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Rhaphidophora decursiva leaves: Phenolic content and antioxidant activity

(Daun *Rhaphidophora decursiva*: Kandungan fenolik dan aktiviti antioksidan)

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Key words: *Rhaphidophora decursiva* (Roxb.) Schott, antioxidant activity, phenolic, FRAP, DPPH, β-carotene

Abstract

Total phenolic content (TPC) and antioxidant activity (AA) of *Rhaphidophora decursiva* (Roxb.) Schott leaves extracted with methanol and water were studied. The TPC was assessed using Folin-Ciocalteu method. Meanwhile, the antioxidant activity was estimated using ß-carotene bleaching, 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and ferric reducing antioxidant power (FRAP) assay. Butylated hydroxytoluene (BHT) was used to compare its AA with the other extracts. It was found that the total AA of methanol extracts was higher than that of BHT. Significant differences were found in the scavenging activity, EC_{50} value and TPC between the water and methanol extracts. No significant relationship ($p > 0.05$) between TPC and AA as measured by β-carotene bleaching and DPPH radical scavenging methods for both water and methanol extracts. However, TPC and AA as measured by FRAP assay did show a significant relationship at $p < 0.01$ level. This study showed that the methanol extracts of the plant possess both higher TPC and AA compared to that of water extracts, and this was supported by the consistent results showed by all antioxidant activity assays. The antioxidant properties of the plant may have originated not only from phenolic but also from the non-phenolic compounds which possess high antioxidant activity.

Introduction

There are a few Chinese communities in Malaysia that believe the plant, *Rhaphidophora decursiva* (Roxb.) Schott, or locally known as *Pa Shu Long* is effective in curing colon cancer. The plant is viewed as the last line of defence among many cancer patients, especially to those who were diagnosed as third stage or last stage. Many have claimed that they recovered miraculously after merely drinking the water boiled with the plant for several months. However, it is yet to be proven scientifically through laboratory research because there has been no proof to verify the reliability of the belief.

Previous research found that *R. decursiva* actually contains antimalarial compounds after performing antimalarial bioassay-directed fractionation, which has led to the isolation of "decursivine", a new active indole alkaloid, from the leaves and stems of *R. decursiva* (Zhang et al. 2002).

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Antioxidants are a broad group of compounds that destroy single oxygen molecules, also called free radicals, in the body, thereby protecting against oxidative damage to cells which causes several diseases such as coronary heart disease, cancer, ageing etc. They are essential to good health and are found naturally in a wide variety of foods and plants, including many fruits and vegetables. The most commonly used antioxidants are vitamin C, vitamin E and β-carotene. Others include grape seed extract, vitamin A, selenium and coenzyme Q_{10}.

In light of Malaysia is rich in various kinds of plant resources, more studies can be carried out to discover plants that have useful medicinal properties. These plants could act as natural antioxidant to replace the synthetic antioxidant such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which have been revealed by Namiki (1990) could act as promoter of carcinogenesis.

Therefore, this study was to discover other properties related to the plant, which might be valuable to the medical field. It was also to determine the total phenolic content and antioxidant activity of water and methanol extracts of *R. decursiva* (Roxb.) Schott leaves.

Materials and methods

Collection of the plant and preparation of sample

The plant, *Rhaphidophora decursiva* (Roxb.) Schott was collected based on convenience sampling from residential area of Bercham in Ipoh, Perak. The leaves were first separated from the stem and washed thoroughly with distilled water. The leaves were then freeze-dried on the following day and stored at –20 °C before further analysis.

Sample extraction

About 2.0 g of freeze-dried sample was weighed and ground to a powder form. Then, 0.2 g of the ground sample was extracted with 40 ml of distilled water or 100% (v/v) methanol according to method by Othman et al. (2007) with slight modification. The mixtures were then filtered through Whatman filter paper. If a clear solution was not obtained, the filtrate was centrifuged at 5,000 rpm for 10 min. The extracts with the concentration of 5 mg/ml were used for all methods of analysis.

Determination of total phenolic content

The total phenolic content was determined according to the colorimetric Folin-Ciocalteu method with gallic acid as a standard (Amin et al. 2004). Total phenolic content was expressed as gallic acid equivalents (GAE).

Determination of total antioxidant activity

ß-carotene bleaching assay Antioxidant activity of the sample was assayed based on ß-carotene-linoleate bleaching assay according to the method described by Amin and Tan (2002) with modifications. ß-carotene (0.2 mg) was dissolved in 1 ml of chloroform. The chloroform was then evaporated at 40 °C by rotary evaporator. After dilution with distilled water, mixture was then vigorously agitated to form an emulsion. Five ml aliquots of the ß-carotene emulsion and 0.2 ml of water or methanol extracts at a final concentration of 1 mg/ml were mixed well then incubated at 45 °C in water bath for 2 h. Butylated hydroxytoluene (BHT) was used as positive control. Oxidation of the ß-carotene emulsion was monitored using spectrophotometer at 470 nm at initial time (t = 0) against a blank, consisting of an emulsion without ß-carotene. The measurement was carried out at 20-minute intervals for 2 h.

Degradation rate (DR) was calculated using the following equation based on Al-Saikhan et al. (1995):

$$\ln (a/b) \times 1/t = DR_{sample} \text{ or } DR_{standard}$$

where ln is natural log, a is the initial absorbance (470 nm) at time 0, b is the

absorbance (470 nm) at 20, 40, 60, 80, 100 or 120 min and *t* is time in minutes when absorbance was taken. The antioxidant activity (AA) was expressed as percentage inhibition relative to control using the following equation:

$$AA = \left[\frac{DR_{control} - DR_{sample\ or\ standard}}{DR_{control}} \right] x\ 100$$

2, 2-diphenyl-2-picrylhydrazyl radical scavenging assay A method according to Lai et al. (2001) with slight modifications was used to test for radical scavenging activity using 2, 2-diphenyl-2-picrylhydrazyl (DPPH). Aliquot of water or methanol extract at 200 µl with the concentration of 0.5, 1.5, 2.5, 3.5, and 5.0 mg/ml and 0.15, 0.50, 1.00, 1.30 mg/ml of ascorbic acid as positive control were prepared. Then, 1 ml of freshly prepared 500 µM DPPH working solution was added into the test tubes and shaken vigorously. The resulting solutions were then left to stand for 20 min. The absorbance at 517 nm by DPPH was then measured by spectrophotometer. A blank (100% methanol) was also prepared having the same conditions as samples. The scavenging effect of the DPPH radical was calculated using the following equation:

Scavenging effect (%) =

$$\left[1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}} \right] x\ 100$$

EC_{50} value, which was defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%, was determined from the plotted graph of scavenging activity against the concentration of water or methanol extracts respectively. Triplicate measurements were carried out, and their scavenging effect was calculated based on the percentage of DPPH scavenged.

Ferric reducing antioxidant power (FRAP) assay The FRAP assay was done according to the method described by Benzie and Strain (1996) with slight modifications. FRAP assay was determined based on the reduction of Fe^{3+} TPTZ to a blue coloured Fe^{2+} TPTZ. The FRAP working solution was prepared freshly by mixing 25 ml of 300 mM acetate buffer, pH 3.6, 2.5 ml of 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixture was then warmed at 37 °C prior to usage. Then, 150 µl of plant extracts with concentration of 1 mg/ml or methanol in the same amount (control) were allowed to react with 2.85 ml of FRAP solution for 30 min in dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The antioxidant potential of sample was determined from a standard curved plotted using iron (II) sulphate ($FeSO_4.7H_2O$) at a concentration range between 200 and 1,00 µM. Results were expressed in µmole Fe/g dried mass (DM).

Statistical analysis

Data was analysed by using SPSS (Statistical Package for Social Science) version 15.0. T-test was used to determine the differences in antioxidant activity and total phenolic content for each sample by two different methods of extraction (water and 100% methanol extracts) at the level of $p < 0.05$. Pearson correlation was used to determine the relationship between total antioxidant activity and total phenolic content.

Results and discussion

The methanol extracts of *R. decursiva* leaves had significantly higher phenolic content compared to water extracts (*Table 1*). The difference was most probably due to the characteristic of the solvent, which could affect the compounds extracted from the plant matrix. This indicated that phenolic compounds of *R. decursiva* leaves were

Table 1. Mean of the total phenolic content of water and methanol extracts of *Rhaphidophora decursiva* expressed as mg gallic acid equivalent (GAE) per 100 g dried weight (DW)

Samples	Total phenolic content (mg GAE/100 g DW)	
	Mean ± SD	% CV
Water extracts	11.74 ± 0.50a	4.26
Methanol extracts	18.24 ± 1.43b	7.84

*Means with different letters were significantly different (p <0.05)

Table 2. Mean of the total antioxidant activity in terms of inhibition of ß-carotene oxidation in *Rhaphidophora decursiva* samples extracted with water and methanol

Samples	Total antioxidant activity (%)	
	Mean ± SD	% CV
Water extracts	81.90 ± 10.34a	12.63
Methanol extracts	91.21 ± 3.86a	4.23
BHT (positive control)	84.42 ± 6.49a	7.69

*Means with same letters were not significantly different (p >0.05)

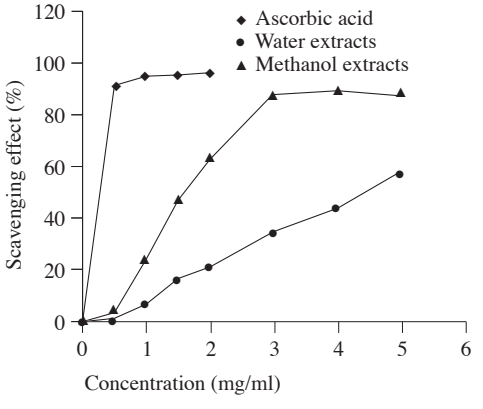

Figure 1. Scavenging effect of methanol and water extracts and ascorbic acid on DPPH radicals. Values were expressed as mean ± standard deviation (n = 3). Ascorbic acid was used as the standard

effectively extracted in methanol than in water.

Due to complex nature of phytochemicals, the antioxidant activities of plant extract cannot be evaluated by a single method. Therefore, commonly accepted assays such as the β-carotene bleaching, DPPH radical scavenging and ferric reducing antioxidant power (FRAP) were employed to evaluate the antioxidant activity of *R. decursiva* leaves extracts.

Methanol extracts had higher antioxidant activity compared to water extracts and also BHT (*Table 2*). However, the antioxidant activity as determined by β-carotene, was not significantly different between methanol and water extracts. Pearson correlation showed that there was no correlation between total antioxidant activity and total phenolic content for both methanol and water extracts as measured by ß-carotene bleaching assay.

The DPPH method was used to evaluate the antioxidant activity of plant extracts because it is one of the most effective methods for evaluating the concentration of radical-scavenging materials activity by a chain-breaking mechanism (Niki 1987). The methanol extracts had significantly higher scavenging activity than the water extracts but lower than ascorbic acid for all concentrations (*Figure 1*). EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of plant extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. The lowest EC_{50} indicates the strongest ability of the extracts to act as DPPH scavengers.

Methanol extracts had a significantly lower (p <0.05) EC_{50} value than water extracts but not as low as ascorbic acid (*Table 3*). However, Pearson correlation showed that there was no significant relationship between EC_{50} value and total phenolic content for both water and methanol extracts (p >0.05).

The ferric reducing antioxidant power (FRAP) assay is another method used to assess antioxidant activity of the plant extracts in this study. FRAP assay which was elaborated by Benzie and Strain (1996)

Table 3. EC_{50} value of water and methanol extracts of *Rhaphidophora decursiva*. EC_{50} value is defined as the amount antioxidant necessary to decrease the initial DPPH radical concentration by 50%

Samples	EC_{50} (DPPH) mg/ml	
	Mean ± SD	% CV
Water extracts	3.60 ± 0.10a	2.78
Methanol extracts	1.18 ± 0.05b	4.24
Ascorbic acid (standard)	0.10 ± 0.00c	0.00

Values were expressed as mean ± standard deviation (n = 3)
*Means with different letters were significantly different (p <0.05)

Table 4. Ferric reducing antioxidant power (FRAP) values of water and methanol extracts of *Rhaphidophora decursiva*

Samples	FRAP value (μM Fe/g dried weight)	
	Mean ± SD	% CV
Water extracts	15.35 ± 1.77a	11.35
Methanol extracts	41.60 ± 6.36a	15.29
Control	3.60 ± 0.28a	7.78

Values were expressed as mean ± standard deviation (n = 3)
*The % CVs were less than 16. Means with same letters were not significantly different (p >0.05)

was originally tailored for the research on human cases only. Nevertheless, Szollosi and Varga (2002) found that this method is also appropriate to measure the total antioxidant activity of medicinal herbs in phytotherapy. The FRAP value however, was not significantly different between the two extracts (*Table 4*). Pearson correlation showed that there was a significant relationship between the antioxidant activity, assayed by FRAP assay and total phenolic content for both water and methanol extracts.

Decker (1997) pointed out that phenolics might influence the antioxidant properties, which can exhibit in a wide range of solubility characteristics. Previous study found that there was a direct relationship between antioxidant activity and total phenolic content in selected fruits and vegetables (Velioglu et al. 1998). However, in this study, there was no correlation between total antioxidant activity and total phenolic content assayed by means of ß-carotene bleaching and DPPH radical scavenging. The negative correlation indicated that phenolic compounds may not be the only compound that contributes to the antioxidant activity.

The antioxidant activity observed by β-carotene bleaching assay and DPPH radical scavenging assay was probably also contributed by other non-phenolic compounds such as ascorbic acid, phytic acid, sterol and carotenoid that might present in the extracts. However, this can only be concluded if profiling and identification of these compounds were carried out.

Nevertheless, FRAP assay did show a significant relationship between total antioxidant activity and total phenolic content for both methanol and water extracts. This indicated that phenolic compound in both plant extracts have strong capability to act as reducing power as shown by FRAP assay. Kriengsak et al. (2006) also reported similar result with methanol extracts of guava in which they found that FRAP assay showed the highest correlation between antioxidant activity and total phenolics, compared to DPPH scavenging assay and other antioxidant assay.

Conclusion

The methanol extract of the leaves of *R. decursiva* (Roxb.) Schott possess higher total phenolic content and total antioxidant activity as measured by all assays when compared to its water extracts. This plant also possesses high antioxidant activity, comparable to that of BHT, which has been popularly and generally known as "antioxidants" in the scientific world. Hence, this study has supported the fact that plant sources possess important antioxidant properties. However, more extensive study (in vivo and in vitro) is needed to determine whether this plant has a role in the prevention of the proliferation of

colon cancer as claimed by many Chinese communities in Malaysia.

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Abstrak

Jumlah kandungan fenolik (TPC) dan aktiviti antioksidan (AA) di dalam daun *Rhaphidophora decursiva* (Roxb.) Schott yang diekstrak dengan metanol dan air suling telah dikaji. Jumlah kandungan fenolik dikaji dengan mengaplikasikan kaedah Folin-Ciocalteu. Manakala, aktiviti antioksidan pula dikaji menggunakan kaedah pelunturan ß-karotena, 2, 2-difenil-2-pikrilhidrazil (DPPH) antiradikal bebas dan kuasa antioksidan penurunan ion ferik (FRAP). AA yang dihasilkan oleh butil hidroksitoluene (BHT) telah dibandingkan dengan AA kedua-dua ekstrak. Didapati jumlah AA ekstrak metanol lebih tinggi daripada BHT. Terdapat perbezaan yang signifikan untuk aktiviti anti radikal bebas, nilai EC_{50} dan TPC antara ekstrak air dengan metanol. Tiada hubung kait yang signifikan ($p > 0.05$) antara TPC dengan AA yang dikaji dengan kaedah pelunturan ß-karotena dan DPPH antiradikal bebas bagi ekstrak air dan metanol. Walau bagaimanapun, ujian FRAP pula menunjukkan hubung kait yang signifikan pada aras $p < 0.01$. Kajian ini menunjukkan bahawa dengan ekstrak metanol tumbuhan ini mengandungi TPC dan AA yang lebih tinggi berbanding dengan ekstrak air, dan ini telah dikukuhkan oleh keputusan konsisten yang ditunjukkan oleh pelbagai ujian AA yang telah dijalankan. Sifat antioksidan tumbuhan ini mungkin bukan sahaja berasal daripada kandungan fenolik bahkan daripada sebatian bukan fenolik yang juga mempunyai AA yang tinggi.

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