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Tyrosinase Inhibitory Efficiency and Antioxidant Activity of Gac Fruit (*Momordica cochinchinensis* Spreng.) Extract

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Abstract

This research evaluated the inhibitory efficiency of gac fruit (*Momordica cochinchinensis* Spreng.) extracts on tyrosinase and antioxidant activity. The results showed that ethanolic extract of aril had the highest tyrosinase inhibitory efficiency (80.57±0.50%), followed by peel extract and seed extract (p<0.05), respectively. The ethanolic extract of aril possessed the strongest tyrosinase inhibition of 1.28 fold compared with that of a standard inhibitor, kojic acid. Water extract of aril revealed the highest content of total phenolic compounds (1.291±0.011 mg GAE/g) and ethanolic extract of seed coat showed the strongest DPPH radical scavenging activity (63.18±0.34%). The inhibitory efficiency on tyrosinase associated with total phenolic content and antioxidant activity of the extract, especially in the ethanolic fraction. Our results can support the development of gac fruit extract for natural whitening products with further clinical study to confirm the efficiency and safety of the extract. It also enhances the economic value and reduces wastes of gac fruit, particularly peel and seed which were underutilized parts of fruit.

Keywords : Antioxidant Activity; Gac fruit (*Momordica cochinchinensis* Spreng.); Tyrosinase.

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1. Introduction

Nowadays, natural extracts are increasingly being used instead of synthetic substances in various industries, especially those related to health and cosmetic product development. Natural extracts are full of phenolic compounds which have many properties, such as antioxidant activity, anti-aging, anti-wrinkles, anti-inflammatory and anti-chronic diseases [1]-[3]. It is interesting that phenolic compounds exhibit tyrosinase enzyme inhibitory properties because of aromatic rings in their molecules. This structure is similar to tyrosine, the substrate that hydroxylated to L-DOPA (3,4-dihydroxyphenylalanine), dopaquinone and melanin, respectively [4]. This process is catalyzed by tyrosinase enzyme. Tyrosinase inhibitor can bind to copper at active site causes the competitive inhibition, leads the reduction of melanin synthesis in melanocyte [5]-[7].

Gac fruit or Fak-Khao (in Thai) (*Momordica cochinchinensis* Spreng.) is in the Cucurbitaceae family, commonly found in Southeast Asia such as Thailand, Vietnam and Malaysia. Immature fruit is yellowish-green, and turns to reddish-orange with yellow pulp and red aril during ripening. Gac fruit enriches with carotenoids, polyunsaturated fatty acids, α-tocopherol (Vitamin E), phenolic and flavonoid compounds, mainly in aril part [8]. Phenolic acids and flavonoids found in gac are gallic acid, *p*-hydroxy benzoic acid, ferulic acid, synapic acid, myricetin, epigenin, quercetin, rutin, luteolin and hesperidin [9]-[10]. These compounds play an important role in the ultraviolet protection and tyrosinase inhibition properties [11], [12].

Therefore, this research was focused on tyrosinase inhibitory efficiency and antioxidant properties of gac fruit extracts for the skin lightening product development. It also reduces wastes of gac fruit, especially peel and seed, and enhances the fruit economic value.

2. Research Methodology

2.1 Materials and Methods

2.1.1 Chemicals

Chemicals used in this research were analytical reagent grade: DPPH (2,2-diphenyl-1-picryllhydrazyl), gallic acid, kojic acid, L-DOPA (3,4-dihydroxy-L-phenylalanine) and tyrosinase from mushroom (2,150 units/ml) were obtained from Sigma-Aldrich (USA). Ethanol was product of BDH (Germany). Ascorbic acid was product of Fisher-Scientific (UK). Disodium hydrogen phosphate and Folin-Ciocalteu phenol reagent were purchased from Loba chemie (India). Sodium dihydrogen phosphate and sodium carbonate were purchased from Univar Ajax Finechem (Australia).

2.1.2 Sample Extraction

Gac fruit samples in ripening period (orange-red peel and yellow pulp) were obtained from Khao Kitchakut, Chanthaburi, Thailand during harvest season in August. Samples were identified by the Department of Plant Science and Landscape, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-Ok Chanthaburi

Campus, Chanthaburi, Thailand. Ripe samples were cleaned, separated into five parts (**Fig. 1.**): peel, pulp, aril, seed coat and seed, and cut into small pieces. Each part was macerated overnight at room temperature using two solvents: water and 70% v/v ethanol (10 g of sample macerated with 20 ml of the solvent) according to A. Abdulqader et al. [10]. The mixture was filtered and centrifuged at 5,000 rpm for 30 min. Supernatant was collected and evaporated using rotary evaporator. Crude extracts were kept in 4 ºC before analysis.

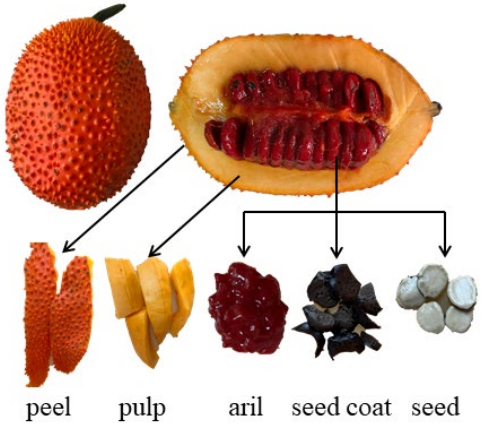

Fig. 1 Gac fruit (*Momordica cochinchinensis* Spreng.) samples (peel, pulp, aril, seed coat and seed)

2.1.3 Tyrosinase Inhibitory Efficiency

The tyrosinase inhibitory efficiency was analyzed by DOPAchrome method modified from A. Manosroi et al. [13]. Briefly, the 0.1 ml of the extract (0.01 mg/ml) or standard kojic acid (0.01 mg/ml) was mixed with 1.8 ml of 20 mM phosphate buffer pH 6.8 (PBS) and 0.1 ml of tyrosinase (100 units/ml). After standing at room temperature for 10 min, the 1 ml of 0.25 mM of L-DOPA was added and left for 20 min. The absorbance at 492 nm was measured and the percentage of tyrosinase inhibition was calculated from the equation (1) compared with standard kojic acid.

$$\%\ Tyrosinase\ Inhibition = \frac{(A-B)-(C-D)\ x\ 100}{(A-B)} \quad (1)$$

When

A = The absorbance of the solution with the enzyme but without the extract or standard kojic acid

B = The absorbance of the solution without the enzyme, extract or standard kojic acid

C = The absorbance of the solution with the enzyme, extract and standard kojic acid

D = The absorbance of the extract and standard kojic acid without the enzyme

2.1.4 Total Phenolic Content and DPPH Radical Scavenging Activity

Total phenolic content was performed using Folin-Ciocalteu phenol reagent as described by M. P. Kähkönen et al. [14] and A. Chanwitheesuk et al. [15]. Briefly, the 1.0 ml of the extract or standard gallic acid was mixed with 5.0 ml of 10%v/v Folin-Ciocalteu phenol reagent and left for 5 min in the dark. Then, 2.0 ml of Na_2CO_3 was added and maintained for 30 min in the dark. The absorbance at 765 nm was determined and total phenolic content was expressed as mg gallic acid equivalent per g of the extract (mg GAE/g extract).

DPPH radical scavenging activity was performed using the method adapted from C. Sanchez-Moreno et al. [16]. The

0.5 ml of the extract or standard trolox (0.005, 0.01, 0.015, 0.02 and 0.025 mg/ml) was mixed with 3.0 ml of 0.05 mM DPPH solution. The mixture was shaken and left in the dark for 30 min. The absorbance was measured at 517 nm and the percentage of DPPH radical inhibition was calculated using the equation (2).

$$\%\ DPPH\ Inhibition = \frac{Actrl - Astd/ext\ x\ 100}{Actrl} \quad (2)$$

When
Actrl = The absorbance of control
Astd/ext = The absorbance of standard ascorbic acid/the absorbance of the extract

2.2 Statistical Analysis

The tyrosinase inhibition activity, total phenolic content and DPPH radical scavenging activity were expressed as mean ± standard deviation of triplicate. The difference among data was analyzed via Duncan's multiple range test at 0.05 of significance (p<0.05). Relationship between total phenolic and tyrosinase inhibitory, as well as DPPH radical scavenging activity and tyrosinase inhibitory efficiency, were determined using r^2 value of the linear regression method.

3. Results and Discussion

The tyrosinase inhibitory efficiency, total phenolic content and DPPH radical scavenging activity of the extracts are demonstrated in **Table 1**.

Table 1 Percentage of tyrosinase inhibition, total phenolic content and DPPH inhibition of gac fruit extract (n = 3)

Gac fruit part		% Tyrosinase inhibition	Total phenolic content (mg GAE/g)	% DPPH inhibition
peel (water extract)	PW	52.20±0.88^{a}	0.379±0.012^{a}	37.42±0.68^{a}
peel (ethanol extract)	PE	73.66±0.99^{b}	0.386±0.014^{a}	58.24±0.68^{b}
pulp (water extract)	PUW	55.18±0.64^{c}	0.269±0.009^{b}	18.04±1.12^{c}
pulp (ethanol extract)	PUE	69.85±0.32^{d}	0.270±0.004^{b}	50.10±2.15^{d}
aril (water extract)	AW	41.44±1.46^{e}	1.291±0.011^{c}	10.64±1.17^{e}
aril (ethanol extract)	AE	80.57±0.50^{f}	1.103±0.012^{d}	29.24±0.86^{f}
seed coat (water extract)	SCW	39.14±0.55^{g}	0.338±0.009^{e}	47.11±1.05^{g}
seed coat (ethanol extract)	SCE	69.87±1.31^{d}	0.458±0.008^{f}	63.18±0.34^{h}
seed (water extract)	SW	51.52±0.18^{a}	0.259±0.013^{b}	36.58±0.54^{a}
seed (ethanol extract)	SE	70.89±1.02^{d}	0.271±0.010^{b}	54.81±0.15^{i}
standard kojic acid (0.01 mg/ml)	-	62.88±1.62^{f}	-	-

The superscript letters ($^{a, b, c,\cdots}$) in each column showed the significant difference (p<0.05)

From the results, the ethanolic extract of aril (AE) showed the strongest tyrosinase inhibition (80.57±0.50%), followed by ethanolic extract of peel (PE)

and seed (SE), respectively. Ethanolic extract of seed coat (SCE) exhibited the highest DPPH radical scavenging activity (63.18±0.34%), followed by peel (PE) and seed extract (SE), respectively. In the contrary, water extract of aril (AW) gave the highest content of total phenolic compounds (1.291 ±0.011 mg GAE/g extract), followed by ethanolic extract of aril (AE) and seed coat (SCE), respectively (p<0.05).

The tyrosinase inhibitory efficiency of different part of gac fruit extract (peel, pulp, aril, seed coat and seed) was conducted using two solvents; ethanol and water. Interestingly, enzyme inhibition, total phenolic content and DPPH radical scavenging activity of ethanolic extract were significantly larger than those of water extract (p<0.05). B. B. Li et al. [17] noted that the combination of water with polar solvent such as ethanol or methanol gave to powerful extraction of phytochemical species. Our result is in agreement with I. Bakhouche et al. [18], who mentioned that tyrosinase inhibition in methanolic extract of *Limonium delicatulum* root was stronger than that of water extract and also gave high phenolics, flavonoids and tannins content, significantly (p<0.05).

The relationships among tyrosinase inhibition, total phenolic content and DPPH radical scavenging activity of the extracts are demonstrated in **Fig. 2**. For the ethanolic extracts, quite good relationship between % tyrosinase inhibition and total phenolic content (r^2 = 0.8574), and between % tyrosinase inhibition and % DPPH inhibition (r^2 = 0.7315) were found. On the other hand, there were no relationship between %

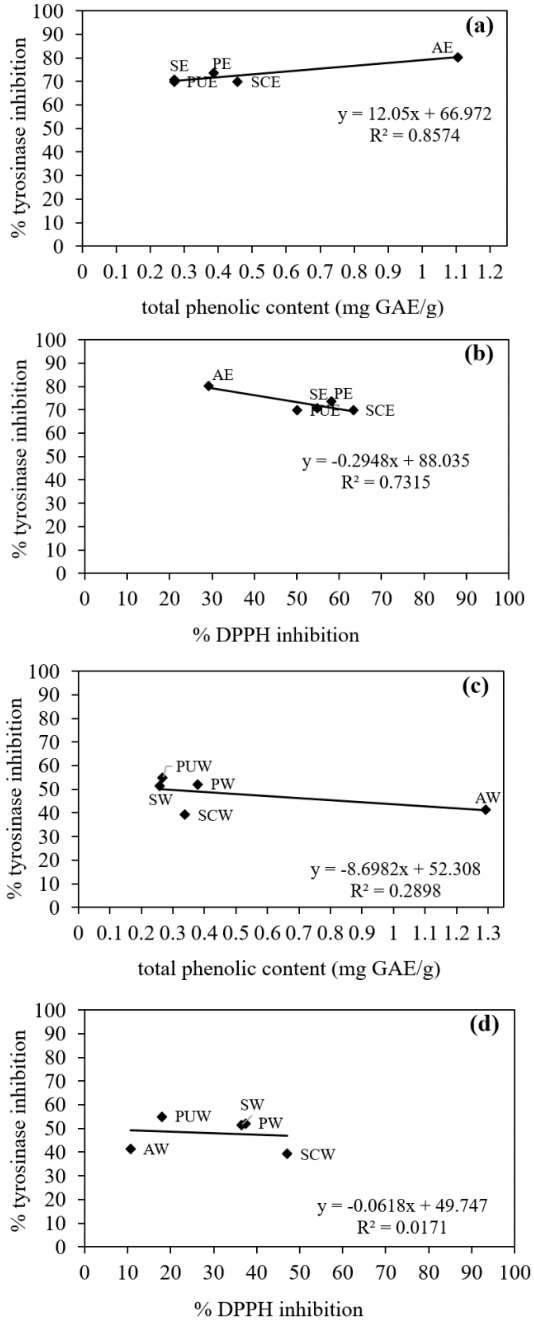

Fig. 2 Relationship between the percentage of tyrosinase inhibition and (a) total phenolic content, (b) percentage of DPPH inhibition in ethanolic extract. The percentage of tyrosinase inhibition and (c) total phenolic content, (d) percentage of DPPH inhibition in water extract.

tyrosinase inhibition and total phenolic content, and between % tyrosinase inhibition and % DPPH inhibition in water extracts (r^2 = 0.2898 and 0.0171, respectively). It was concluded that the extract with high content of phenolic compounds and strong antioxidant activity would be high tyrosinase inhibitory efficiency. This result is in conformity with A. M. Muddathir et al. [19] who found the anti-tyrosinase of some Sudanese medicinal plants extracts had a significant correlation with total phenolic content and antioxidant activity.

The aril, seed and peel of gac fruit showed strong tyrosinase inhibitory efficiency. The ethanolic extract of aril possessed the strongest tyrosinase inhibition of 1.28 fold compared with that of a standard inhibitor, kojic acid (% tyrosinase inhibition = 62.88%). While the ethanolic extract of other parts were between 1.11-1.17 fold compared with standard kojic acid. These findings were correlated with the reports of J. Kubola and S. Siriamornpun [9] and A. Abdulqader et al. [10]. Their researches were noted that in the aril part of gac fruit was a rich source of flavonoids (rutin, myricetin and luteolin), carotenoids (lycopene and β-carotene) and phenolic acids (gallic acid and *p*-hydroxy benzoic acid). These compounds possessed the antioxidant and ultraviolet protection activities [11], [12].

J. Kim et al. [20] reported that pulp and aril extracts of gac fruit, which contained high concentration of phenolic and flavonoid compounds, had anti-melanogenesis activity *in vitro*. The extracts demonstrated the inhibition of melanin synthesis in melan-A cells by inhibiting tyrosinase activity and suppressing p-PKC expression. The authors suggested that pulp and aril extracts of gac fruit could be used in whitening products. According to the researches of H. Baek et al. [21] and Y. Kim et al. [22], many hydroxyl groups in phenolic compounds made their structure similar to tyrosine, which is the substrate in melanin synthesis. Instead of tyrosine, phenolic compounds could bind with tyrosinase active site, resulting in the decrease of enzyme activity and melanin synthesis.

4. Conclusion

The extracts of gac fruit show the strong tyrosinase inhibitory efficiency, especially in the aril, seed and peel, with reference to standard kojic acid. It may be one of the potentially constituent in natural whitening products with further clinical study to confirm the efficiency and safety of the extract.

5. Acknowledgement

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