Malaysian Applied Biology (2023) 52(4): 119-125
https://doi.org/10.55230/mabjournal.v52i4.m024

Research

Flavonoid Content of *Phaleria macrocarpa* Fruit and Its Proximate Compositions

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ABSTRACT

Flavonoids are one of the compounds in phenolic compounds in fruits. Flavonoids have been documented to modulate or modify lipid peroxidation, free radical scavenging activity, and inhibition of hydrolytic and oxidative enzymes. Flavonoids also influence anti-inflammatory action, anti-tumour, anti-hyperglycemia, anti-viral, anti-microbial, and anti-fungal effects. In this research, flavonoid content in *P. macrocarpa* fruits was determined, as well as its proximate compositions. To extract flavonoids in the fruit, *P. macrocarpa* fruits were extracted by the Soxhlet extraction method using aqueous as a solvent. Total flavonoid content in *P. macrocarpa* fruit extract was 89.89 ± 3.71 mg QE/100 mL. Proximate analyses were conducted to determine the fruit's moisture content, ash content, dry matter, crude protein, crude fibre, and crude essential oil. Results obtained for proximate composition were 9.45 ± 2.67% (crude protein), 21.633 ± 1.17 (fibre), and 5.605 ± 0.88 (essential oil). Moisture content in this fruit was 88.401 ± 0.749%, the dry matter was 10.96%, and the ash content was 6.33 ± 3.72%. FTIR analysis shows the extract's functional spectra of phenol, alkane, alkene, and alkyne groups.

Key words: Antioxidants, flavonoid, FTIR, moisture content, *Phaleria macrocarpa*, phenol

Article History
Accepted: 24 July 2023
First version online: 31 October 2023

Cite This Article:
Abd Gani, S.S., Noor Ezzuddin, N.N., Zaidan, U.H., Halmi, M.I.E., Abd Halim, A.N. 2023. Flavonoid content of *Phaleria macrocarpa* fruit and its proximate compositions. Malaysian Applied Biology, 52(4): 119-125. https://doi.org/10.55230/mabjournal.v52i4.m024

INTRODUCTION

Phaleria macrocarpa (Scheff.) Boerl or *Phaleria papuana* Warb var. *Wichmannii* (Val) Back is a popular herbal plant species with medicinal properties. The species originates in Indonesia, locally known as *Mahkota Dewa, Crown of God,* and *Pau*. The species is from the family Tymelacea and is commonly found in the topical areas of Papua Island (Anggraini *et al*., 2015). Although the species originates from Indonesia, due to similarities in climatic conditions, *P. macrocarpa* can also be easily found and cultivated in Malaysia. *P. macrocarpa* can grow into a small tree with a stem, leaves, flowers, and fruits. According to Anggraini *et al*., 2015 the plant's normal height is 1 to 6 m, with a productive age ranging from 10 to 20 years. The fruits are green when unripe and turn red once ripened. One to two brown and anatropous seeds exist in a fruit (Altaf *et al*., 2013). It was documented that *P. macrocarpa* fruits contain various chemical constituents, including saponin, flavonoid, polyphenol, alkaloid, and mangiferin (Kim *et al*., 2010; Altaf *et al*., 2013).

Foods consist of different chemical substituents and compositions. Three major compounds that can be found in foods are; carbohydrates, lipids, and proteins. Other compounds such as minerals, water, enzymes, pigments, flavouring substances, and vitamins are also present. Food water content varies according to the amount of moisture

in the foods. Food moisture content is an important factor in the stability of the extract. The period for the plant to deteriorate highly depends on its moisture content. Fats or lipids contain the most concentrated source of dietary energy. These compounds are responsible for the food's flavour, odour, and texture. Common animal fat sources are beef, while plants are soybean, corn, olive, and sunflower (Prakash *et al*., 2012). Fatty acids are oil's main components, important in multidimensional functions such as metabolic and structural activities. For example, in *P. macrocarpa* fruits, essential oils such as octadecane triclosan, dioctyl ester, and tributyl acetyl citrate are present (Azmir *et al*., 2014).

Deep-coloured vegetables and fruits are good resources for phenolics, including flavonoids, anthocyanins, and carotenoids. For example, the colour of ripe *P. macrocarpa* fruits is vibrant red, which might be due to high flavonoid content. More than 4000 known flavonoids, one class of plant polyphenols, have been found. Dietary flavonoids are usually glycosylated. They can be classified as anthocyanidins, flavanols, flavones, flavanones, and flavonols responsible for orange, red and blue-coloured fruits and vegetables (J. Y. Lin & Tang, 2006). Recent studies have focused on the health functions of phenolics, including flavonoids and anthocyanins (J. Y. Lin & Tang, 2006), (Fidrianny *et al*., 2013), (Qian *et al*., 2004), (B. F. Lin *et al*., 2005), and (Merken & Beecher, 2000).

In this analysis, a proximate analysis of *P. macrocarpa* fruit was done, and the flavonoid content of *P. macrocarpa* fruit extract was determined.

MATERIALS AND METHODS

Materials

All solvents and chemical reagents used to determine proximate analysis in this study were analytical grade and purchased from R&M (UK). These chemicals were petroleum benzene ($C_{10}H_8O_4$), sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), and Kjedahl tablets. In determining the flavonoid content, the chemicals were purchased from Sigma-Alderich (M) (Subang Jaya, Malaysia), including aluminium chloride ($AlCl_3$), quercetin, and ascorbic acid.

Sample collection and preparation

Fresh ripe fruits with the vibrant red skin colour of *P. macrocarpa* were collected from a local farm in Bachok, Kelantan, Malaysia. Collected fruits were washed until no dirt was observed with running tap water and cut thinly before being dried in the oven for 1 week at 60 °C drying oven. Dried *P. macrocarpa* were then ground to form a fibrous sample. Three grams of sample were used for extraction using the Soxhlet extraction method. The extraction was carried out for six hours using water as a solvent. Finally, the crude extract obtained was subjected to spray-dried using NIRO Spray Dryer for further analysis.

Proximate analysis composition of P.macrocarpa

In each analysis test, fresh ripe fruits were used and carried out in triplicate as below. The proximate analysis method used a laboratory manual practical from Veterinary Nutrition (VPP3130).

Dry matter content

Porcelain crucibles were appropriately labelled and placed in the oven at 105 °C for 30 min. The heated crucible was then cooled for 20 min and weighed. Approximately 3 g of fresh fruits were placed into the crucible, and initial weights were collected. The samples were then dried in the oven for 24 h at 105 °C. After the dried samples were cooled, the final weight was recorded and kept in the incubator for ash content analysis. Dry matter content was calculated using Equations 1 and 2.

Equation 1:

$$\text{Percentage of moisture } (\%) = \frac{W_f}{W_i} \times 100$$

W_i = weight sample initial (g)
W_f = weight sample final (g)

Equation 2:
Dry matter (%) = 100 - % Moisture.

Ash content

The remaining dried sample from the previous analysis was placed into the furnace at 550 °C for 4 h. The porcelain containing ashes was allowed to cool in the desiccator, and the final weight was recorded. The percentage of ash content was calculated using Equations 3 and 4.

Equation 3:

$$\text{Percent ash (wet) (\%)} = \frac{(\text{crucible and ash} - \text{empty crucible})}{(\text{crucible and sample} - \text{empty crucible})} \times 100$$

Equation 4:

$$\text{Percent ash (dry) (\%)} = \left(\frac{\text{\% ash wet}}{100}\right) - \text{\% moisture}$$

Crude protein content

Digestion and analysis for protein content were conducted according to Kjedahl's method with some modifications. First, 0.5 g of ground sample was weighed and placed in Kjedahl's flasks. Then, a tablet of catalyst and 12 mL of concentrated H_2SO_4 was added into each flask containing the sample and catalyst and allowed to stand overnight. The following day, the sample was digested using a digestor (FOSS Tecator Digestor Auto, Sweden) with an initial temperature set at 150 °C. After 30 minutes, the temperature was increased to 400 °C for 2 h. The solution was then let to cool until no acid vapour was observed. The crude protein of *P. macrocarpa* was analysed using KjeltecTM 2400 (FOSS, Sweden), and the percentage of crude protein was immediately calculated. Samples were prepared in triplicates.

Crude fibre content

Crude fibre analysis was done using the ashing method. The sample weighed approximately 1.5 g and was transferred into a Berzelius beaker. Approximately 150 mL of H_2SO_4 (0.13M) was added to coat the sample. The beaker containing the sample was heated gently for 30 min. The sample was then filtered and rinsed thoroughly with distilled water. The washed sample, including filter paper, was transferred to the same beaker and re-boiled gently for 30 min using 150 mL of NaOH (0.313M). Subsequently, the sample was filtered using a pre-weighed sintered glass crucible. The sample was washed several times with distilled water and acetone once. The sintered glass contained sample was then placed in the oven overnight at 100 °C. The cooled crucible was then placed into the furnace for ashing for 4 h at 520° C. The weight of the ash obtained was recorded, and the crude fibre obtained was calculated using Equation 5:

$$\text{Crude fibre (\%)} = \frac{\text{weight after ashing} - \text{weight after drying}}{\text{weight of sample}} \times 100$$

Crude essential oil

Crude oil content was determined using the Soxhlet method with minor modifications. Empty round bottom flasks were weighed, washed, and dried in the oven for 1 h at 105 °C. Approximately 3 g of the sample was weighed and placed in a thimble for essential oil extraction. The oil extraction was carried out for 4 h using $C_{10}H_8O_4$ as solvent. To obtain crude oil, solvent and oil was separated using a rotary evaporator until all the solvent were separated. Flask-contained oil was weighed, and the percentage of essential oil contained was calculated using Equation 6:

$$\text{Crude oil (\%)} = \frac{W_f - W_o}{W_s} \times 100$$

W_f = weight of round bottom flask+ oil
W_o = weight empty round bottom flask
W_s = weight of the sample

Total Flavonoid Content in *P. macrocarpa* Fruit Extract

Flavonoid content in *P. macrocarpa*'s fruit was determined by referring method from (Lay *et al*., 2014) with minor modification. First, 100 µL (10 mg/mL) of the extract was mixed with 100 µL 2% $AlCl_3$ dissolved in ethanol and incubated for 30 min at room condition. Then, quercetin concentrations were prepared, starting from 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 ppm. In the same step as the sample, a series of quercetin concentrations were mixed with 100 µL 2% $AlCl_3$ and incubated for 30 min. Flavonoids in the extract and quercetin were measured using a Microplate Reader at 415 nm. Concentrations obtained from quercetin were plotted in a calibration curve and ascorbic acid as a positive control. Flavonoid content was expressed as mg of quercetin/g of extract (mg QE/mL).

Functional group determination in *P. macrocarpa* fruit extract

Determination functional groups in the extract were analysed using Fourier-Transform Infrared-Attenuated Total Reflection (FTIR-ATR). FTIR was used to determine the existence of a functional group in the extract. The method for FTIR analysis was adopted from (Easmin *et al*., 2017). ATR from Perkin Elmer Inc., USA, was used for this analysis. The spectral region was set at 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were measured by Spectrum 10.03.09.0139 software. The spray-dried

sample was directly used without any preparation by placing it on the diamond ATR crystal. The ATR crystal was then carefully cleansed between the measurements using acetone.

RESULTS AND DISCUSSION

Proximate composition of *P. macrocarpa* fruit

In proximate analysis, fresh fruit of *P. macrocarpa* was used. One fruit's crude protein, fibre, and essential oil were 9.45 ± 2.67%, 21.633 ± 1.17, and 5.605 ± 0.88. Moisture content in this fruit was 88.401 ± 0.75%, its dry matter was 10.96%, and its ash content was 6.33 ± 3.72%. Based on the observation, only a small amount of oil formed at the upper layer of solvent during extraction. Therefore, it was proven that this fruit had only slightly more oil content. The second observation was that while slicing the fruits, it was hard to slice due to the fibrous texture of the fruit. During the grinding process, dried fruits were not powdered but fibrous instead. It showed that fibre from the fruits was very subtle, resulting in a high percentage of crude fibre. According to Lay and colleagues (2014), the high fibre content in fruits may give them the best source of phenolic antioxidants. As for food and fruit resources, when the moisture content is higher than 15%, it is most likely to contribute to microbial spoilage (Wyman, 2013). As for the *P. macrocarpa* fruit, the moisture content obtained was 88%, which can be considered high. Hence, it is crucial to completely dry the fruits during sample preparation to prevent fungi and microbial growth. Table 1 is the summary of all proximate composition analyses of the sample.

Table 1. Proximate composition in *P. macrocarpa* fruit

Parameters	Proximate composition (%)
Crude Protein	9.45 ± 2.67
Crude Fiber	21.633 ± 1.177
Essential Oil	5.605 ± 0.882
Moisture Content	88.401 ± 0.749
Dry Matter Content	10.968
Ash Content	6.33 ± 3.72

Total Flavonoid Content in *P. macrocarpa* fruit extract, TFC

Flavonoid content in *P. macrocarpa* fruit extract was determined using the spectrophotometric method with aluminium trichloride. TFC was expressed as quercetin equivalent (mg QE/100 mL extract). In this analysis, quercetin was used as a standard. The standard calibration curve was plotted, and the $y = 0.0255x + 0.133$ ($R^2 = 0.9954$) was obtained. Flavonoid content in *P. macrocarpa* fruit was 89.89 ± 3.71 mg QE/100 mL. Compared to series dilution of quercetin, the flavonoid content of *P. macrocarpa* lies between 50 to 100 mg/mL quercetin concentrations. Therefore, flavonoid content in *P. macrocarpa* can be considered high because it almost reached the highest concentration of quercetin. For better illustration, the position of *P. macrocarpa* in a series of quercetin was plotted in Figure 1. Based on Figure 1, the flavonoid content in *P. macrocarpa* is almost as high as the highest concentration of quercetin. Therefore, it can be proven that *P. macrocarpa* fruit has high flavonoid content. According to Lay *et al*. (2014), they found that the methanolic extract has flavonoid content of ethyl acetate, chloroform, and hexane at 15.62 ± 0.9 mg, 13.11 ± 0.8, 7.04 ± 1.1 and 4.98 ± 0.6 mg, respectively. Based on that study, aqueous extraction was observed to give better flavonoid content since water is one of the most polar solvents.

Higher flavonoid content in plants gives more beneficial effects on cancer and heart diseases. Flavonoid consists of a large group of polyphenolic compounds containing a benzo-γ-pyrone structure that is abundantly present in plants (Kumar & Pandey, 2013). Some researchers reported that flavonoids have various biotic activities, including anti-allergy, anti-inflammatory, anti-viral, anti-proliferative, and anti-carcinogenic activities. Despite its high antioxidant, high flavonoid content in plants can prevent cancer and cardiac diseases (Datta *et al*., 2004). Based on other findings, it was suggested that *P. macrocarpa* fruit extract has natural cancer remedies because of its high flavonoid content. Quercetin is flavonol commonly found in green apples, onions, green tea, lemon barks, and leaves. According to (Rastaon & Tuah, 2016), quantitative analysis of quercetin shows that stalks of *P. macrocarpa* give the highest amount of flavonoids, followed by its fruits, leaves, and seeds extract.

FTIR analysis

FTIR spectroscopy has been widely used to determine functional groups of bioactive compounds of natural products. The importance of IR analysis for qualitative analysis arises from its properties, especially as a fingerprint technique. No bioactive compounds will have similar IR spectra. Figure 2 shows the spectra of spray-dried *P. macrocarpa* fruit. Based on the spectra, several peaks showed medium and strong atom stretching. From the far left, absorbance at 3305.7 cm^{-1} showed medium and broad intensity. The O-H bond in this range was with a compound of hydrogen-bonded alcohol, phenol. The other bond that might have the same stretching wavenumber was N-H, which is amines or amides.

Next, the wavenumber that had been detected was at 1645.43 cm^{-1}, which shows very weak intensity. At this wavenumber, the bond stretched at this range was alkenes (C=C). Finally, there was a sharp and strong intensity at 572.07 - 552.62 cm^{-1}. These spectra show the alkynes bond (-C≡C-H) and alkane bond (C-H) absorbance. Table 2 shows the summary of the atom stretching and its intensity.

Natural flavonoids contain a B-ring phenyl group, which gives the effect of incorporating phenyl bioisosteres (Ravishankar *et al*., 2018). Hence, by the spectra from FTIR, it is proven that the broad O-H bond present is from the phenol group of flavonoids.

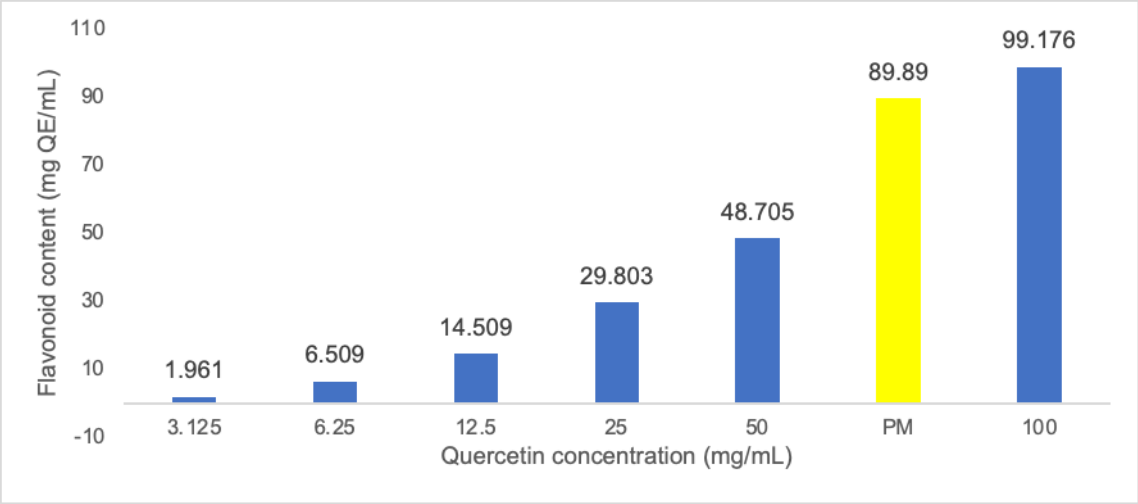

Fig. 1. Total Flavonoid Content (TFC) of different quercetin concentration and Total Flavonoid Content of *P. macrocarpa* extract.

Table 2. The functional group in Spray-dried *P. macrocarpa's* fruit using FTIR

Bond	Types of Compound	Frequency Range, cm^{-1}	Frequency of *P. macrocarpa*, cm^{-1}	Intensity
O-H	Hydrogen-bonded alcohol, phenols	3200-3600	3305.77	Medium,
N-H	Amines, amides			broad
C=C	Alkenes	1610-1680	1645.43	Weak
C≡C	Alkynes	600-700	572.07	Strong
C-H	Alkanes		561.31	sharp
			552.63	

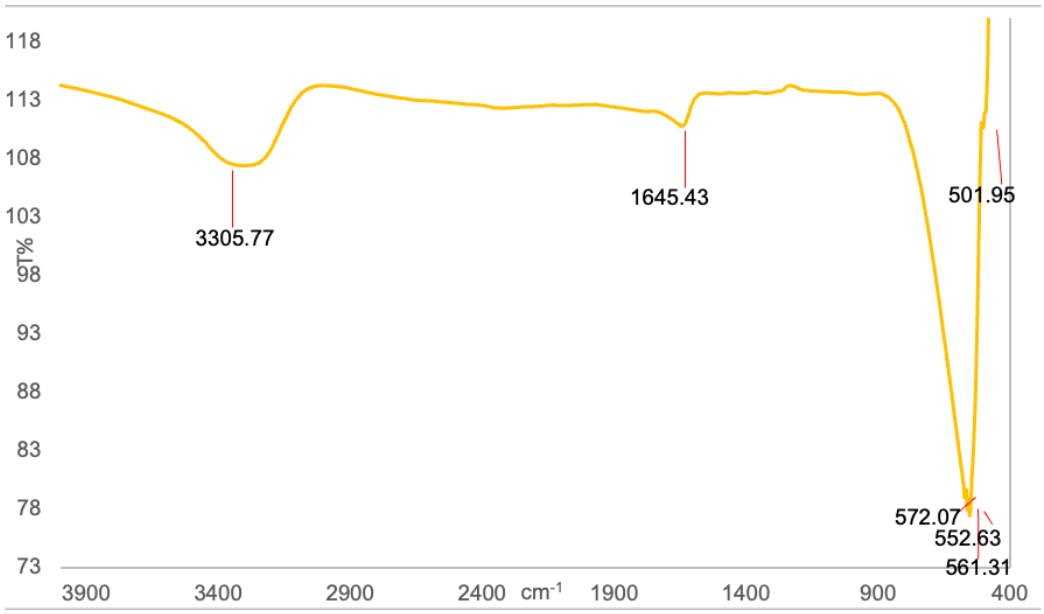

Fig. 2. IR Spectra of Spray-dried *P. macrocarpa's* fruit.

CONCLUSION

To conclude the analysis, flavonoid content in *P. macrocarpa* fruit extract was unexpectedly high because few researchers did the extraction using aqueous extraction. It was shown that water could be substituted for another organic solvent in determining the quantity of the flavonoid since water is more environmentally friendly without reducing the functional properties of the samples. As for proximate analysis, the fruits' composition varied according to their physical properties. Lastly, various functional groups in the extract show the fruits' functional medicinal properties.

ACKNOWLEDGEMENTS

The authors are fully grateful to Halal Product Research Institute, IPPH, Universiti Putra Malaysia, Serdang Selangor, for providing full lab work and analysis facilities and support. Not to forget, our sponsor from Geran Berimpak UPM (9688800).

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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