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# ฤทธิ์ต้านออกซิเดชันและฤทธิ์ต้านแบคทีเรียของสารสกัดเอทานอลจากส่วนต่าง ๆ ของผลฟักข้าว

# Antioxidant and Antibacterial Activities of Ethanolic Extracts from Different Parts of Gac Fruit

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**บทคัดย่อ**

ศึกษาฤทธิ์ต้านออกซิเดชันและฤทธิ์ต้านแบคทีเรียของสารสกัดเอธานอลจากเปลือก เนื้อ เยื่อหุ้มเมล็ด และเมล็ดของฟักข้าว เมื่อทำการทดสอบฤทธิ์ต้านออกซิเดชันจากความสามารถในการจับอนุมูลอิสระ (ดีพีพีเอช) พบว่าสารสกัดจากเนื้อฟักข้าวมีฤทธิ์สูงที่สุด สารสกัดเอทานอลจากเปลือกฟักข้าวมีความสามารถในการเกิดสารประกอบเชิงซ้อนกับไอออนของเฟอรัสได้ดีที่สุด อีกทั้งยังมีความสามารถในการรีดิวซ์ได้ดีที่สุด พบว่าปริมาณโพลีฟีนอลทั้งหมดในสารสกัดจากเปลือกฟักข้าวมีมากที่สุดเมื่อทดสอบด้วยวิธีของโฟลินและสารประกอบฟีนอลในเปลือกฟักข้าวอาจมีส่วนช่วยในการยับยั้งการเกิดออกซิเดชันโดยการเกิดสารประกอบเชิงซ้อนกับไอออนของสารเร่งการเกิดออกซิเดชันรวมทั้งการรีดิวซ์ปฏิกิริยา การประเมินฤทธิ์ต้านแบคทีเรียด้วยการต่อต้านเชื้อก่อโรค 5 ชนิด ด้วยวิธีการซึมผ่านแผ่นกระดาษบนวุ้นแข็ง ผลการศึกษาพบว่าสารสกัดจากเปลือกฟักข้าวมีประสิทธิภาพในการต้านเชื้อ *Staphylococcus aureus* ได้ดีที่สุด โดยสรุปแล้วสารสกัดฟักข้าวจากส่วนที่แตกต่างกันมีฤทธิ์ยับยั้งปฏิกิริยาออกซิเดชันและแสดงความสามารถในการต้านแบคทีเรียด้วย

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## ABSTRACT

The antioxidant and antibacterial properties of ethanolic extracts from gac (*Momordica cochinchinensis* Spreng) fruit peel, pulp, aril and seed were investigated in this study. The antioxidant activities of each ethanolic extract were determined as the 2, 2-diphenyl-1-picrylhdrazyl (DPPH) radical scavenging ability, which pulp extract showed the highest ability. The peel extract showed the highest ability to chelate ferrous ion and the highest reducing power. The peel extract also had the highest value for total phenolic contents, assessed by the Folin Ciocalteu method. Therefore the phenolic compounds in peel extract might contribute to inhibition of oxidation by chelating the prooxidant ions as well as by reducing the reaction. Antibacterial activities were evaluated against five pathogens by the agar disc diffusion method. It was found that the peel extract was the most active against the tested bacteria, especially *Staphylococcus aureus*. In summary, different parts of gac fruit appear to inhibit oxidation reactions and exhibit antibacterial activity.


**คำสำคัญ:** ฟักข้าว ต้านออกซิเดชัน โพลีฟีนอล การเกิดสารเชิงซ้อนกับเฟอรัสไอออน ความสามารถในการรีดิวซ์
**Keywords:** *Momordica cochinchinensis*, Antioxidant, Polyphenol, $Fe^{2+}$-chelating activity, Reducing power

## 1. Introduction

Gac (*Momordica cochinchinensis* Spreng) is a tropical fruit grown in many countries in tropical regions. It may be called by different names, such as Gac (Vietnam), Fak kao (Thailand), Moc Niet Tu (China) and Mak kao (Laos). It is botanically classified in the Cucurbitaceae family and has long been used as a food and traditional medicine in East and Southeast Asia (Iwamoto et al., 1985). The fruit is composed of two main parts: a mesocarp and an endocarp. The mesocarp, which makes up nearly 50% of the weight of the fruit, is thick, spongy, and orange. The endocarp is composed of the soft and sticky arils, with the thickness of about 1-3 mm. This part usually covers the black seed and accounts for around 25% of the fruit weight (Voung, 2000).

Gac fruit aril membrane has been reported as a valuable source of carotenoids (Kubola and Siriamornpun, 2011). The total concentration of lycopene, a powerful antioxidant, in the ripened samples was about 3 mg/g fresh weight (fw), while commercial tomato contains 0-50 μg/g (fw) (Ishida et al., 2004). Gac fruit exhibits a number of health beneficial effects, which are thought to be derived from its bioactive compounds, such as fatty acids, carotenoids and polyphenolics (Aoki et al., 2002). The phytochemical

compounds in gac fruit have antioxidant activities and could reduce the risk of certain types of cancers, including prostate, digestive-tract and lung cancers. They also activate the proper development of the cell membranes and promote healthy vision (Goula and Adamopoulos, 2005; Kubola and Siriamornpun, 2011).

Gac fruit (green fruit or immature fruit) is used in cooking and is commonly consumed as a vegetable. Recently, gac fruit has been produced commercially in Thailand. Only the pulp and aril of ripened fruit are consumed, while other parts are discarded. It has been documented that other parts of gac fruit also show antioxidant activity in methanolic extracts (Kubola and Siriamornpun, 2011). However, mehanolic extract may not be suitable for further application, especially in foods. Therefore, the antioxidant activities of each part should be investigated by extraction using a food favorable solvent. The purpose of the present study was to evaluate the antioxidant activity, metal chelating activity, reducing power and antibacterial activities of the ethanolic extracts from different fruit fractions.

## 2. Materials and methods

### 2.1 Gac fruit fractionation

Gac fruits were cultivated in Amphoe Muang Nong Khai, Thailand. The aged fruits were harvested during November-December 2014. Gac fruits were cleaned and separated into 4 parts including peel, pulp, aril and seed. All parts were dried out in a hot air oven at 50°C for 24 h. The finely ground samples were weighed to calculate the recovery yield of fractionation. All samples were kept in plastic bags in a desiccator until used.

### 2.2 Ethanol extraction

Each finely ground sample (10 g) was mixed with 100 mL of 95% ethanol and kept shaking at room temperature for 24 h. The obtained mixture was then filtered through filter paper (Whatman No. 2). The filtrate was evaporated at (40°C) under a vacuum in a rotary evaporator (BUCHI Rotavapor R-3, Switzerland). The weight of each dried residue from the extraction was divided by the weight of powdered fruit fraction extracted to calculate the yield of extraction. All extracts were re-dissolved in 95% ethanol and stored at -20°C until analysis.

### 2.3 Determination of DPPH radical scavenging activity

The scavenging activity of ethanolic extracts from different gac tissues on the DPPH radical was measured according to the method explained by Kim et al. (2002) with slight modification. Briefly, 1 mL of 0.2 mM DPPH radical solution was mixed with 100 μL of extract or with 100 μL ethanol as the control. The mixtures were vigorously shaken and then left to stand for 30 min at room temperature. The decrease in absorbance at

517 nm caused by the active compounds was averaged over 3 measurements. The percent DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH}\ (\%) = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100$$

The DPPH scavenging activity was expressed as milligram equivalents of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of residue (mg. eq. trolox /g) by calibration to a Trolox standard curve ($y=1.6071x+0.0985$, $R^2=0.9957$).

**2.4 Determination of total phenolic content (TPC)**

TPC was determined by the Folin-Ciocalteu colorimetric method with minor modification (Singleton, 1999). Briefly, 10 μL of the extract was mixed with 2 mL of 2% sodium carbonate and 100 μL of 50% Folin-Ciocalteu's reagent. After incubation for 90 min at room temperature, the absorbance was measured at 760 nm on a Series 1000 UV/visible spectrophotometer, (CECIL Instruments, Cambridge, England). The TPC of each sample was expressed as milligram equivalents of gallic acid per gram of residue (mg eq. gallic acid/g) by comparison to gallic acid standard. All measurements were taken in triplicate. The total phenolic content (TPC) of all samples were calculated with a linear equation based on a gallic acid standard curve ($y=0.004x$, $R^2=0.9978$).

**2.5 Reducing power assay**

The reducing power of ethanolic extracts from gac fruit was determined according to the method described by Choi et al. (2007) with slight modification. Ethanolic extracts (100 μL) were mixed with 900 μL of deionized distilled water. 2.5 mL of 200 mM sodium phosphate buffer, pH 6.6, and 2.5 mL potassium ferricyanide (1%) were added to the mixture. After incubating at 50°C for 20 min, the mixture was mixed with 2.5 mL of 10% (w/v) trichloroacetic acid, and then centrifuged at 2000×g for 10 min. The supernatant (2500 μL) was then mixed with an equal volume of distilled water. Subsequently, 500 μL of ferric chloride solution (0.1%) was added to the mixture. The intensity of blue-green color was measured at 700 nm, with gallic acid serving as a standard. A higher absorbance indicates a higher reducing power. For each sample, three measurements were made and the results were expressed as milligram equivalents of gallic acid per gram of residue (mg. eq. of gallic acid/g). The reducing power of each ethanolic extract was determined and calculated with a linear equation based on a standard curve of gallic acid ($y=0.0022x + 0.0049$, $R^2=0.9938$). All measurements were taken in triplicate.

**2.6 $Fe^{2+}$ chelating activity assay**

The chelating activity for ferrous ions ($Fe^{2+}$) was measured according to the method

previously described by Dinis et al. (1994). Briefly, each ethanolic extract (50 μL) was mixed with 10 μL of ferrous chloride (2 mM) and 20 μL of ferrozine (5 mM) solution for 10 min. The absorbance of the mixture was measured at 562 nm, and compared to EDTA (0-50 μg/mL) as a standard. The ability of a sample to chelate ferrous ion $Fe^{2+}$ is defined as follows:

$$\text{Chelating ability } (\%) = \frac{(A_{Control} - A_{Sample})}{A_{Control}} \times 100$$

where $A_{Control}$ is the absorbance at 562 nm of the control (blank, without extract) and $A_{Sample}$ is the absorbance in the presence of the extract. A lower absorbance indicates a higher chelating power. For each sample, three measurements were made and the results were expressed as milligram equivalents of EDTA per gram of residue (mg. eq. of EDTA /g). $Fe^{2+}$-chelating ability was calculated with a linear equation based on a standard curve using EDTA (y=0.0177x-0.0802, $R^2$=0.9964).

**2.7 Evaluation of antibacterial activity**

The antibacterial activity of crude ethanolic extracts from different gac fruit fractions was evaluated by the disc diffusion method according to Gulluce et al. (2007) with slight modification. The microorganism used in this study (*Serratia marcescens, Bacillus megaterium, Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus*) were all obtained from the Department of Microbiology, Faculty of Science, Khon Kaen University. The bacteria were grown in nutrient broth (NB) at 37°C for 24 h. This inoculum was diluted with sterile water until the optical density ($OD_{600}$) equal to McFarland No. 0.5 ($1.5x10^8$ CFU/ml). Subsequently, this solution was spread on the Mueller Hinton Agar (MHA). Consequently, sterile paper discs with diameters of 5 mm were carefully put on the agar. Thereafter, 20 μL of each extract (100 μg/μL) was loaded on the paper disc. Those agar plates were incubated at 37°C for 24 h. The diameter of the clear zone (including the diameter of the paper disc) around each paper disc was then measured and expressed as inhibition diameter (mm). The negative control was prepared by applying 20 μL of ethanol (95%) on the sterilized paper disc.

**2.8 Statistical analysis**

All experiments results were presented as mean ± SE. One way analysis of variance (ANOVA) was applied for comparison of the mean values. A statistically significant difference was considered at $P < 0.05$. The correlation ($R^2$) between the two variants was analyzed with SPSS software (IBM SPSS Statistic 19 Software).

## 3. Results and discussion

**3.1 Yields of fractionation and extraction**

After dissection of the fruit into 4 parts, the aril made the highest contribution

to the dry weight, followed by the seed portion (Table 1). In contrast, the pulp made up only approximately 11%. Therefore, the aril, which is the most consumable part, contributed the most to the dry mass of the whole fruit. Tinrat et al. (2014) reported that when they dissected the gac fruit, the peel part was the most abundant.

Extraction of the peel yielded a small amount, compared to the other fractions (Table 1). This indicate that the components in the peel may not be extracted by ethanol. It has been reported that methanol and acetone were the most suitable solvents for extraction of unripe and ripe gac fruits (Tinrat, 2014). The differences between the yields of extraction might be attributed to the accessibility and extract ability of different components in the given solvent and ripeness of the fruit (Khamsah et al., 2006; Tawaha et al., 2007).

Based on this data, the aril showed the highest potential for further application if it contained an acceptable level of antioxidant activity or antimicrobial ability. Nonetheless, the antioxidant activities of all four fractions were further evaluated.

**3.2 DPPH assay**

DPPH is a stable free radical widely used to test the ability of extracts to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of a food matrix. The DPPH scavenging activities of the ethanolic extracts are shown in Figure 1. Pulp ethanolic extract demonstrated the strongest radical scavenging activity with the DPPH scavenging activity of 13.55±0.26 mg Trolox equivalents/g extract. A slightly lower activity was obtained with peel with the DPPH scavenging activity of 12.36±0.23 mg Trolox equivalents/g extract, while the lowest DPPH scavenging activities were found in seed and aril (2.05±0.03 and 2.05±0.10 mg Trolox equivalents/g extract), respectively. Our findings are in agreement with those of Gupta et al. (2010) who found the highest antioxidant activity in the pulp juice rather than in the skin of the cherimoya (*Annona cherimola*).

**Table 1** Fractions of dry weight and extraction yield of different parts of gac fruit.

| Fraction | Fraction of dry weight (%) | Yield after extraction (%) |
|---|---|---|
| Peel | 16.50 | 2.20 |
| Pulp | 11.82 | 11.80 |
| Aril | 37.11 | 25.40 |
| Seed | 34.56 | 17.20 |

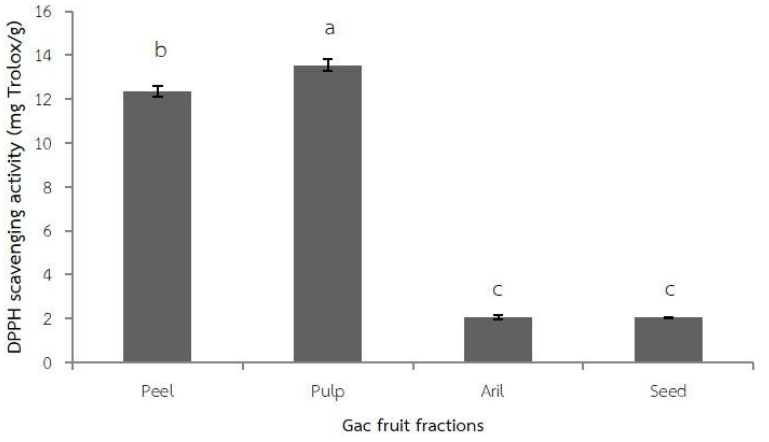


**Figure 1** Scavenging activity of ethanolic extracts from different gac fruit fractions on DPPH radicals. The mean values are plotted the mass of Trolox giving an equivalent response per gram dry extract. Error bars represent the SE (n=3).

**3.3 Total phenolics content**

Figure 2 shows that the TPC of peel (0.90±0.05 mg gallic acid equivalent (GAE) per g dry sample) was higher than that of seed (0.45±0.02 mg GAE/g), pulp (0.36±0.02 mg GAE/g) and aril (0.12±0.01 mg GAE/g). The highest value of TPC in peel compared with pulp was also observed in *Annona squamosal* with 0.121 and 0.015 mg GAE/g dry weight, respectively (Huang et al., 2010). The highest phenolic content was also found in the peel and pulp in other fruits, such as *Psidium guajava* (Bashir et al., 2003) or *Magnifera pajang* (Abu et al., 2009).

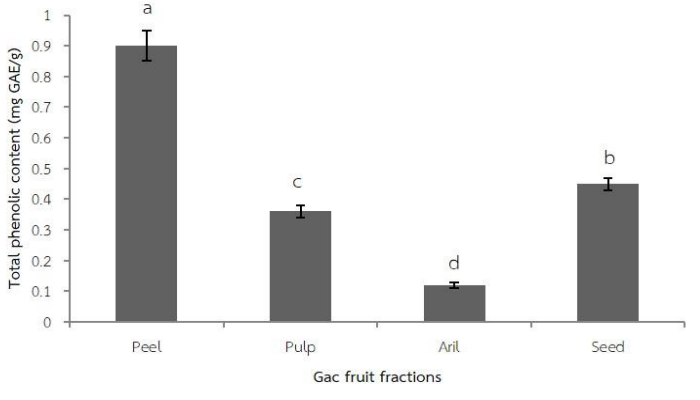


**Figure 2** Total phenolic content of ethanolic extracts from different gac fruit fractions. The error bar represents the standard error (SE, n=3).

### 3.4 $Fe^{2+}$-chelating ability

Measuring the capacity of the fruit extracts to chelate ferrous ions is an important method of assessing its antioxidant potential as ferrous ions are reported to be the most effective prooxidants in foods (Yamaguchi et al., 1988). Peel ethanolic extracts chelated the most iron with $Fe^{2+}$-chelating activity equivalent to 2.18±0.01 mg EDTA/g extract, followed by pulp, seed and aril with $Fe^{2+}$-chelating activities equivalent to 1.91±0.02, 1.06±0.01, and 0.13±0.01 mg EDTA/g extract, respectively (Figure 3). It is interesting that a high correlation exists between $Fe^{2+}$-chelating activity with total phenolics content with an $R^2$ value of 0.799 (Table 2). It is known that phenolic compounds exhibit redox properties (i.e. act as reducing agents, hydrogen donators and singlet oxygen quenchers) and have $Fe^{2+}$-chelating activity (Song and Barlow, 2004; Balasundram et al., 2006).

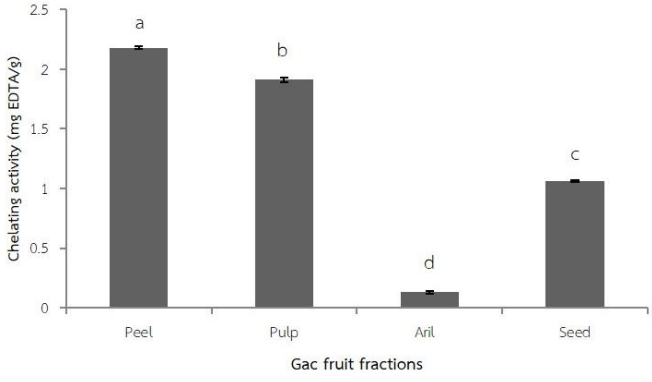


**Figure 3** Chelating activity of ethanolic extracts from different gac fruit fractions. The mean values are plotted as the amount of EDTA giving a response equivalent to 1 g of dry extract. The error bars represent the SE (n=3).

### 3.5 Reducing power

Yen et al., (1993); Siddhuraju et al., (2002) have reported that the reducing power of bioactive compounds was associated with antioxidant activity. Thus, it is necessary to determine the reducing power of the ethanolic extracts from gac to elucidate the relationship between their antioxidant effect and their reducing power. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers (i.e. antioxidants) causes the reduction of the $Fe^{3+/}$ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the $Fe^{2+}$concentration. The ethanolic extracts from peel, pulp, aril, and seed showed reducing powers of 10.54±0.04, 1.35±1.13, 0.91±0.05,

and 0.52±0.06 mg GAE/g extract, respectively (Figure 4). Obviously, the extract from peel was much more effective in reducing the iron than the other fractions of gac fruit. Strong correlations of DPPH scavenging activity with metal chelating activity ($R^2$= 0.892), reducing power with metal chelating activity ($R^2$=0.891), and total phenolic content with metal chelating activity ($R^2$= 0.799) were observed (Table 2). However, only a weak correlation was observed between total phenolic content and DPPH scavenging activity ($R^2$= 0.553). Regarding the reducing power, it is found that the amount of phenolic compounds was high in ethanolic extracts of gac fruit and there was a tight relationship between the amount of total phenolic content and the reducing power. Thus, phenolics present in gac fruit extracts are good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products. These results are in good agreement with the reports of the above-mentioned authors, who showed that the antioxidant properties were concomitant with the development of reducing power.

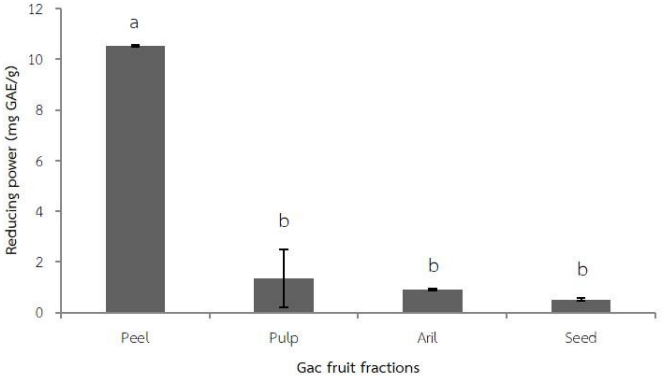


**Figure 4** Reducing power of ethanolic extracts from different gac fruit fractions. Mean values are plotted as the number of mg of gallic acid giving a response equivalent to 1 g dried extract. The error bars show the SE (n=3).

**Table 2** The Pearson correlation coefficient of total phenolic content, chelating activity, reducing power with DPPH scavenging activity, reducing power and chelating activity of different fractions of gac fruit.

| | Correlation ($R^2$) | | |
|---|---|---|---|
| | **DPPH scavenging activity** | **Reducing power** | **Chelating activity** |
| Total phenolic content | 0.553 | 0.891** | 0.799** |
| Chelating activity | 0.892** | 0.643* | - |
| Reducing power | 0.561 | - | - |

**3.6 Antimicrobial activity**

The antimicrobial activities of the crude ethanolic extracts of *M. cochinchinensis* against five bacteria species were evaluated by observing the inhibition zones. The antimicrobial activities of the extracts are shown in Figure 5 and Table 3. It was found that all crude ethanolic extracts showed antimicrobial activity against all five bacterial strains with inhibition zones in the range of 8.13±0.15 - 13.90±0.10 mm. A comparison of the susceptibility of the extracts toward bacterial strains, showed that *Staphylococcus aureus* appeared to be more susceptible to peel extracts than other strains tested. It may be that the different cell wall composition of each species is responsible for the different antimicrobial susceptibilities. The cell wall of *S. aureus* (gram positive bacteria) is composed of peptidoglycan layers combined with the teichoic acid molecules. In gram negative cell wall, the peptidoglycan layer is much thinner, and there is no teichoic acid. Moreover, an outer membrane closely overlies the peptidoglycan layer so that the membrane and layer comprise the cell wall (Alcamo, 2001).

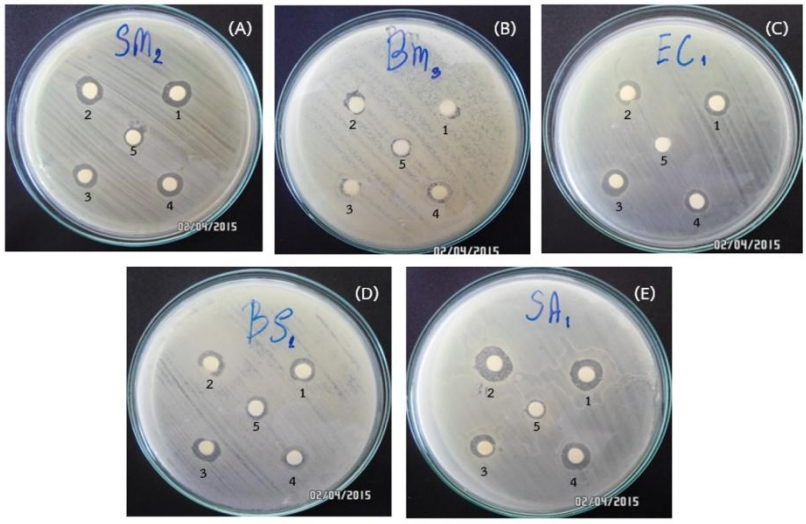


**Figure 5** Disc diffusion inhibition zone of (A) *S. marcescens* (B) *B. megaterium* (C) *E. coli* (D) *B. subtilis* and (E) *S. aureus* tested with 20 μL of 100 μg/μL of ethanolic extracts from (1) peel, (2) pulp, (3) aril, and (4) seed of gac fruit, respectively. Disc 5 is the negative control of 95% ethanol (20 μL).

**Table 3** Antibacterial activity of crude extracts of different fractions from gac fruit.

| Bacteria | Zone of inhibition diameter (mm) | | | | |
|---|---|---|---|---|---|
| | Peel | Pulp | Aril | Seed | Control |
| *Serratia marcescens* | 10.17±0.29$^{b}$ | 8.27±0.25$^{d}$ | 9.10±0.10$^{c}$ | 8.97±0.15$^{c}$ | 7.03±0.06$^{b}$ |
| *Bacillus megaterium* | 9.17±0.15$^{c}$ | 9.07±0.12$^{c}$ | 8.13±0.15$^{d}$ | 10.10±0.17$^{b}$ | 7.90±0.17$^{a}$ |
| *Escherichia coli* | 10.00±0.20$^{b}$ | 10.20±0.20$^{b}$ | 11.10±0.10$^{b}$ | 9.07±0.12$^{b}$ | 6.93±0.06$^{b}$ |
| *Bacillus subtilis* | 9.07±0.12$^{c}$ | 9.13±0.15$^{c}$ | 11.93±0.12$^{a}$ | 10.03±0.06$^{c}$ | 7.90±0.10$^{a}$ |
| *Staphylococcus aureus* | 13.90±0.10$^{a}$ | 11.90±0.10$^{a}$ | 11.90±0.10$^{a}$ | 11.13±0.23$^{a}$ | 7.00±0.00$^{b}$ |

**Remark:** Values are mean±SE, n=3. Means with superscripts having the same letter are not significantly different.

## 4. Conclusion

The present study demonstrated that the ethanolic extract of gac peel possesses potent antioxidant activity and contained a high level of total phenolics, which are well-known antioxidants. The fraction of gac which showed the highest phenolic content in the extract was peel, followed by seed, pulp and aril, respectively. Total phenolic content and DPPH scavenging activity was also found to be correlated with reducing power and chelating activity. The findings of this study suggest that the gac extract contains bioactive compounds, which may have potential to protect against diseases. Therefore, gac is a relevant source of antioxidants, suggesting that food applications and health products can be developed to add value to gac fruit.

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## 6. References

Abu, B.M.F., Mohamed, M., Rahmat, A., and Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Magnifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chemistry 113(2): 479-483.

Alcamo, I.E. (2001). Fundamental of microbiology. 6th ed. New York: Jones and Bartlett Publishers, Inc.

Aoki, H., Kieu, N.T.M., Kuze, N., Tomisaka, K., and Chuyen, N.V. (2002). Carotenoid pigments in Gac fruit (*Momordica cochichinensis* Spreng). Bioscience Biotechnology Biochemistry 66: 2479-2482.

Balasundram, N., Sundram, K., and Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence and potential uses. Food Chemistry 99: 191-203.

Bashir, H.A., Bakar, A, and Abu, G.A. (2003). Compositional changes during guava fruit ripening. Food Chemistry 80: 557-563.

Choi, Y., Jeong, H.S., and Lee, J. (2007). Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry 103: 130-138.

Dinis, T.C.P., Madeira, V.M.C., and Almeida, L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics 315: 161-169.

Goula, A.M., and Adamopoulos, K.G. (2005). Stability of lycopene during spraydrying of tomato pulp. LWT-Food Science Technology 38: 479-487.

Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D. Sokmen, A., Polissiou, M., Adiguzel, A., and Ozkan, H. (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp *longifolia*. Food Chemistry 103: 1449-1456.

Gupta, E.G., Garrett, A., Martinez, A., Robison, R., and O'Neill, K.L. (2010). The antioxidant properties of the cherimoya (*Annona cherimola*) fruit. Food Research International 44(7): 2205–2209.

Huang, W.Y., Cai, Y.Z., Corke, H., and Su, M. (2010). Survey of antioxidant capacity and nutritional quality of selected edible and medicinal fruit plants in Hong Kong. Journal of Food Composition and Analysis 23: 510-517.

Ishida, B.K., Turner, C., Chapman, M.H., and McKeon, T.A. (2004). Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. Journal of Agricultural Food Chemistry 52: 274–279.

Iwamoto, M., Okabe, H., Yamauchi, T., Tanaka, M., Rokutani, Y., and Hara, S. (1985). Studies on the constituents of *Momordica cochinchinensis* Spreng. I. Isolation and characterization of the seed saponins, momordica saponins I and II. Chemical and Pharmaceutical Bulletin 33(2): 464–478.

Khamsah, S.M., Akowah, G., and Zhari, I. (2006). Antioxidant activity and phenolic content of *Orthosiphon stamineus* Benth from different geographical origin. Journal of Sustainability Science and Management 1:14-20.

Kim, D.O., Lee, K.W., Lee, H.J., and Lee, C.Y. (2002). Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. Journal of Agricultural Food Chemistry 50: 3713-3717.

Kubola, J., and Siriamornpun, S. (2011). Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). Food Chemistry 127: 1138-1145.

Siddhuraju, P., Mohan, P.S., and Becker, K. (2002). 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 Chemistry 79: 61-67.

Singleton, V.L., Orthofer, R., and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substances by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152-178.

Song, Y.Y. and Barlow, P.J. (2004). Antioxidant capacity and phenolic content of selected fruit seeds. Food Chemistry 88: 411-417.

Tawaha, K., Alali, F.Q., Gharaibeh, M., Mohammad, M., and El-Elimat, T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species, Food Chemistry 104: 1372–1378.

Tinrat, S., Akkarachaneeyakorn, S., and Singhapol, C. (2014). Evaluation of antioxidant and antimicrobial activities of *Momordica cochinchinensis* Spreng (gac fruit) ethanolic extract. International Journal of Pharmaceutical Science and Research 5(8): 3163-3169.

Tinrat, S. (2014). Comparison of Antioxidant and Antimicrobial Activities of Unripe and Ripe Fruit Extracts of *Momordica cochinchinensis Spreng* (Gac fruit). International Journal of Pharmaceutical Sciences Review and Research 28(1): 75-82.

Voung, L.T. (2000). Under-utilized beta-carotene-rich crops of Viet Nam. Food and Nutrition Bulletin 2(2): 173–181.

Yamaguchi, R., Tatsumi, M.A., Kato, K., and Yoshimitsu, U. (1988). Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agricultural and Biological Chemistry 52: 849-850.

Yen, G.C., Duh, P.D., and Tsai, C.L. (1993). Relationship between antioxidant activity and maturity of peanut hulls. Journal of Agricultural and Food Chemistry 41: 67-70.

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