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Antioxidative Activity and Phenols Content in Five Tropical Lamiaceae Plants

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Abstract

The total antioxidants content in five Lamiaceae plants were examined. Fresh *Plecranthus amboinicus* Lour. Indonesia leaves were collected from Jakarta, Indonesia and leaves of *Plecranthus amboinicus* Lour. Malaysia, *Pogostemon cablin* Benth., *Solenostemon scutellariodes* Red and *Solenostemon scutellariodes* Mix Colour were collected from Kuantan, Malaysia. Leaves were freeze-dried, ground and extracted using methanol. The extracted leaves were determined the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Results showed that the *P. cablin* Benth. leaves exhibited the significant ($p<0.05$) highest degree of antioxidant activity (63.18%). It was followed by *S. scutellariodes* Red (54.81%) and *S. scutellariodes* Mix Colour (50.44%) which both showed significant differences ($p<0.05$). Both of the *P. amboinicus* Lour. Malaysia and *P. amboinicus* Lour. Indonesia displayed the lowest degree of antioxidant activity ($p>0.05$), i.e 47.07% and 45.38%, respectively. The *P. cablin* Benth. showed the total phenols (7.64 mg/g fw) but insignificant with the *P. amboinicus* Lour. Indonesia (7.42 mg/g fw), *S. scutellariodes* Red (7.32 mg/g fw) and *P. amboinicus* Lour. Malaysia (7.23 mg/g fw). Only the *S. scutellariodes* Mix Colour showed significantly ($p\leq0.05$) compared total phenols (5.64 mg/g fw). This research provided a chemical basis for some of the health benefits claimed for in folk medicine and warrant further studies to develop it as a natural functional food.

1. Introduction

Antioxidants are including vitamins and minerals that occur naturally in foods and also synthesis by our bodies (Norman, 2008). They comprise of important compounds which maintaining our health. They function by forming one network that can react to stabilize free radicals which are abundant in human body (Packer and Colman, 1999).

The need for antioxidants in the human body becomes even more important with increased exposure to free radicals. Pollution, cigarette smoke,

drugs, illness, stress and even exercise can increase free radical exposure (Rakesh et al., 2010; Zin et al., 2006). Plants are main sources of antioxidants such as vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens which have been recognized as having ability to reduce the risk of disease (Adom et al., 2005). Antioxidants that derived from plants have been identified as free radical scavengers. Wong et al. (2006) reported that some medicinal plants contain variety classes of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than dietary plants.

In this research, since some Lamiaceae plants are used as traditional remedies, thus we identified the antioxidant potential of these plants. As comparison, some of the Lamiaceae plants used as ornamentals were also explored to find out the antioxidant potential in them so that the benefits of plants are not neglected. *P. amboinicus* (Lour.) Indonesia is previously named as *Coleus amboinicus* Lour. It is called *Torbangun* in Indonesia whereas in Malay community it is called *Ati-ati hijau*. *P. amboinicus* (Lour.) Indonesia is famous as herbal medicine in Malay and Chinese communities, especially to treat cough, nausea, headache and indigestion (Singh and Panda, 2005; Ong, 2008). The *P. amboinicus* (Lour.) Indonesia is already well known in the North Sumatera Island, Indonesia and the leaves are consumed as soup by Bataknese lactating woman to stimulate breast milk production (Damanik et al., 2006).

P. amboinicus (Lour.) Malaysia is found in wild and also cultivated throughout India, Ceylon and Moluccas. The crushed leaves are used as a local application to the head in treating headache and in conjunctivitis; the concentrated juice is applied around the orbit to relieve the pain (Singh and Panda, 2005).

P. cablin Benth. is known as *Nilam* (Malay) and Patchouli (English). The plant has pleasant aromatic scent, has been used widely as perfume in cosmetic industry. Additionally, its essence oil is used to remove scar and overcome dandruff and skin hair problem (Noraida, 2007).

Besides, *S. scutellariodes* Red and *S. scutellariodes* Mix Colour is known also as *Coleus blumei* Benth or *Ati-ati* (Malay). The addition of red and mix colour at the end of their name is because of their colour. This *Ati-ati* juice is drunk to overcome menses problem and to cure stomach ache and heart burn (Noraida, 2007). However, the use of *Ati-ati* in Malaysia is still widely used instead as ornamentals.

2. Materials and Methods

Plant Material

Fresh leaves were collected from Kuantan. Plants were identified by botanist from Herbarium, Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia.

Preparation of Plant Extract

Fresh leaves were washed once before they were placed in the flasks inside the cold trap of the freeze-dryer machine. The cold trap was turned on to freeze the leaves. The leaves were left inside the cold trap until the temperature drop to -80^{o}C. After 24 hours, the leaves were removed from the cold trap and the vacuum pump was on. The flasks containing the leaves were attached into the manifold. The manifold cock was rotated into vacuum state and the drying process took place. Drying process was considered finished when there was no pressure variation and the leaves looked dry. The dried leaves were then ground, sieved (50µ) and stored in the bottle for further analysis.

DPPH Scavenging Activity

The DPPH radical is one of the few stable organic nitrogen radicals, which bears a deep purple colour. This assay is based on the measurement of the reducing ability of antioxidant towards DPPH^{+} free radical. The free radical scavenging ability can be evaluated by measuring the decrease of its absorbance.

Antioxidant assay are based on measurement of the loss of DPPH colour at 517 nm after reaction with test compound and the reaction is measured by a spectrometer. The percentage of the DPPH remaining is calculated as % Scavenging activity as in **Eq. 1**:

$$[(A_A - A_B) / A_A] \times 100\% \quad (1)$$

Where

A_B is absorbance of DPPH* solution with methanol,

A_A is absorbance of a DPPH solution with samples or positive control, i.e BHA or ascorbic acid.

In this assay, a methanolic solution (2 mL) of samples of various concentrations (1.67-10.00 mg/mL) was placed in test tube, and 2 mL of fresh methanolic solution of DPPH (0.004%) was added. These mixtures of solution were kept in the dark for 30 min, and then the optical density was measured at 517 nm using a UV spectrophotometer. Ascorbic acid (AA) and Butylated hydroxy-anisole (BHA) were used as positive control. Absorption of blank which containing the same amount of methanol and DPPH solution (0.004 %) was prepared and measured.

Total Phenols

Total phenols content of leaves were measured using the Folin-Ciocalteu assay (Waterman and Mole, 1994). About 10 mg homogenised leaves were extracted with 10 ml methanol and sonicated for 1 hour. An amount of 0.2 ml of methanolic sample was diluted with distilled water to make up 10 ml solution (ratio 1:10). 0.2 ml from the dilution was added with 1.8 ml distilled water and 20 µl Follin-Ciocalteu. After 4 minutes, 2 ml of 20 % sodium carbonate was added. The mixture was left for 30 minutes, and then the absorbance was measured at 760 nm using a UV-VIS spectrophotometer. Results were expressed as Gallic acid equivalents (GAE) mg/g fresh weight (fw) sample using the **Eq. 2**:

$$C = \frac{c \times V}{M} \quad (2)$$

Where

C is total content of phenols compounds (mg/g) in plant extract expressed in GAE

c is the concentration of gallic acid (mg/ml)

V is the volume of plant extracts (ml)

M is the weight of tissue sample

Statistical Analysis

Statistical analysis was performed with SPSS software using one-way analysis of variance (ANOVA), and differences between means was analyzed using Tukey's method of multiple comparison at $\alpha = 0.05$. Statistical significance was considered at $p < 0.05$.

3. Results and Discussion

In this study, the total antioxidants content of five Lamiaceae plants were determined. The antioxidant properties of the methanol extract of leaves of *P. amboinicus* Lour. Indonesia, *P. amboinicus* Lour. Malaysia, *P. cablin* Benth., *S. scutellariodes* Red and *S. scutellariodes* Mix Colour were examined for DPPH radical scavenging activity according to the method described. The presence of antioxidant in the sample is based on the disappearance of DPPH radical chromogens which can be measured spectrophotometrically at 517 nm.

The results of the screening are shown in Figure 1. In term of antioxidant activity, the *P. Cablin* Benth. leaves exhibited the significant ($p < 0.05$) highest degree of activity (63.18%). It was followed by *S. scutellariodes* Red (54.81%) and *S. scutellariodes* Mix Colour (50.44%) which both showed significant differences ($p < 0.05$). Whereas, the *P. amboinicus* Lour. Malaysia and *P. amboinicus* Lour. Indonesia displayed the lowest degree of antioxidant activity ($p > 0.05$), i.e 47.07% and 45.38% respectively. However comparing to the positive controls, all the leaves recorded lower free radical scavenging activities than the BHA and AA.

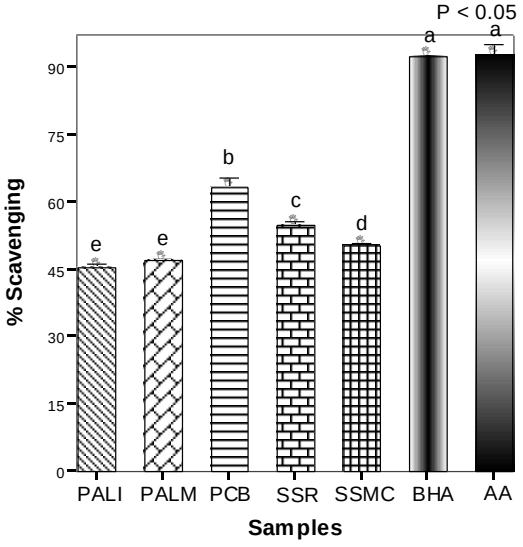

Fig. 1: Figure showed the scavenging activity (%) of five Lamiaceae plants at 0.01 mg/ml using DPPH Assay. Values are average of three independent experiments ± standard deviation. Error bars indicate the standard deviation of three measurements. A significant difference of scavenging

effect (%) between Lamiaceae plants is represented with different lowercase letters; $P < 0.05$. (PALI: *P. amboinicus* Lour. Indonesia, PALM: *P. amboinicus* Lour. Malaysia, PCB: *P. Cablin* Benth., SSR : *S. scutellariodes* Red, SSMC: *S. scutellariodes* Mixed Colour.)

The IC_{50} values of the leaves were depicted in Table 1. The IC_{50} value for each extract, defined as the concentration of extract causing 50 percent of scavenging activity was determined form the curves plotted and tabulated. The IC_{50} was obtained by linear regression equations.

Table 1: IC_{50} values of the five Lamiaceae plants

Leaves	% of Scavenging activity of the Extracts (mg/mL)				
	0.002	***0.003***	***0.007***	***0.01***	***IC 50***
PALI	32.06 ± 1.11	35.62 ±0.30	41.07 ± 1.45	45.38 ± 0.84	0.038
PALM	39.34 ± 0.24	40.75 ± .51	43.60 ± 1.44	47.07 ± 0.39	0.040
PCB	45.74 ± 0.59	49.32 ±1.21	56.61 ± 1.55	63.18 ± 2.24	0.011
SSR	47.63 ± 0.76	50.51 ±0.47	53.42 ± 1.15	54.81 ± 0.84	0.011
SSG	47.55 ± 0.70	48.13 ±1.02	49.87 ± 0.51	50.44 ± 0.54	0.024
BHA	61.55 ± 0.36	75.42 ±1.13	89.73 ± 0.45	92.44 ± 0.10	-0.009
AA	73.48 ± 3.79	82.74 ±1.25	94.43 ± 0.14	92.74 ± 2.37	-0.010

(**PALI**: *P. amboinicus* Lour. Indonesia, **PALM**: *P. amboinicus* Lour. Malaysia, **PCB**: *P. Cablin* Benth., **SSR** : *S. scutellariodes* Red, **SSMC**: *S. scutellariodes* Mixed Colour.)

* The IC_{50} was obtained by linear regression equations.

The values are mean ± standard deviation (n =3)

The bold number is the lower IC_{50} value which reflect greater antioxidant of the extract

Results indicated that the IC_{50} values of the extracts are ranged from 0.04 mg/ml – 0.114 mg/ml (Table 1). Since IC_{50} is a measurement of scavenging activity, a lower IC_{50} value would reflect greater antioxidant of the sample. It means just preparation as low as 0.04 mg/ml concentration will cause 50 percent of scavenging activity.

Furthermore Figure 2 showed that the *P. Cablin* Benth. leaves consistently displayed the highest antioxidant activity and total phenolic content. Antioxidant activity of leaf extracts from medicinal plants have a direct linear relationship with the total phenolic content as described by Fecka and Turek (2008), indicating that the phenolic compounds might be the major contributors to the antioxidant activities of these extracts (Rudnicki, 2007).

The results of the total phenols in leaves of different Lamiaceae plants were actually lower than the previous results by Rasineni et al. (2008). Their research demonstrated that the leaves of *P. amboinicus* Lour. recorded 16.32 mg/g fw compared to the results of in leaves of *P. amboinicus* Lour. Indonesia (7.42 mg/g fw) and *P. amboinicus* Lour. Malaysia (7.23 mg/g fw). The variation in these antioxidant activities may be due to the sample location as Rasineni et al. (2008) collected the samples from Tamilnadu, India.

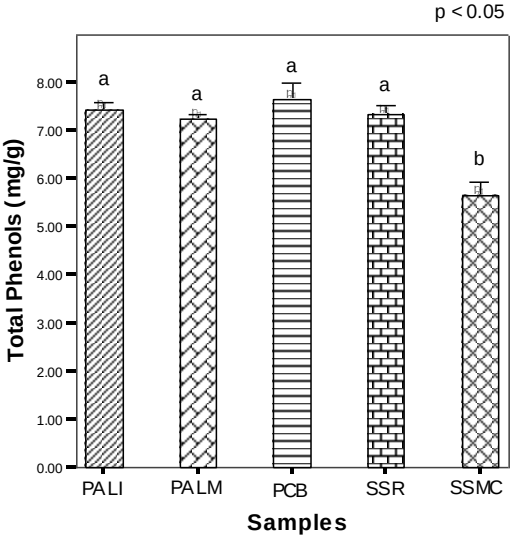

Fig. 2: Total phenolic content based on gallic acid equivalent (GEA) (mg/d fw) in leaves tissues of five Lamiaceae plants, using Folin Ciocalteau Assay. Values are mean of three independent experiments ± S.D. Error bars indicate standard deviation of three measurements. A significant difference of total phenols between Lamiaceae plants is represented with different lower case letters; $P < 0.05$. (**PALI**: *P.*

Amboinicus Lour. Indonesia, **PALM**: *P. amboinicus* Lour. Malaysia, **PCB**: *P. Cablin* Benth., **SSR** : *S. scutellariodes* Red, **SSMC**: *S. scutellariodes* Mixed Colour.)

Our results suggested that the high antioxidant in Lamiaceae plants can be possible used as antioxidant supplement. Research have been shown that high consumption of antioxidant-rich plant will inhibit the oxidation of LDL, and thus slow the process of artherosclerosis and also reduce the risk of cancer and many other diseases (Zin et al., 2006, Atmani et al., 2009). Furthermore, Norman (2008) discovered that Japanese which having the lowest rate of cardiac disease in the world due to their eating habit in high consumption of antioxidant-rich food such as vegetables, fish, sushi and green tea (Norman, 2008).

4. Conclusion

In conclusion, among all the Lamiaceae plants extract analysed, 0.01 mg/ml *P. cablin* Benth. leaves extract showed the highest antioxidant activity (63.18%) by using DPPH assay. However, the results cannot challenge the highest antioxidant activity by positive control; ascorbic acid and BHA which recorded 92.74% and 92.44% respectively. Leaves extract of *S. scutellariodes* Red recorded 54.81% antioxidant activity, followed by leaves extract of *S. scutellariodes* Mix Colour (50.44%). Both of the *P. amboinicus* Lour. Malaysia and *P. amboinicus* Lour. Indonesia displayed the lowest antioxidant activity ($p>0.05$), i.e 47.07% and 45.38%, respectively. From the Follin Ciocalteau method, it showed that the *P. cablin* Benth. leaves extract consistently displayed the highest total phenolic content. It had been shown that the total phenolic content had positive correlation with antioxidant activity. Therefore, the identification of specific phenolic compounds responsible for the high antioxidant activities which can be very beneficial for use as food additives represents one of our future aims.

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