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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *MOMORDICA COCHINCHINENSIS* SPRENG (GAC FRUIT) ETHANOLIC EXTRACT

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Keywords:

Momordica cochinchinensis Spreng., Antioxidant and Antimicrobial activity, MIC and MBC, Total phenolic and flavonoid content, Gac fruit

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ABSTRACT: Ethanolic extracts from three factions (peel, pulp and aril) of Gac fruit were evaluated the antioxidant and antimicrobial activities. The antioxidant activities of plant extracts were also investigated by using DPPH and FRAP assay. The highest antioxidant activities of ethanolic extract from the aril of ripe Gac fruit were 4.87 mg AAE/g FW and 0.016 mg AAE/g FW when determined by the DPPH and FRAP, respectively. Moreover, The ethanolic extract from pulp fraction had the highest the total phenolic and flavonoid content of 0.205 mg GAE/g FW and 0.143mg RE/g FW, respectively. Antimicrobial activities were evaluated by MIC and MBC assay against six pathogenic microorganisms. *E. coli* ATCC 25922 had the most susceptible to ethanolic extracts from peel and pulp factions with MIC value of 1.562 mg/mL. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 was the most susceptible to aril extract with MIC value of 3.125 mg/mL. The MBC value in all extract factions of Gac fruit were > 50 mg/ml. Therefore, the result of Gac fruit from this study will be useful to develop the plant extract as natural therapeutic agents for treatment the disease and health care of human in the future.

INTRODUCTION: Edible plants are integral part of human existence. Over 80% of the world population also replied on the medical plants from their basic health care. *Momordica cochinchinensis* Spreng (Gac) is a medical plant that found in tropical of Asian countries, including Viet Nam, Laos, Thailand, China, Bangladesh and India [1, 2]. It is botanically classified in the *Cucurbitaceae* family.

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In Thailand, gac is called Fak Khao, is one of Thai local herbs plants that are presently interested in human health. The fruit flesh contains red soft and sticky arils covering hard seed. Each parts of them were study and mostly processed into cooking (immature and mature Gac) and cosmetic products (mature Gac) such as cream. Many researches indicated that Gac fruits were the major source of lycopene [2,3] and carotenoid as antioxidant substances [3-7]. They has been reported that to be associated with reduce risk of various cancers like prostate cancer and lung cancer [3,8]. Furthermore, Gac fruit has also potential antimicrobial proterties [9,10]. Therefore, to increase knowledge in the field of antioxidants that had several groups in plants as well as to inhibit the growth of pathogens in

humans. The aims of this research were to evaluate the antioxidant activity by using DPPH radical scavenging activity assay and ferric ion reducing antioxidant power in ethanol crude extract from different parts of riped Thai gac fruits (peel, pulp and aril). In quantitative, total of phenolic compound and flavonoid compounds were measured by using folin-ciocalteu method and aluminum chloride method, respectively and to study antibacterial activity of ethanol crude extract from riped Thai gac fruits against various pathogenic strains by using broth macrodilution method (MIC and MBC) as an alternative natural therapy.

MATERIALS AND METHODS:

Sample Preparation and Extraction: The ripe fruit of Gac were collected from Nakhonpathom Province, in the central region of Thailand, aged of 6 days after harvest (fully ripe; red color). The Gac fruits were thoroughly cleaned and soaked with 70% alcohol for 15 min in order to clean. The different parts of Gac fruits, including peel, pulp and aril were separated and soaked in 95% ethanol in ratio 1:2 for 2 days separately. The filtrates obtained were subsequently concentrated to a small volume under vacuum on a rotary evaporator at 40°C. The concentrated ethanolic extracts were stored at -20°C under dark condition until further analysis. The final weight of the crude extracts were weighted and calculated for the percentage yield.

Determination of total phenolic content: The total content of phenolic compounds (TPC) in the ethanolic extracts of Gac fruit were determined with Folin-Ciocalteu reagent by spectrophotometer [11]. Briefly, 100 µL of all sample extracts in 95% ethanol was mixed with 750 µL of fresh Folin-Ciocalteu reagent diluted (1:10) in distilled water. After standing at room temperature for 5 min, 750 µL of 6% (w/v) sodium carbonate (Na_2CO_3) was added and allowed to completely react for 90 min at the room temperature in the dark condition. And then the absorbance at 725 nm was measured on a spectrophotometer. The phenolic content was expressed in terms of mg of gallic acid equivalent per gram of fresh weight (mg GAE/g FW) using the linear equation based on standard calibration curve of the gallic acid (0.02 - 0.1 mg/mL).

Determination of total flavonoid content: Total flavonoids content (TFC) in the plant sample was measured by aluminium chloride colorimetric assay [2, 12]. This method based on a complex flavonoid-aluminium formation. Briefly, 200 µL sample extract was mixed with 2.3 mL 30% of methanol with aluminium trichloride ($AlCl_3$). The mixture was 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. And then, the absorbance at 506 nm was measured using spectrophotometer against a blank sample consisting of a 200 µL extract solution with 2.3 mL 30% of methanol without $AlCl_3$. Total flavonoid content was expressed in terms of mg of rutin equivalents (RE) per gram fresh weight (mg RE/g FW) using the linear equation based on standard calibration curve of rutin (0.01-0.05 mg/mL)

Determination of DPPH free radical scavenging activity: The antioxidant activities of sample extract were evaluated through free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Omar et al. (2012) [13]. Briefly, 900 µl of 0.1 mM DPPH in methanolic solution was added into 100 µL of samples in methanol. The mixture was thoroughly mixed and left to stand for 15 min in the dark and was recorded at 517 nm using spectrophotometer. Ascorbic acid (0.01-0.05 mg/mL) was used as standard control and was prepared using the similar procedure. The percentage scavenging of free radical by DPPH was calculated in following formular:

DPPH Scavenging effect (%) = [$Abs_{control}$ – Abs_{sample} / $Abs_{control}$] x 100

Determination of ferric reducing/antioxidant power assay (FRAP): The total reducing capacity was determined by using FRAP assay. FRAP assay was performed according to the method of Tabaraki and Ghadiri (2013) [14] with some modification. The FRAP reagent was initially prepared consisting of 300 mM Acetate buffer (pH 6.3), 20 mM 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ solution.

The fresh working solution was warm at 37°C in oven prior to use. An amount of 300 µL fruit ethanolic extracts was added to 2.7 mL of the FRAP reagent in test tubes and mixed well by vortex. After incubation for 30 min, the absorbance was measured at 596 nm by using spectrophotometers. Samples were done in triplicates. The results were expressed in terms of mg of ascorbic acid equivalents (AAE) per gram fresh weight (mg AAE/g FW) based on standard calibration curve of ascorbic acid (0.01-0.05 mg/mL).

Sources and maintenance of organisms: The *in-vitro* antimicrobial activity of all the ethanolic extracts at different concentrations was studied by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods against six pathogenic strains. Gram-positive organisms (*Staphylococcus aureus* ATCC 1216, *Bacillus cereus* DMST 5040) and Gram-Negative organisms (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, ATCC 27853 *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311) were obtained at the laboratory of the Department of Biotechnology, King Mongkut's University of Technology North Bangkok, Thailand. All pathogenic microorganisms were obtained from stock cultures which were maintained on brain heart infusion (BHI, Difco) agar medium at 37°C, then subcultures in brain heart infusion (BHI) broth at 37°C for 24 h and adjust the optical density of 0.5 (10^8-10^9 CFU/mL) at 600 nm by spectrophotometer prior to each antimicrobial test.

Determination of the minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) values were studied for the bacterial strains sensitive to the sample in the broth macrodilution susceptibility assay. Bacterial strains were prepared from overnight broth cultures and suspensions were adjusted to optical density of 0.5 (10^8 to 10^9 CFU/mL) at 600 nm by spectrophotometer. The extracts were first dissolved in 1% DMSO and then diluted to the highest concentration (50 mg/mL) to be tested, and then serially two-fold dilutions were made in a concentration range from 50 to 0.039 mg/mL with BHI in tested tubes, volume being 1 mL and were added 0.5 mL of the inoculums. The total volume in each tube was 1.5 ml. Contents of each tube were mixed on a vortex for 20 s 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). Microbial growth was determined by absorbance at 600 nm. MIC values were determined as the lowest concentration of the extract where absence of growth was recorded after 24 h.

Determination of the Minimum Bactericidal Concentration (MBC): The minimum bactericidal concentration (MBC) was determined by subculturing at one loop full from each tube showing no apparent growth on a BHI agar (Difco) plate. All plates were incubated at 37°C for 24 h. Least concentration of extracts showing no visible growth on subculturing was taken as MBC.

Statistical analysis: All measurements were done in triplicate and data were expressed as mean ± standard deviation. 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.

RESULT AND DISCUSSION:

Extraction yield: The different parts of the ripe Gac fruit were extracted by 95% ethanol in ratio 1:2 for 2 days. After concentration by evaporator (Eyela rotary evaporator N-100, Japan), percentage yield was calculated. In this study, maximum extract yield was peel ethanolic extract of 23.17% and was followed by pulp and aril ethanolic extract of 21.81% and 14.88%, respectively.

Total Phenolic Content and Total flavonoids content: Phenolic compounds are major plant secondary metabolite which has several biological functions including antioxidant and antibacterial activities. The total phenolic contents (TPC) of the tested medicinal plant extracts were demonstrated by using the Folin–Ciocalteau colorimetric method. The total phenolic contents of the all samples were calculated with a linear equation based on a standard curve using galic acid ($y = 5.43x + 0.0286$, $R^2 = 0.991$) (**Figure 1**).

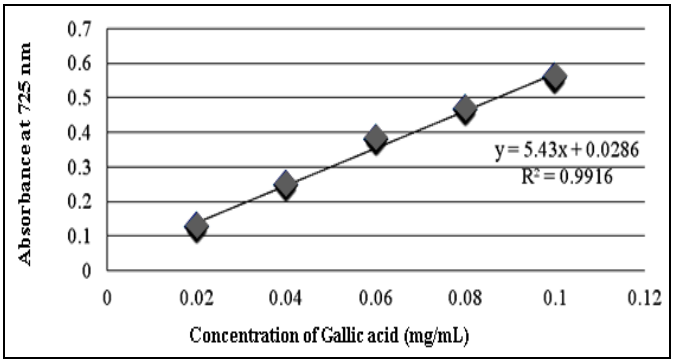

FIG. 1: STANDARD CURVE OF TOTAL PHENOLIC CONTENT USING GALIC ACID AS STANDARD

The amount of TPC in samples was reported as mg of gallic acid equivalent (GAE) per 100 g of fresh sample (Table 1).

Result clearly showed that total phenolic content of pulp ethanolic extract (0.205±0.05 mg GAE/g FW) was higher than that of aril ethanolic extract (0.191±0.06 mg GAE/g FW) and peel ethanolic extract (0.055±0.05mg GAE/g FW)

TABLE 1: ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT FROM DIFFERENT PARTS OF GAC FRUITS

Test	Antioxidant Activity		
	Peel	Flesh	Aril (Seed pulp)
Total Phenolic Content (mg GAE/g FW; %)	0.055±0.05	0.205±0.05	0.191±0.06
Total flavonoids Content (Aluminum chloride method) (mg RE/g FW; %)	0.118±0.06	0.143±0.01	0.084±0.10
Total Antioxidant Capacity			
- DPPH radical scavenging activity assay - (mg AAE/g FW; %)	4.65±0.70	2.41±0.20	4.87±1.58
- Ion Reducing Antioxidant Power (FRAP) (mg AAE/g FW; %)	0.012±0.01	0.012±0.01	0.016±0.01

GAE = Gallic Acid Equivalent; RE = Rutin Equivalent; AAE = Ascorbic acid Equivalent

The concentration of flavonoids in different parts of Gac fruit was determined with aluminum chloride by using spectrophotometric method. The content of flavonoids was expressed in terms of rutin equivalent (the standard curve equation: $y = 0.325x + 0.019$, $R^2 = 0.996$) (Figure 2). The total flavonoid content of pulp ethanolic extract (0.143±0.01 mg RE/g FW) was higher than peel ethanolic extract (0.118±0.06 mg RE/g FW) and aril ethanolic extract (0.084±0.10 mg RE/g FW) (Table 1).

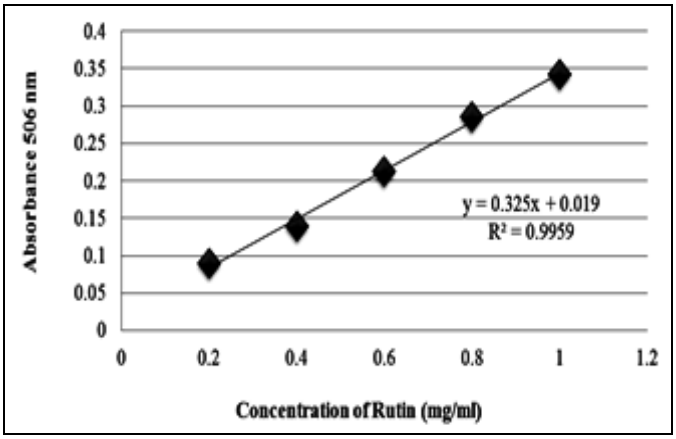

FIG. 2: STANDARD CURVE OF TOTAL FLAVANOID CONTENT USING RUTIN AS STANDARD

From the previous results, the total phenolic compounds content and total flavonoid content depended on solvents polarities such ethanol and hexane. In addition, the total phenolic content and flavonoid content level may be related to many factors such as growing season, dryness, high temperature and solar exposure. And phenols posses a wide spectrum of biological activities[15] that were presented in all the ethanolic extract of ripe Gac fruits.

DPPH radical scavenging activity: The antioxidant activity is influenced by many factors that cannot be assessed by a single method. Therefore, at least two test models have been recommended for the evaluation of antioxidant activity[16]. In this study, the antioxidant activity was determined by DPPH and FRAP radical scavenging activity methods. DPPH assay is widely used to determine the free radical scavenging ability in plant extract. DPPH is a free radical compound and stable in room temperature. Antioxidant compound scavenges free radical by hydrogen donation and reduction of $DPPH^{\bullet}$ (violet color) to DPPH-H (yellowish color) [17, 18].

The antioxidant activity of different parts of ethanolic extract from the *Momordica cochinchinensis* Spreng. was expressed in terms of percentage of inhibition (%) that was calculated with a linear equation based on a standard curve using Ascorbic acid (y = -10.863X + 0.7639, R^2 = 0.993) (Figure 3).

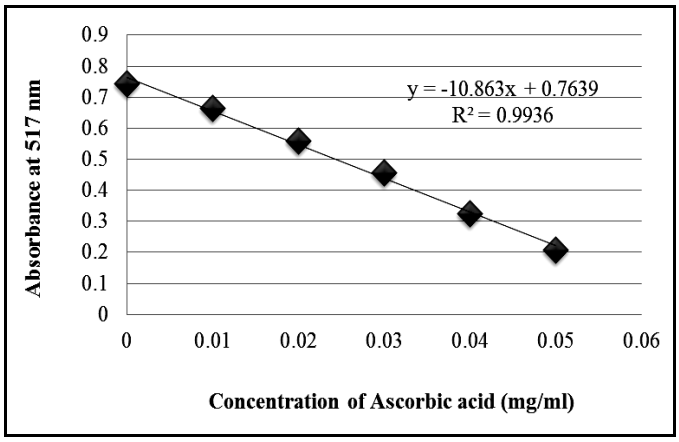

FIG. 3: STANDARD CURVE OF TOTAL DPPH USING ASCORBIC ACID AS STANDARD

In DPPH radical scavenging activity assay, the ethanolic aril extract showed the most antioxidant activity when compared to ethanolic peel and flesh extract. Percentage of scavenging activity of ethanolic extract indicated that aril extract with 4.87±1.58 mg AAE/g FW had higher antioxidant than that of peel and pulp ethanolic extract (4.65±0.70 and 2.41±0.20mg AAE/g FW, respectively) (Table 1).

FRAP assay: The reducing potential of the ethanolic extracts was determined by the ferric reducing antioxidant power (FRAP) method and was calculated with a linear equation based on a standard curve using Ascorbic acid (y = 18.77X + 0.0425, R^2 = 0.997) in terms of mg of Ascorbic acid equivalents (AAE) per gram fresh weight (mg AAE/g FW; %) (Figure 4). In Frap assay, the reducing potential of peel and pulp ethanolic extract was the same (0.012±0.01 mg AAE/g FW). And the result clearly showed that the ferric reducing antioxidant power of seed pulp ethanolic extract had the highest when comparing with other parts of extracts (Table 1).

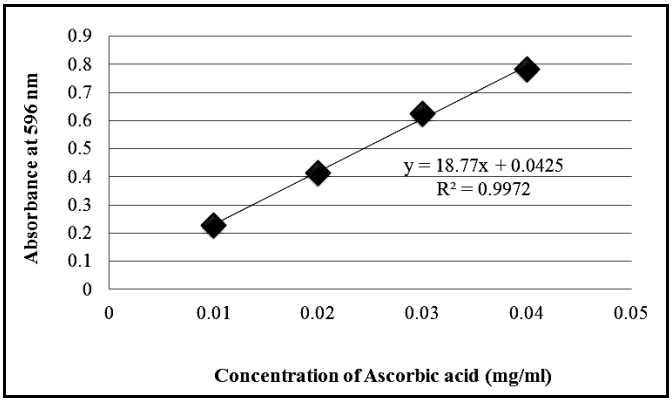

FIG. 4: STANDARD CURVE OF TOTAL FRAP USING ASCORBIC ACID AS STANDARD

Antimicrobial activities (MIC and MBC): The antimicrobial activity of ethanolic extracts from ripe fruit of Gac against different pathogenic strains was calculated by broth macrodilution assay and was qualitatively assessed by the presence MIC and MBC values in **Table 2**.

TABLE 2: ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT FROM DIFFERENT PARTS OF GAC FRUIT BY BROTH MACRODILUTION ASSAY

Tested Strains	Peel		Pulp		Aril (Seed pulp)	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
***Escherichia coli* ATCC 25922**	1.562	>50	1.562	>50	3.125	>50
***Staphylococcus aureus* ATCC 1216**	3.125	>50	6.25	>50	6.25	>50
***Bacillus cereus* DMST 5040**	6.25	>50	3.125	>50	12.50	>50
***Pseudomonas aeruginosa* ATCC 27853**	3.125	>50	6.25	>50	3.125	>50
***Salmonella typhimurium* ATCC 13311**	6.25	>50	3.125	>50	12.50	>50
Klebsiella pneumoniae	12.50	>50	6.25	>50	6.25	>50

Active of ethanolic extract of Gac fruit showed a potent antimicrobial activity against both Gram-positive and Gram-negative bacteria. The different parts of ethanolic extract from Gac fruit had MIC values of range from 1.526 to 12.5 mg/mL and had also MBC values of >50 mg/mL against pathogenic strains. Among the Gram positive ones, ethanolic extract of peel had the low MIC values (1.562 mg/mL) against *E. coli* ATCC 25922 and Among the Gram negative ones, pulp ethanolic extract of ripe Gac fruit had the low MIC values (3.125 mg/mL) against *Bacillus cereus* DMST 5040. On the basis of MIC and MBC values, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 1216, *Pseudomonas aeruginosa* ATCC 27853 was more sensitive than *Klebsiella pneumoniae*, *Salmonella typhimurium* ATCC 13311 and *Bacillus cereus* DMST 5040. Aril ethanolic extract had microbicidal activity against all pathogenic strains but it was showed lower antimicrobial activity than that of other part extracts (peel and pulp).

A comparison of the susceptibility of the extracts toward pathogenic strains, including Gram-positive and Gram-negative strains, showed that *E. coli* ATCC 25922 as Gram-negative strain revealed to be more susceptible to peel and pulp ethanolic extracts than other tested strains. This possibly means that the different compounds in cell wall of each pathogenic strains had effective to antimicrobial activity. Gram-positive bacteria has peptidoglycan layers combined with teichoic acid molecules in cell wall, while has much thin peptidoglycan layer and non- teichoic acid in Gram-negative cell wall [19].

The data of this study clearly indicated that the different parts of ethanolic extract from Gac fruit (*Momordica cochinchinensis* Spreng.) had antibacterial activity against all of microorganisms in gram-positive and gram-negative and antibacterial activity depended on among concentration of samples against tested strains. Rojas et al. (2003) has been reported that the using 95% ethanol as solvents for dissolving the crude extracts always gave negative results [20]. In other words, ethanolic plant extract did not influence in the antimicrobial activities. However, in this study, the ethanolic extracts seemed to have effective compounds. And there are many researches showed that the ethanolic extract from other species of *Cucurbitaceae* has antimicrobial acitivities [21-24].

CONCLUSIONS: The antioxidant and antimicrobial activities of ethanolic extract from *Momordica cochinchinensis* Spreng. (Gac fruit) were evaluated. Seed pulp extract with ethanol solution exhibited higher antioxidant activities in 2 antioxidant assays performed (DPPH and ferric reducing antioxidant power (FRAP)). And flesh extract presented the highest the total phenolic and flavonoid contents. From these result of present study concluded that ethanolic extract of ripe Gac extract could be a sources of these natural constituents as antioxidant compounds. The antimicrobial activity of ethanolic extracts from peel, pulp and aril of ripe Gac fruit were assessed by using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) method. These extracts showed good activity against six pathogenic strains, including Gram-positive and negative. *E. coli* ATCC had the most susceptibility to peel ethanolic extract of ripe Gac fruit.

The present study provides additional data for supporting the use of *Momordica cochinchinensis* Spreng. (Gac fruit) extracts as natural antimicrobial and antioxidant agents for product developments in food and pharmaceutical industries.

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