TRANSACTIONS ON SCIENCE AND TECHNOLOGY

Quantitative Analysis of Quercetin in Various Parts of *Phaleria macrocarpa* (Scheff.) Boerl Extracts

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Received: *6 April 2016*
Revised: *20 April 2016*
Accepted: *3 May 2016*
In press: *20 May 2016*
Online: *30 June 2016*

Keywords:
Phaleria macrocarpa; Phytochemicals; Flavonoids; Quercetin; High-performance liquid chromatography

Abstract

Phaleria macrocarpa, which is also known as Mahkota Dewa, is one of native Indonesian plants. There are a number of findings associate this plant with anti-oxidant, anti-microbial, and anti-cancer attributes. This study is the first stage of on-going research to perform phytochemical analysis and to identify the antimicrobial property of *P. macrocarpa* against pathogenic bacteria and yeast. In particular, quantitative analysis of flavonoids of various parts of *P. macrocarpa* was the main purpose of this study. Phytochemical screening of *P. macrocarpa* leaf, stalk, fruit, and seed showed the existence of flavonoid. The amounts of flavonoid quercetin in various parts of *P. macrocarpa* has been determined by reversed-phase high-performance liquid chromatography (RP-HPLC) with UV detection using 15 cm × 4.6 mm, 10 µm particle, Ascentis™ C_{18} column. The quantitative analysis of quercetin of *P. macrocarpa* crude extracts revealed that stalk contained the highest amount of quercetin (1670.40 ± 13.48 µg ml^{-1}), followed by fruit (1426.72 ± 22.17 µg ml^{-1}), leaf (494.47 ± 30.46 µg ml^{-1}), and seed extracts (313.22 ± 61.81 µg ml^{-1}). The presence of phytochemicals in *P. macrocarpa* may be responsible for its anti-microbial and anti-oxidant activities and may serve as a substitute for synthetic drugs.

Introduction to the subject

Phaleria macrocarpa, commonly known as God's crown, "Mahkota Dewa", or "Pau" is a herbal plant that originates from Papua, Indonesia which belongs to a family of Thymelaseae. The plant is relatively small with about 1.5 to 3 meters and can be grown throughout the year. Extracts of *P. macrocarpa* are reported for a number of pharmacological activities, including anti-tumor, anti-hyperglycemia, anti-inflammation, anti-diarrheal, vasodilator, anti-oxidant, anti-viral, anti-bacterial, and anti-fungal effect.

The fruit of *P. macrocarpa* is empirically believed as a potent medicine to treat some diseases such as high blood pressure, diabetes, gout and so forth (Andrean *et al*., 2014). Moreover, various investigations reported that secondary metabolites of this plant such as tannin, saponin, phenolic compounds, flavonoid and alkaloid play a major role as anti-oxidant, anti-inflammatory, and anti-microbial agents (Hendra *et al*., 2011).

Quercetin is a flavonol, which is a plant-derived flavonoid, used as a nutritional supplement largely found in fruits and vegetables (Figure 1). It is mainly found in many often consumed foods including green apple, onion, green tea, lemon, as well as many seeds, flowers, barks, and leaves

(Phani *et al*., 2010). Moreover, according to Spencer *et al*., (2008), quercetin is thought to have potent anti-oxidant, anti-diabetic, anti-tumor, anti-viral, and anti-inflammatory benefits. Therefore, in this study, qualitative and quantitative analysis of flavonoid quercetin from medicinal plant *P. macrocarpa* were investigated.

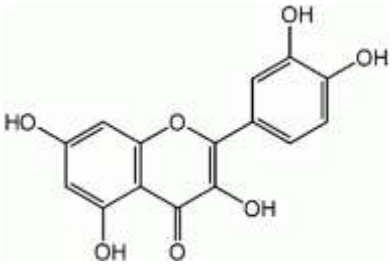


Figure 1. Molecular structure of Quercetin

Methodology

Sample collection

Fresh *P. macrocarpa* leaf, stalk, fruit, and seed were obtained from Mahkota Heritage Plantation, Kg. Sarang, Kota Belud, Sabah. All parts of this plant were washed with water, dried, and grinded prior to extraction. After that, each finely ground samples were placed in an air-tight container and stored at room temperature.

Sample extraction

5 grams of each plant samples was extracted with methanol for 6 hours by using a Soxhlet extractor. The extracts were then filtered using Whatman filter paper (No.1), concentrated in vacuum under reduced pressure using rotary evaporator at 40°C. The concentrated extracts were freeze-dried for 5 days and the final yields were calculated. These crude extracts were kept in sterile universal bottles, under refrigerated condition, prior to analysis.

Qualitative phytochemical screening

The phytochemical screening was performed to determine the presence of bioactive compound implementing chemical methods, and by adopting standard protocols as described by Harborne *et al*., (1999) with some modifications.

Detection of flavonoid (alkaline reagent test)

About 0.5 ml test solution was treated with a few drops of 2M sodium hydroxide solution and observed for intense yellow coloration, which disappeared on the addition of diluted 2M hydrochloric acid.

Standard preparation

Stock solution of standard quercetin was prepared in HPLC grade methanol (200 µg ml^{-1}). A series of five different concentrations of flavonoid standard were then prepared ranged from 5 µg ml^{-1} to 25 µg ml^{-1}. The calibration curve was obtained by plotting these five data points. Linear regression of the

peak area of quercetin standard versus the concentration was performed, to determine the slope, intercept, and correlation coefficient of the calibration curve.

Chromatographic conditions

RP-HPLC analysis of different parts of *P. macrocarpa* was carried out by Ascentis™ C_{18} column at 210 nm. The mobile phase was acetonitrile:water (95:5 v/v) at a flow rate of 1.5 ml min^{-1}. The injection volume of sample was 10 µl and the column oven temperature was maintained at 30°C. Each extract was injected in duplicate. The chromatographic peaks of the analytes were confirmed by comparing their retention times with those of the reference standards.

Result and discussion

5 grams of *P. macrocarpa* leaf, stalk, fruit, and seed extracts yield solid residue weighing 0.36 g (7.2% w/w), 0.27 g (5.4% w/w), 1.14 g (22.8% w/w), and 1.95 g (39% w/w) respectively as illustrated in Table 1. The phytochemical screening of various parts of *P. macrocarpa* showed positive results where the presence of flavonoids was observed (Table 2). According to a study conducted by Hendra *et al*., (2011), he reported that quercetin was found as flavonoid compound in pericarp and seed of *P. macrocarpa*.

The quantitative analysis of flavonoid from *P. macrocarpa* crude extracts were determined by RP-HPLC on 15 cm × 4.6 mm, 10 µm particle, Ascentis™ C_{18} column. A calibration curve used for the quantification of the flavonoid is shown in Figure 2. Good precision was obtained, as R^2 value was 0.99. Thus, identification of flavonoid quercetin was made by comparing their retention times with those of the standards. Figure 3 shows the separation of flavonoid quercetin of various part *P. macrocarpa* crude extracts.

Table 1. Extraction yield of various parts of *P. macrocarpa* extracts

P. macrocarpa Parts	Extraction Yield (g)	Percentage Yield (%) , w/w
Leaf	0.36	7.2
Stalk	0.27	5.4
Fruit	1.14	22.8
Seed	1.95	39

Table 2. Phytochemical screening of flavonoid

Extracts of *P. macrocarpa*	Flavonoid
Leaf	+
Stalk	+
Fruit	+
Seed	+

(+) = presence of phytochemical constituent

(−) = absence of phytochemical constituent

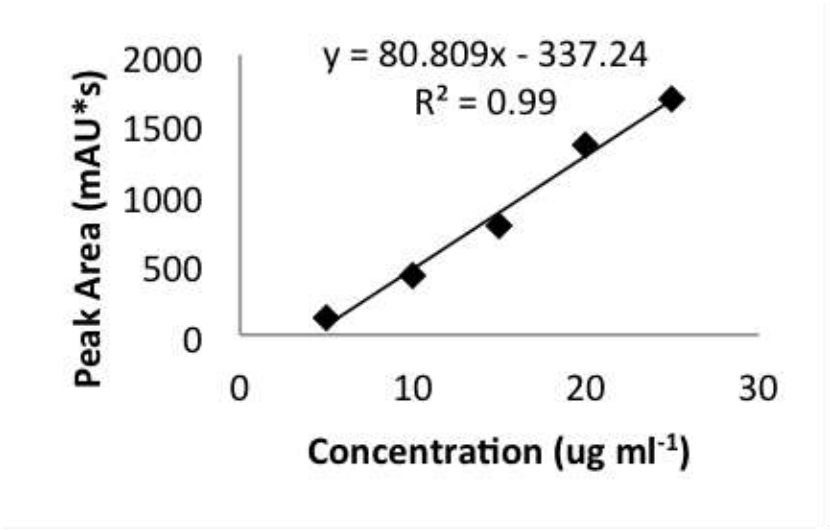
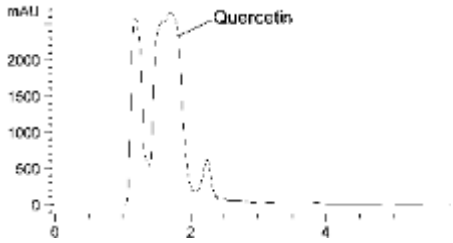


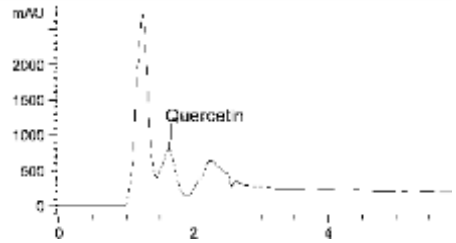
Figure 2: Calibration curve of quercetin standard

(a) Quercetin content of leaf of *P. macrocarpa*.



(b) Quercetin content of stalk of *P. macrocarpa*.

(c) Quercetin content of fruit of *P. macrocarpa*.



(d) Quercetin content of seed of *P. macrocarpa*.

Figure 3. RP-HPLC chromatogram of quercetin in different parts of *P. macrocarpa* crude extracts; detection was at 210nm.

In this study, it was found that the amount of quercetin varied in different parts of *P. macrocarpa* crude extracts, as illustrated in Figure 4. From the bar chart, crude extract of *P. macrocarpa* stalk contained the highest amount of quercetin which was 1670.40 ± 13.48 µg ml^{-1}, approximately five times greater than that in *P. macrocarpa* seed. Apart from that, the quercetin content was also found in appreciable amounts in *P. macrocarpa* fruit, which was 1426.72 ± 22.17 µg ml^{-1}, followed by leaf

which was 494.47 ± 30.64 µg ml^{-1}. Extract that contained the least amount of quercetin was seed with the concentration of 313.22 ± 61.81 µg ml^{-1}.

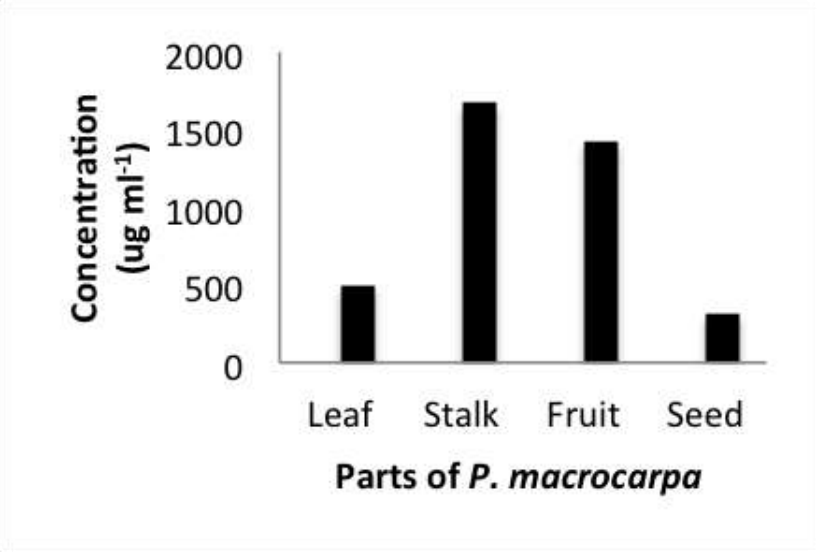


Figure 4: Quercetin content in different parts of *P. macrocarpa* crude extracts

To date, very limited sources of research have been done on *P.macrocarpa* stalk. However, there are many previous investigations which have discovered significant amounts of flavonoid in *P. macrocarpa* fruits, leaves, and seeds. Phytochemical tests showed that secondary metabolites such as flavonoids, glycosides, saponin glycosides, phenolic compounds, steroids, tannins, and terpenoids were present in different extracts of *P. macrocarpa* fruits (Lay *et al*., 2014). Flavonoids, polyphenols, saponins, and tannins which obtained from the other plants are known to have antimicrobial activity (Harborne *et al*., 1999).

In another research conducted by Hendra *et al*., (2011), he found that the contents of flavonoid quercetin were greater in *P. macrocarpa* seed (45.20 ± 0.003 µg/g) than that in mesocarp (31.80 ± 0.002 µg/g). Such differences are largely distributed to factors such as agroclimatic region, post-harvest handling, genetic variability and stage of plant development (Pakade *et al*., 2013).

Conclusion

P. macrocarpa plant is an excellent source of flavonoids. The qualitative and quantitative analysis of flavonoids of *P. macrocarpa* crude extracts revealed that stalk extract contained the highest amount of quercetin, followed by fruit, leaf, and seed extracts (stalk > fruit > leaf > seed). The results of this present research will be further investigated for their anti-microbial and anti-fungal effects.

Acknowledgements

The authors wish to acknowledge UMS Research Grant Scheme, SBK 0123-STWN-2014 entitled "Rhizospheric Microbial Potentials of Pittosporum resiniferum (Petroleum Nut)".

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