2012 International Conference on Environment, Chemistry and Biology
IPCBEE vol.49 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V49. 14

Bioactive Phenolic Compounds and Antioxidant Activity of Selected Fruit Peels

Khairusy Syakirah Zulkifli[1], Noriham Abdullah[1+], Aminah Abdullah[2], Nurain Aziman[1] and Wan Saidatul Syida Wan Kamarudin[1]

[1] Faculty of Applied Sciences Universiti Teknologi MARA 40450 Shah Alam, Selangor, Malaysia

[2] School of Chemical Science and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Abstract. Fruit peels are the major agro-industrial wastes in canned fruit manufacture and fruit juice processing which make it worth to investigate on their bioactive phenolic compounds. This study discussed on the total phenolic content (TPC), the quantification of eight individual bioactive phenolics using HPLC and evaluation of antioxidant activitu using DPPH scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assays on *Psidium guajava* (guava), *Mangifera indica* var. Chakonan (Chakonan mango), *Citrus sinensis* var. Navel (Navel orange) and *Malus sylvestris* (Granny Smith apple) peels. Results showed that fruit peels are significantly rich in phenolic acids, with gallic acid and chlorogenic acid present in considerably high concentrations. *M. indica* peel extract contained the highest total phenolic content with 537.70 mg GAE/ g extract weight and also possessed strongest antioxidant activity in both antioxidant assays. Results also showed strong correlations between the presence phenolics with the antioxidant activity. The study herein revealed that fruit peels represent an excellent source of high natural antioxidants, particularly phenolic acids which may be applied in pharmaceutical, food and cosmetic industries.

Keywords: Fruit peels, phenolic compounds, HPLC, antioxidant activity

1. Introduction

For the past decades, efforts have been made extensively to improve methods and find alternative ways to utilise fruits and vegetables wastes therapeutically. Until today, agro-industrial wastes often been used as animal feeds or fertiliser. Recently, scientists are able to develop high value products from these by-products such as cosmetics, medicines, and foods and the recovery seems to be economically attractive [1]. The idea of utilising fruit by-products mainly the peels, have slowly gaining popularity especially when researchers found that peels possessed better biological activities than other fractions [2].

Previously, several studies have highlighted the potential uses of fruit peels as a rich source of natural antioxidants mainly due to their phenolic constituents such as carotenoids, flavonoids, phenolic acids and anthocyanins [3]. Recent study also confirmed substantially higher amount of phenolic compounds and ascorbic acids in the peel than in pulp for most of the fruits [4]. Phenolic compounds in the peels play an important role as a protector for inner material from insects and microorganisms. They also contributed for the colour and appearance of the fruits [5]. With an increased pressure on finding alternative ways to utilise food waste, together with scarce information on individual bioactive phenolic compounds and huge research interest on antioxidant activity are expected to widen the opportunities for commercial exploitation.

The aim of this study is to assess total phenolic content, quantify individual bioactive phenolic compounds and evaluate antioxidant activities of local *Psidium guajava* (guava) and *Mangifera indica* var.

+ Corresponding author. Tel: +60123317037
E-mail address: noriham985@salam.uitm.edu.my

Chakonan (Chakonan mango) and also imported *Citrus sinensis* var. Navel (Navel orange) and *Malus sylvestris* (Granny Smith apple) peels.

2. Materials and Methods

2.1. Plants Material

Fresh fruits with no apparent physical or microbial damage were obtained from local market in Shah Alam, Selangor, Malaysia. Samples were local fruits, *Psidium guajava* (guava) and *Mangifera indica* var. Chakonan (Chakonan mango) as well as imported fruits of *Citrus sinensis* var. Navel (Navel orange) and *Malus sylvestris* (Granny Smith apple).

2.2. Extraction Procedure

Fresh peels were ground before boiled in water with 1:20 peel to water ratio. They were homogenized before filtered under vacuum with Whatman No. 1 filter paper. The supernatants were further concentrated at 60℃ by rotary vacuum evaporator (Buchi distillation R-210, Switzerland). The samples were lyophyilised (Christ Alpha 1-4 LD Plus, Germany) and kept at -20℃ until further use [6]

2.3. Determination of Total Phenolic Content (TPC)

Gallic acid was used as a standard with concentration ranged from 100 to 500 ppm. Both samples and standards were mixed with Folin-Ciocalteu reagent (1:1), 7.5% (w/v) sodium carbonate before measured using UV-Vis spectrophotometer at 760 nm absorbance [7]. Each measurement was repeated five times and expressed as mg gallic acid equivalent per g extract weight (mg GAE/g extract weight).

2.4. Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP assay was assessed using a UV-Vis spectrophotometer at 593 nm [8]. The sample extracts and standard were mixed with 8.7 mL of working FRAP reagent and incubated in the dark for 1 hour at 50℃ before analysed. FRAP reagent was freshly prepared by mixing 10 mM TPTZ solution with 20 mM $FeCl_3.6H_20$ and 300 mM acetate buffer (pH3.6) with ratio 10:10:100 and were incubated at 37℃ for 10 minutes. Calibration curve was prepared by using trolox as standard (100-500ppm). The results were expressed as millimolar per 100 gram extract weight (mM/100g).

2.5. DPPH Free Radical Scavenging Capacity

The sample extracts and standards (butylated hydroxylanisole and ascorbic acid) with various concentrations were mixed with ethanolic solution of DPPH [9]. After standing for 20 min in the dark, the mixtures were measured at 517 nm using UV-Vis spectrophotometer. The percentage of remaining DPPH against the sample and standard concentration was plotted to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50% (EC_{50}). Based on the EC_{50} parameter, the result was expressed in terms of μg extract per gram standard equivalent DPPH in the reaction medium.

2.6. HPLC Analysis of Phenolic Compounds

Sep-Pak C18 cartridge was preconditioned with methanol, acetonitrile and distilled water. After rinsing with acidified water and distilled water, extract with pH2.0 (acidified using 1 N HCl) was loaded into the cartridge. Acidified water and distilled water were added to wash the extract before eluted with methanol and acetonitrile. The elution was filtered with 0.45 μm Advantec filter and phenolic compounds were analyzed by using a Reversed Phase-HPLC instrument (Agilent 1200 model G1311A) equipped with a diode array detector [10]. The detection was performed at three different wavelengths: 280, 320, and 370 nm. The mobile phase consisted of acidified water as solvent A and acetonitrile as solvent B. The flow rate was 1.0 ml/min and the injection volume was 20 μL.

2.7. Statistical Analysis

Results were expressed as mean values ± standard deviation. Comparisons were performed by analysis of variance (ANOVA). Statistical analyses were run using SAS software. The correlations among the data were calculated using Pearson's correlation coefficient (r) and $P<0.05$ was considered as significantly different.

3. Results and Discussion

3.1. Total Phenolic Content of Selected Fruit Peels

The total phenolic content of the extracts were determined using Folin-Ciocalteu (FC) colorimetric method. The TPC of respective fruit peel extracts is shown in Table 1. *M. indica* peel exhibited the highest TPC while *M. sylvestris* showed the lowes. Meanwhile, there was no significant different between *C. sinensis* and *P. guajava*. Aqueous extraction were used due to their wide solubility selection and from toxicological point of view, water is much safer than other organic solvents such as methanol, acetone and chloroform. Phenolic compounds are mostly polar, thus increasing solvent polarity will increased the extraction yields [11]. In addition, boiling condition will increased the solubility of phenols and enhanced the breakdown of high molecular weight phenolics into free form which also led for the extraction of more polyphenols [5, 12].

Table 1. Total phenolic content and antioxidant activity of selected fruits peels

TYPES	TPC (mg GAE/g extract weight)	EC_{50} (mg/ml)	FRAP (mM/100g)
Citrus sinensis	277.00 ± 18.90^{C}	0.564 ± 0.006^{b}	20.03 ± 1.464^{c}
Malus sylvestris	216.11 ± 7.72^{D}	0.606 ± 0.008^{a}	14.48 ± 1.423^{d}
Mangifera indica	537.70 ± 10.01^{A}	0.138 ± 0.014^{d}	37.51 ± 0.621^{a}
Psidium guajava	499.88 ± 22.71^{B}	0.443 ± 0.007^{c}	33.41 ± 1.819^{b}
Ascorbic Acid	-	0.046 ± 0.001	-
BHA	-	0.115 ± 0.002	-
Trolox	-	-	44.45 ± 1.571

Values, mean ± SD (n = 5, determinations in triplicate). Data with different letters in the same column are significantly different at $p< 0.05$

3.2. Antioxidant activity of selected fruit peels

DPPH assay is used to determine the scavenging potential of antioxidant extract based on its capability as hydrogen donator and electron transfer. A lower value of EC_{50} corresponds to a higher antioxidant activity of the plant extract [13]. The scavenging activity of aqueous extracts were in the order of *M. indica* > *P. guajava* > *C.sinensis* > *M. sylvestris* (Table 1). The potent scavenging activities of all fruit peel extracts may be contributed by their phenolic compounds, as we managed to identify strong correlation with the total phenolic content ($r^2 = 0.723$).

The reducing antioxidant power of sample extract depends on its electron transfer ability towards the FRAP reagent [14]. Table 1 showed the FRAP value is in order of *M.indica* > *P. guajava* > *C. sinensis* > *M. sylvestris* where *M. indica* extracts possessed ferric reducing activity by almost three fold of activity as compared to *M. sylvestris* extract. Our findings however, showed significantly higher result than the previous work by other researchers where they reported that FRAP value for *P. guajava, M. indica* and *C. sinensis* peel extracts were 10.24 mM/100g, 10.13 mM/100g and 5.69 mM/100g respectively using water extraction [15] . Meanwhile, *M. sylvestris* peel extracts was in the ranged of 7.28 to 12 mM/100g by using ethanol as a solvent [16]. The FRAP values were found to be strongly correlated with the total phenolic content ($r^2 = 0.960$) indicating that most extracted phenolic compounds have good redox property.

3.3. Bioactive phenolic compounds in selected fruit peels

A total of eight phenolic compounds including four flavonoids, were indentified and quantified as shown in Table 2. The results showed that bioactive phenolic compounds varied in different plant tissues. Among the phenolics, gallic acid and chlorogenic acid present in high concentration whereas other compounds including flavonoids only present in trace amounts. The gallic acid content in fruit peels were in the order of: *M. indica* < *P. guajava* < *C. sinensis* < *M. sylvestris*. Previous studies have demonstrated the strong antioxidant activities possessed by gallic acid [17]. Some researchers claimed that the antioxidant activity of plants mainly depends on the amount of gallic acid present. Fruits are the main source of hydroxycinnamic acid and its derivatives, including ferulic, sinapic, p-coumaric, chlorogenic and caffeic acid [18]. Among them, chlorogenic acid was claimed to be the most important as they present in huge amount and sometimes it may be present as the predominat phenolic compound. As shown in Table 2, the amount of

chlorogenic acid in *M. indica* peel was found to be almost 10-fold higher than in *P. guajava* peel and it was also the predominant phenolics in *M. indica* and *M. sylvestris* peels. Previous studies also found chlorogenic acid as the most pronounced phenolics present in the peels of various apple, guava and mango cultivars [19, 20]. Meanwhile, other phenolic acids (ferulic and sinapic acids) were found highest in citrus fruits. Furthermore, ferulic acid is claimed to be the major constituents of citrus's phenolics [18].

Table 2. Individual bioactive phenolic compounds in the peels of selected fruits analysed by HPLC

Compounds (mg/100g)	*P.guajava*	*M. indica*	*M. sylvestris*	*C. sinensis*
Gallic Acid	131.13 ±6.43^{b}	152.20 ±0.14^{a}	42.56 ±0.42^{c}	127.74 ±1.64^{b}
Chlorogenic Acid	51.26 ±2.42^{d}	456.85 ±0.55^{a}	175.57 ±2.04^{b}	124.09 ±0.50^{c}
Sinapic Acid	1.05 ±0.48^{d}	22.31 ±1.02^{b}	1.80 ±0.12^{c}	430.6 ±0.46^{a}
Ferulic Acid	0.22 ±0.005^{d}	0.28 ±0.03^{c}	2.31 ±0.004^{b}	3.26 ±0.93^{a}
Myricetin	13.26 ±0.31^{c}	26.13 ±0.18^{b}	6.79 ±0.01^{d}	46.54 ±0.85^{a}
Quercetin	1.30 ±0.20^{b}	1.34 ±0.06^{b}	0.57 ±0.50^{c}	17.02 ±0.49^{a}
Naringenin	ND	ND	ND	1.27 ±0.40^{a}
Kaempferol	2.68 ±0.60^{c}	ND	17.04 ±0.01^{b}	26.71 ±3.03^{a}
Total	200.90 ±10.45^{d}	659.11 ±1.98^{a}	246.64 ±3.10^{c}	777.23 ±8.3^{b}

*The values of individual compounds were with the mean ± standard deviation (n=3). Data with different letter in the same row is significantly difference at the level $p < 0.05$.
* ND –Not detected

Several studies claimed that flavonoids are the main contributor for plant's antioxidant activity [21, 22]. From Table 2, among the fruit peels, *M. sylvestris* peel was found to contain the lowest amount of flavonoids (myricetin and quercetin). It has been known that 90% of total phenolics in apple peels consist of cinnamic acid derivatives and flavonols (catechin and epicatechin) [23]. The total phenolic compounds were in the order of *C. sinensis* > *M. indica* > *M. sylvestris* > *P. guajava* and weakly correlated with the antioxidant activities. As mentioned before, different phenolics exhibited different antioxidant potential, and other compounds may influenced the antioxidant activities [17, 18]. The low content of phenolic compounds especially gallic acid and quercetin in *M. sylvestris* peel may be the reason for its low antioxidant activities and on the other hand, the strong antioxidant activity possessed by *M. indica* peel may be contributed by the abundance of gallic acid and chlorogenic acid.

4. Conclusion

The study highlighted the composition of individual bioactive phenolic compounds varied among fruit peels analysed which indirectly influenced their antioxidant activity. This study showed that phenolic acids especially gallic acid and chlorogenic acids were the most abundant phenolic compounds present in the peel. Present study also showed that *Mangifera indica* peel had the most promising antioxidant agent as potent as the synthetic antioxidants BHA. Further work on the effect of fruits acidity and colour pigment on antioxidant activity is necessary.

5. Acknowledgement

The authors would like to acknowledge the Institutes of Postgraduates Studies and Faculty of Applied Sciences, Universiti Teknologi MARA, Malaysia, for the Excellent Fund and facilities provided to conduct this research.

6. References

[1] I. S. Ashoush and M. G. E. Gadallah. Utilization of Mango Peels and Seed Kernels Powders as Sources of Phytochemicals in Biscuit**.** *World Journal of Dairy & Food Sciences*. 2011, 6 (1): 35-42.

[2] Y.Y. Lim, T.T. Lim and J.J. Tee. Antioxidant properties of guava fruit: comparison with some local fruits. *Sunway Academic Journal.* 2006. 3: 9–20.

[3] J.K. Moon and T. Shibamoto. Antioxidant assays for plant and food components. *J. Agric. Food Chem.* 2009, 57: 1655–1666.

[4] V. Goulas and G.A. Manganaris. Exploring the phytochemical content and the antioxidant potential of Citrus fruits grown in Cyprus. *Food Chem*. 2012, 131: 39–47.

[5] S.M. Jeong, S.Y. Kim, D.R. Kim, S.C. Jo, K.C. Nam, D.U. Ahn and S. Lee. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *J. Agric. Food Chem.* 2004, 52: 3389-3393

[6] P.D. Duh, Y.Y. Tu and G.C. Yen. Antioxidant activity of aqueous extract of harn jyur (*Chrysanthemum morifolium* Ramat), *Lebensmittel-Wissenschaft Technol.* 1999, 32: 269-277.

[7] V.L. Singleton and J.A. Rossi. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vit.* 1965, 37:144-158.

[8] I.F.F. Benzie and J.J. Strain. The ferric reducing ability of plasma (FRAP) as a measure of 'Antioxidant Power': The FRAP assay. *Anal. Biochem.* 1996, 239: 70-76.

[9] D. Heimler, P. Vignolini, M.G. Dini and A. Romani. Rapid tests to assess the antioxidant activity of *Phaseolus* vulgaris L. dry beans. *J. Agric. Food Chem.* 2005, 53: 3053-3056.

[10] A.A. Boligon, T.F. De Brum, J.K. Frolhich, A.L.F. Froeder and M.L. Athayde. HPLC/DAD profile and determination of total phenolics, flavonoids, tannins and alkaloids content of Scutia buxifolia Reissek stem bark. *Res. J. Phytochem.* 2012, 6(3): 84-91.

[11] W. Boonchum, Y. Peerapornpisal, D. Kanjanapothi, J. Pekkoh, C. Pumas, U. Jamjai, D. Amornlerdpison, T. Noiraksar and P. Vacharapiyasophon.. Antioxidant activity of some seaweed from the gulf of Thailand. *Int. J. Agric. Biol.* 2011, 13: 95-99.

[12] R. Sathishkumar, P.T.V. Lakshmi and A. Annamalai. Effect of drying treatment on the content of antioxidants in Enicostemma *littorale* blume. *Res. J. Med. Plants*. 2009, 3: 93-101.

[13] P. Maisuthisakul, R. Pongsawatmanit and M.H. Gordon. Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (*Cratoxylum formosum Dyer*). *Food Chem.* 2007, 100: 1620-1629.

[14] Y.C. Chung, C.T. Chang, W.W. Chao, C.F. Lin and S.T. Chou. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis*. *J. Agric. Food Chem.* 2002, 50: 2454–2458.

[15] C. Guo, J. Yang, J. Wei, Y. Li, J. Xu and Y. Jiang. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutr. Res.* 2003, 23: 1719–1726.

[16] A. Valavanidis, T. Vlachogianni, A. Psomas, A. Zovoili and V. Siatis. Polyphenolic profile and antioxidant activity of five apple cultivars grown under organic and conventional agricultural practices. *Int. J. Food Sci. Tech.* 2009, 44: 1167–1175.

[17] X.N. Chen, J.F. Fan, X. Yue, X.R. Wu, and L.T. Li. Radical Scavenging Activity and Phenolic Compounds in Persimmon (*Diospyros kaki* L. cv. Mopan*). J. Food Sci.* 2008, 73: 24-28.

[18] K. Robards, P.D. Prenzler, G.Tucker, P. Swatsitang and W. Glover. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem*. 1999, 66: 401-436.

[19] R. Veberic, M. Trobec, K. Herbinger, M. Hofer, D. Grill2 and F.Stampar. Phenolic compounds in some apple (*Malus domestica* Borkh) cultivars of organic and integrated production. *J. Sci. Food Agric.* 2005, 85:1687–1694

[20] S. Kondo, M. Kittikorn and S. Kanlayanarat. Preharvest antioxidant activities of tropical fruit and the effect of low temperature storage on antioxidants and jasmonates. *Postharvest Bio. Technol.* 2005, 36: 309-318

[21] H.M. Merken and G.R. Beecher. Measurement of food flavonoids by high-performance liquid chromatography: a review. *J. Agric. Food Chem.* 2000, 48(3): 577-599.

[22] O.K. Chun, S.J. Chung and W.O. Song. Estimated dietary flavonoid intake and major food sources of U.S. adults. *J. Nutr.* 2007. 137: 1244–1252.

[23] M.J. Amiot, M. Tacchini, S. Aubert, and J. Nicolas. Phenolic composition and browning susceptibility of various apple cultivars at maturity. *J. Food Sci*. 1992, 57: 958-962.