Journal of Tropical Resources and Sustainable Science

journal homepage: jtrss.org

Phytochemical and Antioxidant Potential of Four Traditional Malaysian Medicinal Plants

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Received 5 January 2017
Accepted 17 April 2017
Online 2 June 2017

Keywords:
Antioxidant activity, Phytochemical, *Albizia myriophylla, Garcinia opaca, Oxalis barrelieri, Tacca cristata*.

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Abstract

Phytochemicals and antioxidants can be found at different levels in medicinal plants that associated for the use in chronic diseases treatment. The objective of the present study was to investigate the phytochemical and antioxidant properties of *Albizia myriophylla*, *Garcinia opaca var. dumosa*, *Oxalis barrelieri* and *Tacca cristata*, traditional medicinal plants used in healthcare by aborigines or 'Orang Asli' from Kelantan, Malaysia. The total antioxidant capacity was estimated by ABTS (2,2' azinobis-(3-ethybenzthiazoline-6-sulfonic acid)), DPPH (1,1-diphenyl-2-picrylhydrazyl radical) and FRAP assay. The results showed that the four selected plants had significant differences in their total phenolic content (ranging from 45.92 ± 2.01 to 613.37 ± 8.92 mg GAE/g dry weight), in their total flavonoid content (ranging from 6.03 ± 1.03 to 19.29 ± 2.90 mg CE/g dry weight), in their tannins (ranging from 28.23 ± 7.39 to 64.99 ± 7.27 mg TAE/g dry weight), in their alkaloids (ranging from 0.40 to 3.27%) and in their saponins (ranging from 4.31 to 13.15%). The TEAC values of the extracts ranged from 112.12 ± 3.16 to 237.20 ± 1.55 µmol Trolox/g dry weight, from 8.38 ± 2.09 to 219.36 ± 23.48 µmol Trolox/g dry weight and from 136.83 ± 5.24 to 220.93 ± 48.71 µmol Trolox/g dry weight, for ABTS, DPPH and FRAP respectively. TEAC values determined by ABTS, DPPH and FRAP were well correlated with phenolics, flavonoids and tannins contents. *Garcinia opaca* demonstrated the highest total phenolics and tannins while *Oxalis barrelieri* showed the highest total flavonoid and alkaloid compared to other plants studied. *Garcinia opaca* also had shown the highest antioxidant potential, followed by *Oxalis barrelieri.* This study had showed that, *Garcinia opaca var. dumosa* and *Oxalis barrelieri* can be promoted as the sources of phenolics antioxidant and radical scavengers.

1. Introduction

For a few past decades, functional food industries have been experiencing a tremendous growth, and functional foods have been accepted in many countries by the public due to the demand for healthier foods products (Yao et al., 2012). Consumers believed that functional food offer great potential for health improvement and prevention of diseases when ingested as part of a balanced diet (Sarkar, 2007). Nowadays, there are many medicinal plants that have been commercialized as the functional foods. There has been increasing interest in the use of medicinal plants as many of them have been proved to successfully aid in various diseases leading to mass screening for their therapeutic components (Atanasov, 2015).

Medicinal plants contain high density of important nutrients such as minerals, vitamins and fiber which can prevent certain diseases as well as strengthen body tissue and improve nerve system (Fadzelly et al., 2006). Besides that, medicinal plants also are known to be sources of phenolic compounds such as flavonoids, phenolic acids and phenolic diterpenes (Pietta et al., 1998). Moreover, medicinal plants contain phytochemicals and natural antioxidant that can scavenge free radical that associated with chronic diseases such as cancer, artherosclerosis, diabetes and other diseases (Fadzelly et al., 2006).

Phytochemicals are present in a variety of plants used as important component of both human and animal diets (Okwu & Emenike, 2006). Phytochemical are widely distributed as plant constituents and possess many ecological and physiological roles. Alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins and lignans are phytochemicals that can be find in plants (Okwu, 2005). Many of these phytochemicals possess significant antioxidant capacities (Pietta et al., 1998; Zheng & Wang, 2001).

Natural product research is often based on ethnobotanical information and many of the drugs used today were developed from medicinal plants employed in native societies (Heinrich, 2003). Recently, there has been a growing interest in the herbal treatments of diabetes. Hasan et al. (2011) reported that 64.9% from Malaysian diabetic patients was found to use herbal medicine. *Albizia myriophylla, Garcinia opaca var. dumosa, Oxalis barrelieri* and *Tacca cristata* are medicinal plants that are believed to have antidiabetic properties (Nurraihana et al., 2016).

eISSN Number: 2462-2389

A. myriophylla is known as tebu gajah in Malaysia. Traditionally, *A. myriophylla* was used as antitussive (root), tonic (wood), digestant (flower), menstrual stimulant (leaves), expectorant (wood and root), and demulcent (wood and root). Saad et al. (2012) reported that aqueous extract of *A. myriophylla* at 5 mg/kg and 25 mg/kg, respectively showed hypoglycemic activity in streptozotocin-nicotinamide induced diabetic rats with no obvious toxicological effect on the liver and kidney. *G. opaca* is known as beraus or manggis hutan in Malaysia. G. opaca was reported to be administered after childbirth as a preventive medicine and have been prescribed to relieve itching. It also has antioxidant activity (Lim, 2015). While, *T. cristata* which is also known as *Tacca integrifolia* was used to control blood pressure and treat diabetes, hemorrhoid and kidney pain (Ong, 2004; Noraida, 2005). *O. barrelieri* is known as belimbing tanah in Malaysia. *O. barrelieri* has been claimed to have effect on antifungal and free radical scavenging activities (Cavin et al., 1999). Enoch et al. (2007) reported that administration of 500 mg/kg and 1000 mg/kg aqueous and ethanolic extracts of *O. barrelieri* on Sprague-dawley rats produced significant reductions of glycemia in both non-diabetic and diabetic rats. To date there is a lack of data published on these plants especially on their chemical and nutritional composition.

The aim of this study were to investigate chemical composition and antioxidant activities through determination of total phenolics, flavonoids, tannins, alkaloids and saponins, as well as ABTS, DPPH and FRAP assay of the four selected medicinal plants. These data will provide some useful information for people's healthy dietary and the potential application of new materials as food products preparation in functional foods.

2. Materials and Methods

2.1. Plants

Four plants were studied, namely, *Albizia myriophylla*, *Garcinia opaca*, *Oxalis barrelieri* and *Tacca cristata*. The part of plants that were used in this study were based on their reported ethnomedical uses (Nurraihana et al., 2016). All plant materials were collected from Gua Musang and Jeli, Kelantan. The plants were identified by taxonomist of Herbarium Unit, School of Biology Science, Universiti Sains Malaysia. The voucher number of *A. myriophylla*, *O. barrelieri* and *T. cristata* were 11466, 11468 and 11467, respectively. Fresh plant samples were cleaned, air-dried and ground into the powder using laboratory blender (Model 8010S, Waring) and stored in an airtight container until further usage.

2.2. Plant Extraction

According to the traditional use of the herbs reported by Nurraihana et al. (2016), aqueous extracts of herbal components were studied. Dried and ground samples were boiled at 100°C in distilled water for 10 min at a solid to solvent ratio of 1:20 (w/v) (Palasuwan et al., 2005). The extract was filtered (Whatman No.1) and the filtrate was subjected to freeze-dry to remove water. The extract was stored in vial at 4°C for future usage.

Table 1: Part of the plants used for extraction

No.	Plant	Family	Part of Used
1.	*Albizia myriophylla*	Leguminosae	Roots
2.	*Garcinia opaca var.dumosa*	Clusiaceae	Leaves
3.	*Oxalis barrelieri*	Oxalidaceae	Whole plant
4.	*Tacca cristata*	Taccaceae	Whole plant

The part of plants that were used are based on their reported ethnomedical uses (Nurraihana et al., 2016)

2.3. Total Phenolic Content

Total phenolic content was determined according to the method of Miliauskas et al. (2004). 0.2 N Folin-Ciocalteu reagent (5 ml) was added to 1 ml aliquots of the extracts (1 mg/ml) prior to vortex-mixing of the mixture. Then, the mixture was incubated for 3 min at room temperature. Subsequently, 4 ml of 7.5% Na_2CO_3 solution in water was added to the mixture and the content was thoroughly mixed. The mixture was further incubated for 60 min at room temperature. Finally, the absorbance was measured at 765 nm by using UV-Vis spectrophotometer (Model Cary-100 Bio, Varian). The calibration curve using Gallic acid (0.01, 0.03, 0.25, 0.5 and 1 mg/ml) was obtained in the same manner as above except that the absorbance was read after 30 min. The mean (±SD) results of triplicate analyses were expressed as mg Gallic acid equivalents per g plant material (mg GAE/g).

2.4. Total Flavonoid Content

Total flavonoid content was determined by using method of Sakanaka et al. (2005). 250 µl extract (1 mg/ml) or (+)-catechin standard solution (0.005, 0.03, 0.05, 0.07 and 0.1 mg/ml) was added with distilled water (1.25 ml) and $NaNO_2$ (75 µl, 5% (w/v)) prior to the incubation at room temperature for 6 min. Subsequently, 150 µl of 10% $AlCl_3$ solution was added and the mixture was further incubated for 5 min before the addition of NaOH (0.5 ml, 1 M). Thereafter, distilled water (275 µl) was added and vortex-mixed. Finally, the absorbance was measured at 510 nm in spectrophotometer and the measurement was compared to a standard curve of catechin and expressed as mg catechin equivalents per g plant material (CE/g).

2.5. Tannins

Tannins were determined using method of Paaver et al. (2010) 0.1 g of a dry plant sample was transferred to 100 ml flask. 50 ml of distilled water was added and boiled for 30 min. After filtration with filter paper (Whatman No.1), the solution was further transferred to a 500 ml flask and mark up to the mark. 0.5 ml aliquots were finally transferred to test tubes and mixed with 1 ml 1% $K_3Fe(CN)_6$ and 1 ml 1% $FeCl_3$. Then, distilled water was added until 10 ml volume. After five min, the solutions were measured spectrophotometrically at 720 nm. The actual tannin concentrations were calculated on the basis of the optical absorbance values obtained for the standard solutions using tannin acid in range 5 - 200 µg/ml. The results of tannins were expressed in terms of tannic acid in mg/g of dry extract (mg TAE/g).

eISSN Number: 2462-2389

2.6. Saponin

Saponin determination was done using the method of Eleazu et al. (2012) with minor modification. Saponin extraction was done using acetone and methanol subsequently. The acetone was used to extract crude lipid from the samples while the methanol was used for the extraction of the saponin. 2.0 g of the sample was folded into a thimble and put in the Soxhlet extractor (SER 148/6, Velp Scientifica, Italy) fitted with the flask containing 100 ml of acetone and the sample was extracted for 3 hours. After that, the flask was dismantled and another flask containing 100 ml of methanol was fitted to the extractor and extraction was carried on for another 3 hours. The weight of the flask before the extraction was taken. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was then allowed to cool and the weight of the flask taken.

$$Saponin = \frac{A - B}{SM} (100)$$

Where,
A = mass of flask and extract; B = mass of empty flask. SM = sample mass

2.7. Alkaloids

Saponin determination was done using the method of Okwu (2005). 5 g of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the filtrate was concentrated using water bath to one-quarter of the original volume. Ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60°C for 30 min, transferred into desiccators to cool and then weighed. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analysed. The experiment was done in triplicates.

2.8. Antioxidant Activities

2.8.1. ABTS Assay

ABTS free radical decolorization assay was done according to Jing et al. (2010) with minor modification. The pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM $K_2S_2O_8$. The mixture was allowed to stand for 16 h in the dark at room temperature. The solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.2 units at 734 nm. The plant extracts was separately dissolved in 95% methanol to yield a concentration of 1 mg/ml. The aliquot of 0.2 ml of methanolic test solution of each sample (different concentrations) was added to 1 ml of ABTS free radical cation solution instead of 2 ml of ABTS as previous study. The absorbance, monitored for 5 min was measured spectrophotometrically at 734 nm using a spectrophotometer. Appropriate solvent blanks were run in each assay. The experiment was carried out in triplicate. The results were expressed in µmol Trolox equivalent (TE)/g of dry weight samples.

2.8.2. DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) Free Radical Scavenging Assay

DPPH scavenging activity of the extracts was carried out as described by Han et al. (2008) and Lim et al. (2007) with minor modification. In this study, volume of samples and the concentration of DPPH were changed. 0.2 ml of different concentrations of samples in 95% methanol was added to 1 ml of 0.1 mM methanol solution of DPPH. The absorbance at 517 nm was measured after solution had been allowed to stand at room temperature for 30 min in the dark. The experiment was carried out in triplicate. The results were expressed in µmol Trolox equivalent (TE)/g of dry weight samples.

2.8.3. FRAP (Ferric Reducing/ Antioxidant Power) Assay

This procedure was done according to Jing et al. (2010). The working FRAP reagent was produced by mixing 300mM acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio prior to use and heated to 37°C in water bath. A total of 3.0 ml FRAP reagent was added to a cuvette and blank reading was then taken at 593 nm using spectrophotometer. A total of 100 µl selected plant extracts and 300 µl distilled water was added to the cuvette. After addition of the sample to the FRAP reagent, a second reading at 593 nm was performed after 4 min. The change in absorbance after 4 min from initial blank reading was compared with standard curve. The FRAP values for the samples was taken determined using this standard curve. The results were expressed in µmol Trolox equivalent (TE)/g of dry weight samples.

2.9. Statistical Analysis

Data were expressed as mean ± standard deviation. Data were subjected to 1-way analysis of variance (Kruskal-Wallis) and Mann Whitney U test was applied to determine significant difference between groups using SPSS 20. P value less than 0.05 was considered statistically significant. Correlations among data obtained were calculated using Spearman's correlation.

3. Results and Discussion

3.1. Phytochemical Constituents

We examined the total contents of phenolics, flavonoids, tannins, alkaloids and saponins of the aqueous extracts from four selected medicinal plants (Table 2). The amount of total phenolics varied widely among four plant materials and ranged from 45.92 to 613.37 mg GAE/g dry weight. The highest level of phenolics was found in *Garcinia opaca* (613.37 mg GAE/g), which was higher than *Garcinia mangostana* reported by Surveswaran et al. (2007) with 5.10 mg gallic acid/100 g. Phenolic compounds are known to exhibit strong antioxidant activities (Ozgen et al., 2010). They can scavenge free radicals due to their redox properties that allow them to act as reducing agents. Besides the antioxidant properties, the phenolics are reported to inhibit alpha-amylase, sucrose, as well as the action of sodium glucose-

eISSN Number: 2462-2389

transporter 1 (S-GLUT-1) of the intestinal brush border, hence their antidiabetic action (Tiwari & Rao, 2002). The flavonoids contents were ranged from 6.03 to 19.29 mg CE/g dry weight and the highest level of flavonoids was found in *Oxalis barrelieri*, which was higher than *Oxalis corniculata* reported by Borah et al. (2012) with 0.81 mg/g. Flavonoids are important for human health due to their high pharmacological activities as radical scavengers (Burda & Oleszek, 2001). Flavonoids also possess antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, and anticancer activity, whilst some flavonoids exhibit potential for anti–human immunodeficiency virus functions (Yao et al., 2004).

Tannins content ranged from 28.23 to 64.99 mg TAE/g. *Garcinia opaca* was found to contain the highest level of tannins (64.99 mg TAE/g), which was higher than the tannins content in fruit rind of *Garcinia mangostana* as reported by Pothitirat et al. (2010) with 46.83 g tannic acid equivalents/100 g extract. Tannins are known to be useful in preventing cancer and treatment of inflamed or ulcerated tissues (Okwu & Emenike, 2006). It also has been reported to have antimicrobial activity (Scalbert, 1991).

Saponins content ranged from 4.31 to 13.15%. The quantification of total saponin revealed that *Tacca cristata* was clearly the species presenting significantly higher contents of these compounds. This content was higher than saponin content in *Tacca leontopetaloides* as reported by Ubwa et al. (2011), which was 35.0 mg/kg for peels, 31.5 mg/kg for leaves and 34.5 mg/kg for stems. In addition, there was a significant difference between all the samples at $p < 0.05$. Saponins have been shown to help reduce the risk of cancer, have antitumor and antimutagenic activity. It also has antioxidants properties and help lowering cholesterol in the body (Ubwa et al., 2011). Tiwari & Rao (2002) reported that tannins and crude saponins possess potent S-GLU-1 mediated inhibition of glucose transport, hence antidiabetic activity.

The alkaloids content ranges from 0.40% to 3.27% with a significant difference between all the samples at $p < 0.05$. *Oxalis barrelieri* was also found to contain the highest level of these compounds (3.27%), which was higher than alkaloids content in antidiabetic medicinal plants reported by Atangwho et al. (2009), which was 2.84% in *Azadirachata indica*, 2.13% in *Vernonia amygdalina* and 1.97% in *Gongronema latifolium*. Alkaloids have been reported to possess analgesic, antimalarial, antiseptic and bactericidal activities. However, it could be toxic to cells (Facchini, 2001; Wintola & Afolayan, 2011).

The ranking of the phenolics content was similar to the trend of tannins content: *Garcinia opaca* > *Oxalis barrelieri* > *Tacca cristata* > *Albizia myriophylla*. While, the trend of total flavonoids content was similar to trend of alkaloids content: *Oxalis barrelieri* > *Garcinia opaca* > *Tacca cristata* > *Albizia myriophylla*. Whereas the trend of saponins content was different with other chemical compositions: *Tacca cristata* > *Garcinia opaca* > *Oxalis barrelieri* > *Albizia myriophylla*

Table 2: The total phenolics, flavonoids, tannins, alkaloids and saponins of aqueous extracts of selected medicinal plants

Plants	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	Tannins (mg TAE/g)	Alkaloids (%)	Saponins (%)
Albizia myriophylla	45.92 ± 2.01	6.03 ± 1.03	28.23 ± 7.39	0.40 ± 0.06	4.31 ± 0.53
Garcinia opaca	613.37 ± 8.92	17.04 ± 0.57	64.99 ± 7.27	1.84 ± 0.42	7.56 ± 1.58
Oxalis barrelieri	64.30 ± 1.50	19.29 ± 2.90	42.59 ± 10.23	3.27 ± 0.33	7.17 ± 1.15
Tacca cristata	51.31 ± 1.88	7.99 ± 0.49	41.50 ± 7.56	1.58 ± 0.64	13.15 ± 1.48

Results are expressed as mean ± SD of three sample replicates

3.2. Antioxidant Activity

The antioxidant capacities of all plant extracts used in the current study were determined with DPPH, ABTS and FRAP assays. The free radical scavenging activity determined by ABTS varied from 0.11 to 0.24 mmol TE/g dry weights (dw) and the values determined by DPPH ranged from 0.0084 to 0.22 mmol TE/g dw (Table 3). In the DPPH and ABTS methods, the antioxidant capacity of *Garcinia opaca* was stronger than that of other plants. For each antioxidant assays, the levels of individual Kruskal Wallis tests revealed highly significant difference among the plants (Table 3). For DPPH, pair wise comparison shows that there is significant different between each plant group ($P \leq 0.05$). For ABTS, pair wise comparison shows that there is significant different between each plant group ($P \leq 0.05$).

In the FRAP assay, the ability of plant extracts to reduce Fe^{3+} to Fe^{2+} ranged from 0.14 to 0.67 mmol TE/g dw (Table 4). The reducing power of the plant extracts decreased in the following order: *Oxalis barrelieri* > *Garcinia opaca* > *Tacca cristata* > *Albizia myriophylla.* The levels of individual Kruskal Wallis tests revealed highly significant difference among the plants (Table 3). Pair wise comparison shows that there is significant different between each plant group ($P \leq 0.05$) except between *Garcinia opaca* and *Tacca cristata* ($P = 0.513$) and between *Garcinia opaca* and *Oxalis barrelieri* ($P = 0.127$).

Table 3: Antioxidant capacity of plant extracts. Results are expressed as mmol Trolox/g dry weight (mean ± SD of three sample replicates)

Plants	ABTS	DPPH	FRAP
Albizia myriophylla	0.14 ± 12.15	0.0084 ± 2.09	0.14 ± 5.24
Garcinia opaca var.dumosa	0.24 ± 1.55	0.22 ± 23.48	0.67 ± 16.10
Oxalis barrelieri	0.21 ± 19.63	0.11 ± 2.13	0.22 ± 48.71
Tacca cristata	0.11 ± 3.16	0.072 ± 0.46	0.16 ± 9.84

eISSN Number: 2462-2389

3.3. Correlation among Three Antioxidant Assays

Correlation analysis was used to explore the relationship amongst different antioxidant variables measured for all plant extracts used (Table 4). ABTS and DPPH were highly correlated with total phenolics and tannins content. The data of the correlations with total phenolics content were 0.836 and 0.961; the data of correlations with tannins content were 0.789 and 0.775, respectively. These results indicated that phenolic and tannin compounds are major contributor to the radical scavenging activity of these plant extracts. There was moderate correlation between total flavonoids and ABTS (r = 0.575) and between flavonoids and DPPH (r = 0.721). These could happened as only flavonoids of a certain structure and particularly hydroxyl position in the molecule determine antioxidant properties and these properties depend on the ability to donate hydrogen or electron to a free radical (Miliauskas et al., 2004). FRAP was moderately correlated with total phenolics, flavonoids and tannins content; the data of the correlations were 0.611, 0.607 and 0.586, respectively. This implies that these plants contain compounds that are low capable of reducing oxidants (ferric ions). Among the methods used for quantifying antioxidant activities, the correlation between ABTS and DPPH were high (R = 0.804), while correlation of FRAP with ABTS and DPPH were moderate with R values, 0.582 and 0.618, respectively. Saponins was moderately correlated with antioxidative activities determined in all assays while alkaloids was poor correlated with antioxidative activities determined in all assays. These findings indicated that the antioxidant activities of the plant extracts do not depend on the presence of tannins and saponins.

Table 4: Spearman's correlation coefficients of antioxidant activities, total phenolics, total flavonoids and tannins contents

Trait	ABTS	DPPH	FRAP
DPPH	0.804**		
FRAP	0.582*	0.618*	
Total phenolics content	0.836**	0.961**	0.611*
Total flavonoids content	0.575*	0.721**	0.607*
Tannins content	0.789**	0.775**	0.586*
Alkaloids content	-0.343	0.154	-0.093
Saponins contents	0.318	0.514*	0.500

ns = non-significant, * = significant at $P < 0.05$ and ** = significant at $P < 0.01$, respectively.

4. Conclusion

In the present study, *Garcinia opaca* and *Oxalis barrelieri* were rich in polyphenolic constituents and demonstrated the strong antioxidant abilities. That plant, rich in phenolic-flavonoid compound could be a good source of natural antioxidants. Moreover, good correlation existed between antioxidant activity and total phenolics, total flavonoid and tannin, confirming that those plant likely to contribute to the antioxidant activity of the extracts. Consequently, those extracts of plants could be a valuable source for the development of antioxidant food that important implications for human health.

Acknowledgement

Thanks to Universiti Sains Malaysia (USM) for Short-term grant (304/PPSK/661311021) to support this work.

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eISSN Number: 2462-2389