Bioactive Compounds and Antioxidant Activities from Pomegranate Peel and Seed Extracts

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Abstract

The aim of this study was to investigate the contents of bioactive compounds (total phenolic (TPC), flavonoid (TFC) and proanthocyanidin (TPAC) contents) and their antioxidant activities (DPPH, and ABTS radical scavenging activities and ferric reducing antioxidant power (FRAP)) of pomegranate peel and seed, which are wastes from fruit juice processing. Peel and seed of pomegranate were extracted by three different solvents (water, 95% ethanol and 70% acetone) at ratio of 1:10 (w/v) by shaking at 150 rpm for 6 h at 25 °C. The results showed that the greatest amount of TPC, TFC and TPAC were obtained from peel extract with acetone extraction (1.140 ± 0.007 mg GAE/g extract, 0.249 ± 0.008 mg QE/g extract and 0.097 ± 0.006 mg CE/g extract, respectively). However, the tendency of antioxidant activity was slightly different from their bioactive contents. The highest DPPH radical scavenging activity was found in acetone peel extract (2.956 ± 0.002 mg TEAC/g extract) and the greatest ABTS activity was obtained from acetone seed extract (3.319 ± 0.016 mg TEAC/g extract). Moreover, FRAP had the highest capacity in acetone extract of peel (7.078 ± 0.028 mg TEAC/g extract). The results suggested that the pomegranate juice processing wastes, including peel and seed might be used as an active ingredient in several non-food products.

Keywords: Antioxidant, Bioactive compound, Pomegranate, Proanthocyanidin, Waste Utilization

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1. Introduction

The skin age tends to be lost its natural elasticity and become thinner, more fragile and laxer and effect on a wrinkled appearance. The process of skin aging is a complex biological process influenced by various endogenous (genetic, endocrine, oxidative stress) and exogenous factors (ultraviolet radiation, environmental pollution and life style) (Naidoo and Birch-Machin, 2017). Thus, several skin anti-aging strategies have been continuously developed. There can be summarized in three kinds of methods; basically including, protecting the skin from external environmental stimuli; scavenging intracellular free radicals; and repair or supplement nutrition to skin cells. The use of cosmetological care (skin care, sun protection, aesthetic non-invasive procedures) or topical agents (antioxidants and cell regulators) is the first treatment to improve functioning skin barrier and promote healthy skin (Ganceviciene *et al.*, 2012). Nowadays, the active ingredient in anti-aging product is commonly composed of two main groups, including antioxidant agents and the cell regulators. Polyphenol, Flavonoid and vitamin, are known as antioxidant compounds, could reduce collagen degradation. The cell regulators, such as retinol, peptide and growth factor (GF) have direct effects on collagen metabolism and elastin enhancing that preventing wrinkle formation. However, synthesis antioxidants can cause adverse side effect. For example, butylated hydroxytoluene (BHT) or dibutylhydroxytoluene, a chemically derivative of phenol, is used as antioxidant in many cosmetic products but it causes skin irritation at high concentration. Thus, the findings of natural antioxidants have been studied for replacement on use of synthetic antioxidants. Natural antioxidants were found in many fruits such as blueberry, grapes and pomegranate fruit.

Pomegranate (*Punica granatum* L. var. *Ganesha*) fruit was typically in season from February to April. The pomegranate tree has been cultivated since ancient times from Himalayas in Northern India throughout the Mediterranean region of Asia including Thailand (Morton, 1987). Nowadays, pomegranate, especially pomegranate juice has become popular due to its nutritional value and health benefit. Furthermore, pomegranate has historically been identified as a rich source of polyphenolic compounds and hydrolysable tannins with several bioactivities, particularly anti-oxidant properties that can help anti-ageing (Diwakar *et al.*, 2012). Pomegranate extract has also been used as bioactive compound in cosmetic products to improve the appearance of wrinkled skin by reducing inflammation and forestalling further damage (Fowler *et al.*, 2010). During processing of pomegranate juice, seed and peel are generated as wastes which are normally used for production of animal feed and fertilizer. Thus, this study aimed to extract the bioactive compounds from seed and peel of pomegranate by different solvents (water, 95% ethanol and 70% acetone) and to determine antioxidant activities.

2. Materials and Methods

2.1 Chemicals and reagents

All chemicals and solvents were analytical grade, cosmetic ingredients were cosmetic grade. Acetone, dimethylsulfoxide (DMSO), ethanol (95%), ferric chloride, hydrochloric acid (37%), methanol, sulphuric acid, vanillin was purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), dibasic phosphate and monobasic phosphate. Potassium acetate (CH_3COOK) and potassium persulphate ($K_2S_2O_8$) were purchased from Ajax Finechem (Seven Hills, Australia) . ABTS(2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) , DPPH(2,2-diphenyl-1-picrylhydrazyl) , catechin, folin-ciocalteu, gallic acid, 2,4,6-tris(2-pyridyl) -1,3,5-triazine (TPTZ) , quercetin, and trolox were purchased from Sigma (St. Louis, MO, USA).

2.2 Sample preparation

Pomegranate (*Punica granatum* L.var. *Ganesha*) fruits were obtained from local market of Chiang Rai province, Thailand. The samples, including peel and seed were separated from fruit. Their peels were cut into small pieces. Both peel and seed were dried by hot air oven (Memmert, model 100–800, Germany) at 50 °C until dry. Dried samples were milled as powder by Rotor and Beater mill (Retsch, model SK300, Germany) and kept at 4 °C until used.

2.3 Extraction of bioactive compounds

Pomegranate peel and seed powder were extracted with three different solvents; water, 95% ethanol and 70% acetone, at a ratio 1:10 (w/v) using an incubator shaker (SHELLAB, model SSI3, USA) at 150 rpm for 6 h. The extracts were filtrated by Whatman filter paper No.1 and residue organic solvents (ethanol and acetone) were removed by rotary evaporator (EYELA, model n-1100, Japan) at 50 °C. The extract samples were lyophilized (Labconco, model 71061 6L, USA) and kept at -20 °C until future used.

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

TPC was measured to the method of Negi *et al. (*2003*)* with a slight modification. Firstly, 20 µL of pomegranate peel and seed extracts *(*1mg*/*ml*)* were mixed with 100 µL of 0.2 M Folin-Ciocalteu reagent for 1 min. Then, 80 µL of 7.5*% (*w*/v)* sodium carbonate were added into the reaction mixture. After incubation at room temperature for 30 min, the absorbance of mixture was measured at 765 nm by microplate reader *(*Biotek, Epoch, USA*)*. Gallic acid was used as a reference standard, and the results were expressed as mg Gallic acid equivalents *(*GAE*)/*g extract.

2.5 Determination of total flavonoid content (TFC)

TFC was measured by slightly modified from Li *et al.* (2006). Briefly, prepared 25 µL of sample, were mixed with 80 µL of 7.5% (w/v) Na2CO3, 140 µL of DI water, 5 µL of 10% (w/v) AlCl3 and 5 µL of 1M CH3 COOK. The mixtures were incubated in the dark at room temperature for 30 min. The absorbance was measured at 415 nm using a microplate reader (Biotek, USA). TFC was calculated using quercetin as standard and expressed as mg quercetin equivalents (QE)/g extract.

2.6 Determination of total proanthocyanidin content (TPAC)

TPAC was determined by the method modified from Zhenbin *et al.* (2011). Sample or catechin (standard) (20 µL) were added into 50 µl of 1% vanillin and 50 µL of 25% H2SO4 in methanol. The mixtures were incubated for 15 min in the dark at room temperature. The absorbance was measured at 500 nm by using a microplate reader. TPAC of samples were expressed as mg catechin equivalents (CE)/g extract.

2.7 Determination of DPPH radical scavenging activity

A 190 µL of 0.1 mM DPPH in ethanol was added into 10 µL of sample (1 mg/mL). The reaction was performed in the dark at room temperature for 30 min and the absorbance was measured at 515 nm. Trolox was used as standard. Absorbance of sample were calculated to be % inhibition.

$$\% \text{ inhibition} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100$$

Where, A control = the absorbance of the control solution without antioxidant agent, A sample = the absorbance of sample to be tested. DPPH radical scavenging activity of samples were reported as mg trolox equivalent antioxidant capacity (TEAC)/g extract.

2.8 Determination of ABTS radical scavenging activity

The ABTS radical assay was adapted from Thaipong *et al.*, (2006). Briefly, ABTS radical solution was prepared by mixing 7 mM ABTS with 2.45 mM K2S2O8.Then, the ABTS working solution was prepared by mixing ABTS radical solution with 50 mM phosphate buffer (pH 7) at a ratio of 1:20 (v/v). A 10 µl of samples was added into 190 µL of the ABTS working solution. The reaction was performed in the dark at room temperature for 15 min and the absorbance was determined at 734 nm. Trolox was used as standard. Results were reported as mg Trolox equivalent antioxidant capacity (TEAC)/g extract.

2.9 Determination of ferric reducing antioxidant power (FRAP)

FRAP measurement of extract was adapted from Benzie and Stain (1996). FRAP solution was prepared by mixing 3 mL of 10 mM TPTZ (in 40 mM HCl), 3 mL of 3.2 mM $FeCl_3$ solution and 30 mL of 300 mM sodium acetate buffer (pH 3.6). A 10 µL of sample was added

into 190 µL of FRAP solution. The reaction was performed in the dark at room temperature for 15 min and the absorbance was measured at 593 nm. Trolox was used as standard. FRAP was calculated as mg trolox equivalent antioxidant capacity (TEAC)/g extract.

2.10 Formulation of cosmetic product

The base formula of gel product was developed (Table 1). Pomegranate extract that had the highest TPC, TFC and antioxidant activities was chosen as active ingredient. The physical property (e.g. Appearance, Color, Odor, pH of product) of gel product was determined.

Table 1. The base formula of gel product

Ingredients	%w/w
DI water	Q.S. to 100
Carbopol 940	0.5
Glycerin	2.0
Dimethicone	1.0
Cyclopentasiloxane	6.0
Butylene glycol	2.5
Propylene glycol	2.0
Tetrasodium EDTA	0.1
Pomegranate extract	5.0
Phenoxyethanol	0.2
Fragrance	0.2
Triethanolamine or citric acid	Q.S.

Note: *Q.S. stands for "quantity sufficient"

2.11 Statistical analysis

All obtained data were expressed as mean ± standard deviation. The data were statistically analysed by analysis of variance (ANOVA) and Tukey's HSD test for post-hoc analysis using IBM SPSS 21 for Windows. The comparison was considered at the significance level of $p < 0.05$.

3. Results and Discussion

3.1 Extraction yield and solubility of crude extract

The extraction yield of crude extracts from pomegranate fruit was shown in table 2. Pomegranate peel with extracted with acetone exhibited the highest yield, while pomegranate seed extracted with ethanol showed higher yield than that extracted with acetone extract. Pomegranate peel extract had higher yield than pomegranate seed extract in the same solvent (Table 2).

Table 2. Extraction yield of pomegranate peel and seed extracts

Part of plant	Solvent	% yield
Peel	Water	34.3
	Ethanol	26.1
	Acetone	50.0
Seed	Water	8.0
	Ethanol	11.9
	Acetone	10.0

3.2 Total phenol content (TPC)

TPC was significantly different between samples extracted from various solvents for each pomegranate part ($p < 0.05$) (Table 3). Acetone extract from pomegranate peel exhibited the highest TPC. Moreover, acetone extracts of seed and peel showed higher TPC than water and ethanol extracts when compared to the same part of plant. Furthermore, peel extract also had greater TPC than seed extract for each extraction solvent. The lowest TPC was observed in water extracts of peel and seed ($p < 0.05$).

3.3 Total flavonoid content (TFC)

TFC was significantly different between three various solvents for each pomegranate part ($p < 0.05$). Acetone extract of pomegranate peel exhibited the highest TFC as shown in Table 2. Among three extraction solvents extract, acetone extracts of pomegranate seed and peel had higher TFC than other extracts with the same part of plant ($p < 0.05$). While the lowest TFC was found in water extracts ($p < 0.05$). Pomegranate peel extract generally had higher TFC than seed extract, ($p < 0.05$). The tendency of TFC was similar to the results of TPC.

3.4 Total proanthocyanidins content (TPAC)

TPAC was significantly different between three various solvents for each pomegranate part ($p < 0.05$) as shown in Table 3. The highest TPAC was found in acetone extract of pomegranate peel. Similarly, pomegranate seed had lower TPAC than pomegranate peel extract when compared with the same solvent. Thus, pomegranate peel had higher proanthocyanidin content than pomegranate seed.

Table 3 Total phenolic, flavonoid and proanthocyanidin contents of pomegranate peel and seed extracts

Part of plant	Solvent	Bioactive compounds		
		Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)	Total proanthocyanidin content (mg CE/g extract)
Peel	Water	0.938 ± 0.014^{c}	0.147 ± 0.006^{b}	0.030 ± 0.007^{bc}
	Ethanol	1.011 ± 0.010^{b}	0.134 ± 0.005^{b}	0.048 ± 0.005^{b}
	Acetone	1.140 ± 0.007^{a}	0.249 ± 0.008^{a}	0.097 ± 0.006^{a}
Seed	Water	0.084 ± 0.002^{f}	0.027 ± 0.004^{d}	0.023 ± 0.006^{bc}
	Ethanol	0.136 ± 0.001^{e}	0.042 ± 0.009^{d}	0.018 ± 0.010^{bc}
	Acetone	0.175 ± 0.007^{d}	0.069 ± 0.004^{c}	0.001± 0.000^{e}

Note: Values are expressed as Mean ± S.D (n=5).

Different letters in the same column indicate significant difference at $p < 0.05$ (ANOVA, Tukey's HSD test).

Table 4 *In vitro* antioxidant activities of pomegranate peel and seed extracts

Part of plant	Solvent	Antioxidant activity		
		DPPH assay (mg TEAC /g extract)	ABTS assay (mg TEAC /g extract)	FRAP assay (mg TEAC /g extract)
Peel	Water	2.069 ± 0.019^{c}	1.169 ± 0.010c	6.060 ± 0.026c
	Ethanol	2.685 ± 0.036^{b}	0.000 ± 0.010e	6.983 ± 0.018^{b}
	Acetone	2.957 ± 0.002^{a}	0.000 ± 0.010e	7.078 ± 0.028^{a}
Seed	Water	0.034 ± 0.006^{e}	0.366 ± 0.010^{d}	0.560 ± 0.004^{f}
	Ethanol	0.066 ± 0.009^{e}	1.420 ± 0.021^{b}	0.935 ± 0.009^{e}
	Acetone	0.512 ± 0.024^{d}	3.320 ± 0.016^{a}	1.903 ± 0.013^{d}

Note: Values are expressed as Mean ± S.D (n=5).

Different letters in the same column indicate significant difference at $p < 0.05$ (ANOVA, Tukey's HSD test).

3.5 Antioxidant activities

3.5.1 DPPH assay

Both pomegranate seed and peel extract had varying DPPH radical scavenging activity between each solvent ($p < 0.05$) as shown in Table 4. Acetone extract pomegranate peel with exhibited the highest DPPH radical scavenging activity ($p < 0.05$). Comparison among three extraction solvents, acetone extract also had higher DPPH radical scavenging activity than other extracts with the same part of plant. Pomegranate peel extract showed higher DPPH radical scavenging activity than pomegranate seed extract.

3.5.2 ABTS assay

The tendency of ABTS result was slightly difference from bioactive compound results and DPPH activity result with significantly difference ($p < 0.05$) as shown in Table 4. Pomegranate seed with acetone extract exhibited the highest ABTS scavenging activity (3.320 ± 0.016 mg TEAC/g extract, $p < 0.05$) followed by pomegranate seed with ethanol extract (1.420 ± 0.021 mg TEAC/g extract, $p < 0.05$). While pomegranate peel with DI water extracts showed higher ABTS scavenging activity (1.169 ± 0.016 mg TEAC/g extract) than other solvent ($p < 0.05$), Pomegranate peel with acetone and ethanol extract had no ABTS scavenging activity.

3.5.3 Ferric reducing antioxidant power (FRAP)

The tendency of FRAP, DPPH and ABTS radical scavenging activities. Acetone extract of pomegranate peel demonstrated the highest FRAP ($p < 0.05$) (Table 4). Pomegranate extract with acetone extraction had higher FRAP than other solvent with the same part of plant. Furthermore, pomegranate peel extract showed higher FRAP than another pomegranate seed extract for all solvent extraction.

3.6 Formulation of cosmetic

According to bioactive compound and antioxidant activity results, the pomegranate peel with acetone extraction mostly showed higher values than other solvent extractions. Thus, it was chosen as active ingredient in gel product by adding in the base formula as shown in Table 1. The result showed that characteristics of gel product with acetone extract of pomegranate peel were clear and translucent when applied on the skin. This product also had light texture and good spreadability. The pH value of gel product was 5.5 which is appropriate to the skin. Therefore, pomegranate peel extract could be used as active ingredient for cosmetic products.

4. Conclusion

The results showed that the greatest TPC, TFC and TPAC were obtained from peel extract with acetone extraction. However, the tendency of anti-oxidant activity was slightly different from their bioactive contents. The highest DPPH radical scavenging activity was found in acetone peel extract while the greatest ABTS radical scavenging activity was noticeable in acetone seed extract. Moreover, FRAP was the highest in ethanol and acetone extracts of peel. Peel of pomegranate with acetone and ethanol extraction exhibited the highest bioactive compound and antioxidant activity. Thus, pomegranate waste especially peel might be used as alternative source of natural antioxidant in non-food products.

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