Comparative Study on the Extraction of Bioactive Secondary Metabolites from Pomelo and Pineapple Peels Extract

O Nurdalilah\textsuperscript{1,a)}, Y P Teoh\textsuperscript{1,2,b)}, Z X Ooi\textsuperscript{3,c)} and S T Sam\textsuperscript{4,d)}

\textsuperscript{1}Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Malaysia Perlis (UniMAP), P.O Box 77, D/A Pejabat Pos Besar, 01000 Kangar, Perlis, Malaysia.
\textsuperscript{2}Department of Petrochemical Engineering, Faculty of Engineering and Green Technology, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia.
\textsuperscript{3}Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia.
\textsuperscript{4}School of Bioprocess Engineering, Universiti Malaysia Perlis (UniMAP), Kompleks Pusat Pengajian Jejawi 3, 02600 Arau, Perlis, Malaysia.

\textsuperscript{a)}nurdalilahothman@gmail.com
Corresponding author: \textsuperscript{b)}teohyp@utar.edu.my
\textsuperscript{c)}zhongxian.ooi@gmail.com
\textsuperscript{d)}stsam@unimap.edu.my

\textbf{Abstract.} In this study, a phytochemical analysis and in vitro analysis of antioxidant assay conducted for two different types of fruit peels extract which are pomelo peel (PP) and pineapple peel (PIP). For phytochemical analysis, total phenols content (TPC), total flavonoids content (TFC) and total tannins content (TTC) and for antioxidant assay, DPPH radical scavenging activity and ferric reducing ability power (FRAP) were determined using spectrophotometric methods. From the analysis, it was found that PP extract rich in TPC, TFC, TTC, DPPH antioxidant scavenging activity and FRAP assay compared to PIP with the values 6.3133 mg GAE/g of extract, 4.6675 mg QAE/g of extract and 0.1593 mg GAE/g of extract respectively. For antioxidant assays, PP extract showed higher percentage of antioxidant scavenging activity compared to PIP extract with 54.95% and also the increase in FRAP when the concentration of extract increased. Thus, PP was chosen to be conducted in a green synthesis of nanoparticles which can be applied in either pharmaceutical or environmental fields.

\section{1. Introduction}
A fast-growing economic in emerging-developing countries has encouraged an increased solid waste generation in that specific zone. Along with the economic development and business movement and consumption rate, daily generation and volume rate of municipal solid waste will accelerate. It has been proven that the higher advancement in economics, the higher the amount of municipal solid waste produced \cite{1}. Approximately, 30000 tons of municipal solid wastes are generated daily, covering 83\% of the country’s waste generation, including agro wastes \cite{2}. It is estimated that 15\% of
the total waste generated in Asia is agro waste, with agricultural waste generation in Malaysia at approximately 0.122 kg/cap/day in 2009 which is projected to reach 0.210 kg/cap/day by 2025 [3].

Global experienced a remarkable upsurge in production of fruit. Past decade, about 3% of the output has been growing at an annual rate. Malaysia besides Thailand, India and China recorded as one of the larger fruit production in Asia and predicted to increase in next few years ahead. Mohamed (2010) reported that 8.5% of Gross Domestic products contributed by agriculture came from industrial crops with 61% and agriculture food crops with (39%) respectively [4]. For the agriculture crops, they are divided into two categories which are crop residues and agro-industrial residues. Peels, husks hulls, bagasse are the example of agro-industrial residues. In Malaysia, the agricultural land covered only 20% for cultivated food crops while the fruit processing produced a great quantity of waste at remaining 80% land were poorly harvested or left to decay on the land.

Due to large amount of water content in agro waste, they are in wet and easily to be fermentable. These agro wastes may lead to the harbourage for insects and few types of pollutions such as odor, soil and serious environmental problems if they were no further processed. and also can contribute to serious environmental pollution if not processed further [5]. Besides, the statistical data provided from Fruit and Agricultural Statistics recorded Malaysia produced about 16% of pineapple and 2% of grapefruit include pomelo while the others are watermelon(13%), oranges(4%), banana (31%) and tropical fresh fruits include durian and rambutan (26%) and mangoes, mangoosteen and guavas (8%) [6]. Non-edible plants produced are believed to be rich in bioactive secondary metabolites and they are cheap renewable feedstocks [7].

Bioactive secondary metabolites also known as bioactive compounds which other than primary metabolites are believed help to increase plants overall ability to survive and to overcome local challenges by allowing them to interact with surrounding [8]. Edible and non-edible plants such as vegetables, fruits, herbs and other plant materials are known to be rich in bioactive secondary metabolites. Seeds, peels, husks and among others are generated every year in the form of wastes and are poorly harvested or left to decay on the land. These bioactive secondary metabolites can be divided in three main categories which are terpenes and terpenoids (approximately 25 000 types, alkaloids (approximately 12 000 types and phenolic compounds (8000 types) and can be extracted using conventional or non-conventional extraction techniques [9].

Recently, researchers were found and able to develop high value added products such as cosmetics and medicines from these by-products and it seems that the recovery was to be economically attractive [10]. The intention of utilizing fruit wastes have slowly gaining popularity mainly the peels which some fruits represents almost 30% of the total weight. More recently, food enriched with fruit peels have been developed since the increasing interest natural sources of bioactive secondary metabolites as approach in functional food. However, the potential of the fruits peels are highly depended on their chemical composition. Hence in this study, the comparative study had been investigated on the pomelo peels (Citrus maxima) and pineapple peels (Ananas comusus) with the aim of exploiting the potential of high value products from these peels.

2. Procedure

2.1. Materials
Agricultural residues such as pomelo peels (PP) and pineapple peels (PIP) were obtained from a local food stall at Kangar, Perlis, Malaysia.

2.2. Chemicals
Methanol was purchased from Merck, n-hexane, Folin-Ciocatue reagents, sodium carbonate (Na₂CO₃), gallic acid (C₆H₆O₇), COOH), potassium ferricyanide (K₃[Fe(CN)]., tannic acid (C₇₆H₅₀O₃₅), sodium nitrite (NaNO₂), aluminium chloride hydrate (AlCl₃.2H₂O), quercetin (C₃₄H₄₆O₁₁), 1-diphenyl-2-picrylhydrazyl (DPPH), sodium phosphate buffer (NaH₂PO₄), trichloroacetic acid (C₂HCl₃O₇) and ascorbic acid (C₆H₈O₆) were purchased from Bendosen.
2.3. **Sample Pre-treatments**

Agricultural residues collected and washed, dried in oven for 24 hours at 60 °C. The sample then were ground to 125 µm were collected and defatted using n-hexane (ratio of solid/liquid 1/10, w/w) at room temperature and then the solvent was evaporated.

2.4. **Extraction of Fruit Peels**

The extraction of phenolic compounds was carried out using solvent 80% methanol. The samples were added to a solvent 1:10 mixed well and kept at room temperature, for 3 days under constant stirring. The mixture was centrifuged at 6,000 rpm for 15 min and the supernatant was filtered through a filter paper. Then, the solvent was evaporated in a rotavapor. The extraction yield was expressed as dry matter percentage.

2.5. **Total Flavonoids Content (TFC)**

Total phenols content determined using method described by Stankovi (2011) [11]. The sample was re-dissolved in the extraction medium. To 0.5 ml methanolic extract, 2.5 ml 10% of Folin - Ciocalteu reactive, and 2.5 ml of 7.5 % aqueous solution of Na₂CO₃ were added. The mixture was kept for 30 min in the dark at room temperature. The absorbance was read at 765 nm using a UV/Vis spectrophotometer (UV-6450; Jenway, UK). Gallic acid (10 - 50 ppm) were used for constructing the standard curve, and the results were expressed as g of gallic acid equivalents (GAE)/100 g of extract.

2.6. **Total Tannin Content (TTC)**

Total tannins content determined by using method described in Tambe and Bhambar (2014) [12]. 0.1 ml methanolic extract, 0.5 ml of Folin - Ciocalteu reactive, 7.5 ml distilled water and 1 ml of 35 % aqueous solution of Na₂CO₃ were added in test tube. Then 0.9 ml of distilled water was added. The mixture kept for 30 min in the dark at room temperature. The absorbance read at 725 nm using a UV/Vis spectrophotometer (UV-6450; Jenway, UK). Gallic acid (20 - 100 ppm) was used for constructing the standard curve, and the results were expressed as g of gallic acid equivalents (GAE)/100 g of extract.

2.7. **DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay**

DPPH radical-scavenging activity were performed by the method described by Atker (2010) [13]. For each determination, the stock solution (1mg/ml) was diluted with solvent. An aliquot of each dilution (0.5 mL) was mixed with methanolic solution of DPPH (5 mL, 0.06 mM). The mixtures were shaken vigorously and incubated at 37 °C in the dark for 30 min. At the same time, a control containing solvent (0.5 mL) and methanolic solution of DPPH (5 mL, 0.06 mM) were run. The absorbance measured at 517 nm against methanol as a blank. The percentage of DPPH scavenging was calculated as follows: DPPH radical scavenging activity (%) = [(Abscontrol - Abssample) / Abscontrol] x 100.

2.8. **Ferric Reducing Ability Power (FRAP) Assay**

Ferric Reducing Ability Power determined by using method described Barku et al., (2014) [14]. In 2.5 ml of methanolic extract at various concentration (31.25 -1000 ppm) were mixed to 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixture were placed in water bath at 50 °C for 20 min. Then 2.5 ml of 10 % TCA (w/w) was added to mixture. The mixtures were centrifuged at 3000 rpm for 10 minutes. This will follow by the addition of 2.5 ml of distilled water and 0.5 ml of 0.1 % of ferric chloride. The absorbance recorded at 700 nm after 5 min. The antioxidant activity was calculated from the calibration curve of ascorbic acid (31.25 – 1000 ppm). Increased absorbance of the mixture indicates increased reducing power.
3. Results and Discussion
In this recent year, a great attention has been focused on the agro-industrial wastes, especially those containing residual phenols from used plant raw material. PP and PIP are example of the important dietary sources of antioxidant properties [12, 13].

Calorimetric analysis of phenols, flavonoids and tannins content indicated that the methanolic extract of PP had highly amounts of TPC, TFC and TTC compared to PIP and this in agreement with Romelle et al., (2016) who studied the chemical composition of selected fruit peels [17]. The polyphenols, flavonoids and tannins content in PP extract was $6.3133 \text{ mg/g}$ of dry weight of extract, expressed as gallic acid equivalents, $4.6675 \text{ mg/g}$ of dry weight of extract, expressed as quercetin equivalents and $0.1593 \text{ mg/g}$ of dry weight of extract, expressed as gallic acid equivalents respectively. A significant lower of the TPC, TFC and TTC in WP which the values were $1.6205 \text{ mg/g}$ of dry weight of extract, expressed as gallic acid equivalents, $0.2675 \text{ mg/g}$ of dry weight of extract, expressed as quercetin equivalents and $0.0164 \text{ mg/g}$ of dry weight of extract, expressed as gallic acid equivalents respectively. The $r^2$ for standard curve of gallic acid in TPC is 0.9997, standard curve of quercetin in TFC is 0.9996 while for standard curve of gallic acid in TTC is 0.9918. The standard curve for TPC, TFC and TTC showed in Figure 1, Figure 2 and Figure 3 respectively. In biosynthesizing of nanoparticles, phenolic compounds play an important role for reduction and stabilization [18].

![Total Phenols Content (TPC)](image1.png)

**FIGURE 1.** Graph Absorbance at 765 nm (Abs) against Concentration of Gallic Acid ($\mu$g/ml).

![Total Flavonoids Content (TFC)](image2.png)

**FIGURE 2.** Graph Absorbance at 415 nm (Abs) against Concentration of Quercetin ($\mu$g/ml).
The antioxidant scavenging activity through the reaction between antioxidant molecules and radical results in the scavenging of the radical by hydrogen donation caused the decrease in absorbance of DPPH radical. In this research, the PP extract was found to be higher in DPPH scavenging activity with 54.95% while in PIP was only 26.24%. Table 1 showed there is a significant linear correlation was confirmed between the phenolic contents and the antioxidant scavenging activity of the fruit peels extract. The highest content of phenolic compounds indicated that these compounds contribute to the higher antioxidant scavenging activity [15, 16].

TABLE 1. TPC, TFC, TTC and DPPH Antioxidant Assay in Fruit Peels Extract.

| Fruits         | TPC (g GAE/mg of extract) | TFC (g QAE/mg of extract) | TTC (g GAE/mg of extract) | DPPH antioxidant assay (%) |
|----------------|---------------------------|--------------------------|--------------------------|----------------------------|
| Pomelo peels (PP) | 6.3133                    | 4.6675                   | 0.1593                   | 54.95                      |
| Pineapple peels (PIP) | 1.6205                    | 0.2675                   | 0.0164                   | 26.24                      |

The PP extract showed the highest ferric reducing power ability compared to PIP extract at various concentration. For the iron reducing power assay, it was found that there was a tight relationship between the amount of phenolic contents and ferric reducing power ability. There is also a linear relationship between the phenolic contents and ferric reducing ability power [21]. Other than that, previous results showed higher in antioxidant scavenging activity also results in higher ferric reducing power ability power of bioactive secondary metabolites. These tight relationship of antioxidant properties have a good agreement with [18, 19]. Thus, higher in phenolic contents also results in antioxidant scavenging activity as well as ferric reducing ability power. However, in this studied, the ability of reducing power of the methanolic extracts for both PP and PIP were significantly lower than the synthetic antioxidant of standard ascorbic acid shown in Figure 4.
4. Conclusion
As the conclusion, it could be concluded that pomelo peels extract has higher TPC, TFC, TTC, DPPH scavenging activity and also ferric reducing power ability compared to pineapple peels extract. Besides the phenolic contents in peel itself, methanol act as solvent which has the highest polar solvent also played a vital role in extraction process. From the collected data for this analysis, it can be concluded that pomelo peel extract has potential to be conducted for further research mainly in synthesizing nanoparticles which these high value-added products can be applied either in pharmaceutical, food and also environmental scopes.

Acknowledgements
The authors would like to acknowledge the financial support from the Fundamental Research Grant Scheme (FRGS) under a grant number of FRGS/1/2015/TK02/UNIMAP/02/05 (Grant Account No.: 9003-00528) from the Ministry of Higher Education.

References
[1] C. Riber, “Waste to Energy in Malaysia,” Ramboll Waste-to-Energy, 2012.
[2] S. H. Fauziah and P. Agamuthu, “Municipal Solid Waste Management in Malaysia: Strategies in Reducing The Dependency on Landfills,” J. Chem. Inf. Model., vol. 53, p. 160, 2012.
[3] C. Bories, M. E. Borredon, E. Vedrenne, G. Vilarem, and P. Agamuthu, “Challenges and Opportunities in Agro-waste Management: An Asian Perspective What is AgroWaste?,” J. Environ. Manage., vol. 143, pp. 186–196, 2009.
[4] M. S. Mohamed and M. Y. Rokiah, “Tropical fruits and vegetables in Malaysia: Production and impact on health,” no. August 2006, pp. 1–5, 2010.
[5] R. Shalini and D. K. Gupta, “Utilization of pomace from apple processing industries: A review,” J. Food Sci. Technol., vol. 47, no. 4, pp. 365–371, 2010.
[6] E. Vladimir and E. K. Joseph, “Production Statistics - Crops, Crops Processed,” 2011.
[7] S. M. Omar, G. C. Azucena, and S. V. Raul, “Agricultural residues as a source of bioactive natural products,” Phytochem. Rev., vol. 11, no. 4, pp. 447–466, 2012.
[8] S. Vijayalaxmi, S. K. Jayalakshmi, and K. Sreeramulu, “Polyphenols from different agricultural residues: extraction, identification and their antioxidant properties,” J. Food Sci. Technol., vol. 52, no. May, pp. 1–9, 2014.
[9] J. Azmir, I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, F. Sahena, M. H. A. Jahurul, K. Ghafoor, N. A. N. Norulaini, and A. K. M. Omar, “Techniques for extraction of bioactive compounds from plant materials: A review,” J. Food Eng., vol. 117, no. 4, pp. 426–436, 2013.
[10] I. S. Ashoush and M. G. E. Gadallah, “Utilization of Mango Peels and Seed Kernels Powders as Sources of Phytochemicals in Biscuit,” *World J. Dairy Food Sci.*, vol. 6, no. 1, pp. 35–42, 2011.

[11] M. S. Stankovi, “Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts,” *Kragujev. J. Sci.*, vol. 33, no. July, pp. 63–72, 2011.

[12] V. D. Tambe and R. S. Bhambar, “Estimation of total phenol, tannin, alkaloid and flavonoid in hibiscus tiliaeuid linn. wood extracts,” vol. 2, no. 4, pp. 41–47, 2014.

[13] M. S. Akter, M. Ahmed, and J. B. Eun, “Solvent effects on antioxidant properties of persimmon (Diospyros kaki L. cv. Daebong) seeds,” *Int. J. Food Sci. Technol.*, vol. 45, no. 11, pp. 2258–2264, 2010.

[14] V. Y. A. Barku, B. Y. Opoku, A. E. Owusu, and E. F. Mensah, “Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of Amaranthus spinosus,” *Asian J. Plant Sci. Res.*, vol. 3, no. 1, pp. 69–74, 2013.

[15] U. K. Ibrahim, N. Kamarrudin, M. U. H. Suzihaque, and S. A. Hashib, “Local Fruit Wastes as a Potential Source of Natural Antioxidant: An Overview,” 2016.

[16] W. Suttirak and S. Manurakchikorn, “In vitro antioxidant properties of mangosteen peel extract,” *J. Food Sci. Technol.*, vol. 51, no. 12, pp. 3546–3558, 2014.

[17] F. D. Romelle, A. Rani, and R. S. Manohar, “Chemical composition of some selected fruit peels,” *Eur. J. Food Sci. Technol.*, vol. 4, no. 4, pp. 12–21, 2016.

[18] A. Dzimitrowicz, P. Jamróz, G. C. diCenzo, I. Sergiel, T. Kozlecki, and P. Pohl, “Preparation and characterization of gold nanoparticles prepared with aqueous extracts of Lamiaceae plants and the effect of follow-up treatment with atmospheric pressure glow microdischarge,” *Arab. J. Chem.*, 2016.

[19] G. Piluzza and S. Bullitta, “Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area,” *Pharm. Biol.*, vol. 49, no. 3, pp. 240–7, 2011.

[20] M. M. Rashad, A. E. Mahmoud, M. M. Ali, M. U. Nooman, and A. S. Al-Kashef, “Antioxidant and anticancer agents produced from pineapple waste by solid state fermentation,” *Int. J. Toxicol. Pharmacol. Res.*, vol. 7, no. 6, pp. 287–296, 2015.

[21] N. Babbar, H. S. Oberoi, D. S. Uppal, and R. T. Patil, “Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues,” *Food Res. Int.*, vol. 44, no. 1, pp. 391–396, 2011.

[22] J. A. Larrauri, P. Rupérez, L. Bravo, and F. Saura-Calixto, “High dietary fibre powders from orange and lime peels: Associated polyphenols and antioxidant capacity,” *Food Res. Int.*, vol. 29, no. 8, pp. 757–762, 1996.

[23] Y. Q. Ma, J. C. Chen, D. H. Liu, and X. Q. Ye, “Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel,” *J. Food Sci.*, vol. 73, no. 8, 2008.