity of 10.420±0.200 mm (100 mg/ml) inhibition zone against *S. aureus* ATCC 1612 followed by aril acetone extract that exhibited 10.330±0.260 mm (100 mg/ml) inhibition zone against *P. aureginosa* ATCC 27853 (Table 3).

On the other hand, the *B. cereus* DMST 5040 and *S. typhimurium* ATCC 13311 were resistance to various solvents of different parts of gac fruit extracts. Innun (2013)[23] report that the flesh and aril extract of gac fruit with 95% ethanol had no inhibition effect on *S. aureus* and *E. col* strains while *E. coli* ATCC 25922 and *S. aureus* ATCC 1216 showed sensitive to gac fruit extracts with methanol in this study. All parts of unripe and ripe fruit

extracts (10 and 100 mg/ml) showed better antibacterial activity against *P. aureginosa* ATCC 27853 when compared with Streptomycin (S10) standard (7.40±0.00 mm; data not shown) except aril hexane extract of ripe fruit (10 mg/ml). At 100 mg/ml, peel and flesh methanolic and flesh acetone extracts of ripe fruit had higher inhibition zone (9.25-10.43 mm) than positive control (S10; 7.00±0.00 mm) against *S. aureus* ATCC 1612. And peel and flesh acetone extracts of unripe fruit at 10 and 100 mg/ml has also higher inhibition zone (7.83-10.33 mm) than positive control (S10; 7.70±0.00 mm) against *E. coli* ATCC 25922. These results are interested that crude extracts of unripe and ripe gac fruit had potential antibacterial activity and may be used as an alternative to antimicrobial agents.

The MIC analysis of plant extracts showed the optimum bacteriostatic concentration for methanol crude extracts of the tested plants. The MIC of all fruit extracts was studied from the range of 100.00 to 0.195 mg/ml. Among the tested microorganisms, the MIC and MBC values were 50 and 100 mg/ml, respectively, except *B. cereus* DMST 5040 and *S. typhimurium* ATCC 13311 (>100 mg/ml) (Table 4). On the basis of MIC and MBC values, *P. aeruginosa* ATCC 27853, *K. pneumoniae*, *E. coli* ATCC 25922 and *S. aureus* ATCC 1216 showed higher sensitivity than *B. cereus* DMST 5040 and *S. typhimurium* ATCC 13311. The results of the MBC value showed that the unripe fruit extracts seemed to be more effective than the ripe fruit extracts against the tested microorganisms in this study. Only the unripe peel extract with acetone represented the MBC value of 100 mg/ml against *E. coli* ATCC 25922. Gac juice could not present the potential antimicrobial activity in both assays.

On comparing the antimicrobial activity of all extracts with period growth, the unripe fruit extract showed more antibacterial activity than the ripe fruit extract with acetone. This is suggestive that ripening may have transformed certain bioactive components which are responsible for the antibacterial activity of the fruit. But the ripe fruit extracts showed more antibacterial activity than the unripe fruit extracts with methanol and acetone. The polarity of bioactive compounds make plant extracts more readily extracted by organic solvents and using organic solvent does not negatively affect to bioactivity against pathogenic strains. Gram positive strains were more susceptible to plant extracts than Gram negative strains. *P. aureginosa* ATCC 27853 was found the most susceptible to the all extracts. This is probably due to the differences in chemical position and structure of the cell wall.[24]

Table 3: The antimicrobial activity of ripe gac fruit extracts by agar disc diffusion method

Bacterial strains	Clear zone of ripe gac fruit extracts (mm±SD)					
	Methanol		Acetone		Hexane	
	10 mg/ml	100 mg/ml	10 mg/ml	100 mg/ml	10 mg/ml	100 mg/ml
Peel						
B. cereus DMST 5040	R	R	R	R	R	R
S. aureus ATCC 1216	9.33 ± 0.26	10.42 ± 0.20	R	R	R	R
E. coli ATCC 25922	8.08 ± 0.20	9.33 ± 0.26	R	R	8.67 ± 0.26	9.83 ± 0.26
P. aeruginosa ATCC 27853	7.42 ± 0.20	8.58 ± 0.20	8.25 ± 0.27	9.92 ± 0.20	7.68 ± 0.25	9.33 ± 0.26
S. typhimurium ATCC 13311	R	R	R	R	R	R
K. pneumoniae	6.92 ± 0.20	8.17 ± 0.26	7.75 ± 0.27	9.92 ± 0.38	R	R
Flesh						
B. cereus DMST 5040	R	R	R	R	R	R
S. aureus ATCC 1216	7.75 ± 0.27	9.25 ± 0.27	8.58 ± 0.20	10.33 ± 0.26	R	R
E. coli ATCC 25922	R	R	R	R	R	R
P. aeruginosa ATCC 27853	7.75 ± 0.27	9.08 ± 0.20	8.25 ± 0.27	10.25 ± 0.27	7.42 ± 0.20	8.42 ± 0.20
S. typhimurium ATCC 13311	R	R	R	R	R	R
K. pneumoniae	7.83 ± 0.26	8.92 ± 0.20	7.75 ± 0.27	9.92 ± 0.38	7.83 ± 0.26	9.08 ± 0.20
Aril						
B. cereus DMST 5040	R	R	R	R	R	R
S. aureus ATCC 1216	R	R	R	R	7.83 ± 0.26	8.58 ± 0.20
E. coli ATCC 25922	R	R	R	R	R	R
P. aeruginosa ATCC 27853	7.58 ± 0.20	9.67 ± 0.26	8.67 ± 0.26	10.33 ± 0.26	6.92 ± 0.20	7.83 ± 0.26
S. typhimurium ATCC 13311	R	R	R	R	R	R
K. pneumoniae	7.420 ± 0.20	8.25 ± 0.27	R	R	7.92 ± 0.20	8.75 ± 0.27

R = Resistance; data not shown clear zone at 1 mg/ml of gac fruit extract

Table 4: The MIC levels of gac fruit extracts against some pathogenic strains by broth macro dilution method

Bacterial strains		MIC value (mg/ml)								
		Methanol			Acetone			Hexane		
		Peel	Flesh	Aril	Peel	Flesh	Aril	Peel	Flesh	Aril
Unripe										
Gram (+)	*B. cereus* DMST 5040	>100	>100	>100	>100	>100	>100	ND	ND	ND
	S. aureus ATCC 1216	>100	>100	50	>100	50	50.00	ND	ND	ND
Gram (-)	*E. coli* ATCC 25922	>100	>100	>100	50	50	>100	ND	ND	ND
	P. aeruginosa ATCC 27853	50	>100	50	50	>100	>100	ND	ND	ND
	S. typhimurium ATCC 13311	>100	>100	>100	>100	>100	>100	ND	ND	ND
	K. pneumoniae	>100	>100	50	>100	50	>100	ND	ND	ND
Ripe										
Gram (+)	*B. cereus* DMST 5040	>100	>100	>100	>100	>100	>100	>100	>100	>100
	S. aureus ATCC 1216	>100	100	>100	>100	50.00	>100	>100	>100	50.00
Gram (-)	*E. coli* ATCC 25922	50.00	>100	>100	>100	>100	>100	50.00	>100	>100
	P. aeruginosa ATCC 27853	50.00	100	50.00	50.00	50.00	>100	>100	50.00	50.00
	S. typhimurium ATCC 13311	>100	>100	>100	>100	>100	>100	>100	>100	>100
	K. pneumoniae	50.00	100	50.00	50.0	50.00	>100	>100	50.00	50.00

ND = Not detected

CONCLUSION

The result in this study showed that different parts of fruit of *M. cochinchinensis* (Spreng) fruit in different solvents contained the bioactive compounds such as phenolics and flavonoids which have variable antimicrobial and antioxidant activities. The result proved methanol and acetone to be the choice solvent for the extraction of bioactive compounds from gac fruit. The ripe fruit extracts showed more antibacterial activities than the unripe fruit extracts. But the unripe fruit extracts showed more antioxidant activities than the ripe fruit extracts. This is suggestive that ripening may have transformed certain bioactive components which are responsible for the antibacterial or antioxidant activity of the fruit. All parts of unripe and ripe gac fruit extracts in different solvents showed broad spectrum of antibacterial activities. Consequently this extract is suitable as a new potential source of a natural preservative in pharmaceutical and food/feed industries.

Acknowledgements: I would like to thank the research funding of Applied Science Faculty, King Mongkut's University of Technology North Bangkok (No. 5747102) for financial support. And we also thank the Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok for supplying all of the chemicals and equipment needed in this work.

REFERENCES

1. Vuong LT, Franke AA, Custer LJ, Murphy SP, *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated, J. Food Comp. Anal., 19, 2006, 664-668.
2. Kubola J, Siriamornpun S, Phytochemical and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng.), Food Chem., 127, 2011, 1138-1145.
3. Tuyen CK, Minh HN, Paul DR, Sophie EP and Constantinos S, Gac fruit: Nutrient and Phytochemical Composition, and Options for Processing, Food Rev. Int., 29, 2013, 92-106.
4. Vuong LT, Franke AA, Custer LJ, Murphy SP, *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated, J. Food Comp. Anal., 19, 2006, 664-668.
5. Nhung DTT, Bung PN, Ha NT, Phong TK, Changes in lycopene and beta carotene contents in aril and oil of gac fruit during storage, Food Chem., 121, 2010, 326-331.
6. Materska M, Perucka I, Antioxidant activity of the main phenolic compounds Isolated from Hot pepperfruit (*Capsicum annuum* L.), J. Agric. Food Chem., 53, 2005, 1750-1756.

7. Marinova D, Ribarova F, Atanassova M, Total phenolics and total flavonoids in Bulgarian fruits and vegetables, J. Univ. Chem. Technol. Metallurgy, 40, 2005, 255-260.
8. Akowuah GA, Ismail Z, Norhayati I, Sadikun A, The effects of different extraction solvents of varying polarities of polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity, Food Chem., 93, 2005, 31-317.
9. Benzie IFF, Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP assay, Anal. Biochem., 239, 1996, 70–76.
10. Kim J, Marshall MR, Wie C, Antibacterial activity of some essential oil components against five foodborne pathogens, J. Agric. Food Chem., 43, 1995, 2839-2845.
11. Oke F, Aslim B, Biological potentials and cytotoxicity of various extracts from endemic *Origanum minutiflorum* O. Schwarz & P.H. Davis., Food Chem. Toxicol., 48, 2010, 1728-1733.
12. Li BB, Smith B, Hossain MM, Extraction of phenolics from citrus peels: I. Solvent extraction method, Separate. Purificat. Technol., 48, 2006, 182–188.
13. Govindarajan R, Singh DP, Rawat AKS, High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent Ayurvedic drug, J. Pharmaceut. Biomed. Anal., 43, 2007, 527-532.
14. Khamsah SM, Akowah G, Zhari I, Antioxidant activity and phenolic content of *Orthosiphon stamineus* Benth from different geographical origin, J. Sustain. Sci. Manage., 1, 2006, 14-20.
15. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T, Antioxidant activity and total phenolic content of selected Jordanian plant species, Food Chem., 104, 2007, 1372–1378.
16. Materska M, Perucka I, Antioxidant activity of the main phenolic compounds Isolated from Hot pepper fruit (*Capsicum annuum* L.), J. Agric. Food Chem., 53, 2005, 1750-1756.
17. Oboh G, Puntel RL, Rocha JBT, Hot pepper (*Capsicum annuum*, Tepin and Capsicum chinese, Habanero) prevents Fe^{2+}- induced lipid peroxidation in Brain: *in vitro*, Food Chem., 102, 2007, 178- 185.
18. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA, Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies, Food Chem., 84, 2004, 551-562.
19. Butsat S, Siriamornpun S, Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice, Food Chem., 119, 2010, 606-613.
20. Siddhuraju P, Mohan PS, Becker K, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp, Food Chem., 79, 2002, 61-67.
21. Luximon-Ramma A, Bahorun T, Soobrattee AM, Aruoma OI, Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in Extracts of *Acacia fistula*, J. Agric. Food Chem., 50, 2005, 5042-5047.
22. Ömer B, Mahfuz E, Fatma G, Total phenolic compounds and antioxidant capacity of leaf, dry fruit and fresh fruit of feijoa (*Acca sellowiana*, Myrtaceae), Journal of Medicinal Plants Research, 4, 2010, 1065-1072.
23. Innuna A, Antimicrobial activity of Gac fruit (*Momordica cochinchinensis*), Proceeding - Science and Engineering, 2013, 1–6.
24. Alcamo IE, Fundamentals of microbiology, 6th ed., Jones and Bartlett Publishers, Inc., New York, 2001.

Source of Support: Nil, **Conflict of Interest:** None.