ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF ORTHOSIPHON STAMINEUS BENTH FROM DIFFERENT GEOGRAPHICAL ORIGIN

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Abstract The antioxidant activity and the total phenolics content of methanol extracts of Orthosiphon stamineus, Benth (Lamiaceae) obtained from different localities were determined. The antioxidant properties were evaluated by measuring scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and bleaching rate by β-carotene/linoleic acid system. The extracts from all locations showed antioxidant activity comparable to that of pure quercetin and higher than that of widely used synthetic antioxidant butylated hydroxyanisole (BHA). Some of the locations exhibited significant differences (P<0.05) from one location to another of antioxidant value in both assays. Total phenolics content of the extracts were determined to correlate with their antioxidant activity. However, at 2 mg/mL, there was poor correlation between the total phenolic content and antioxidant activity by DPPH radical scavenger and β-carotene/linoleic acid bleaching method (r=0.42 and 0.048, respectively). Therefore, the antioxidant activity of Orthosiphon stamineus extracts is not solely due to the phenolics compounds.

KEYWORDS: Orthosiphon stamineus, Total phenolics, Free radical scavenging.

Introduction

Production of free radicals and other reactive species in cells and body tissues has been linked to aging and more than one hundred disease states (Maxwell, 1995). Antioxidant treatment may terminate the attack of reactive species and reduce the risk of coronary heart diseases, cancers and other aging associated diseases (Rice-Evans, 1996). Dietary antioxidants serves as one of the source of protection that the body needs to protect against the damaging effects of reactive species (Prior and Cao, 1999). In addition, antioxidant is an important food additives to enhance the quality, stability and safety of food products. As such, novel natural antioxidant with desired physicochemical properties is in high demand for their application as nutraceuticals as well as food additives (Yu & Zhou, 2004).

Orthosiphon stamineus, better known as Misai Kucing by the locals is rich in flavonoids (Sumaryono et al., 1991). Most flavonoids are bioactive compounds due to the presence of phenolic group in their molecule (Apati, 2003: Havsteen, 2002). Twenty phenolic compounds were isolated from this plant including nine lipophilic flavones, two flavonol glycosides and nine caffeic acid derivatives (Sumaryono et al., 1991). Consequently, there are numerous commercial products related to this plant with antioxidant claims on it. However, Prior and his coworkers (1998) found that considerable variability of antioxidant activity depends on several factors including geographical origin.

The objective of this study is to compare the antioxidant activity of O. stamineus from nine localities in Malaysia and one locality in Indonesia. The antioxidant activity was evaluated by DPPH radical scavenger activity and β-carotene/linoleic acid system. The content of the phenolic compounds of O. stamineus extracts were also determined to correlated with their antioxidant activity.

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Material and methods

Sample preparation

Samples used in this study were obtained from ten different localities as shown in Table 1. The leaves of *O. stamineus* were dried in conventional oven at temperature not exceeding 50°C. The dried leaves were ground into powder. One gram of each powder samples was extracted with 100 mL methanol for four hours with continuous stirring using magnetic stirrer at 40°C. The extracts were filtered through filter paper (Whatman No. 1) with a Buchner filter under vacuum, and then cooled to room temperature. Aliquots of the extracts were kept in refrigerator at -20°C prior analysis. The UV analyses were carried out using Perkin-Elmer Lambda 45 (USA) UV spectrophotometer.

Determination of total phenolic contents

The concentration of total phenols in the extracts was determined by using Folin-Ciocalteu reagent and external calibration with caffeic acid (Sigma, St Louis USA). Extract solution (0.2 mL) and Folin-Ciocalteu reagent (0.2 mL) were added and the contents mixed thoroughly. After four minutes, 15% sodium carbonate (1 mL) was added, and the mixture was allowed to stand for two hours at room temperature. The absorbance was measured at 760 nm. The concentration of the total phenolics was measured as mg of caffeic acid equivalent by using an equation obtained from the caffeic acid calibration curve.

Determination of free radical scavenging activity using DPPH assay

The method for estimating free radical scavenging activity of the methanol extracts was adapted from that of Hatano *et al.* (1988) with some modifications. Two mL methanolic solution of DPPH (0.1 mM) was mixed with 200 µL of extract (0.2 mg/mL) at various concentrations in methanol and made up to a final volume of 3 mL. After 40 minutes standing, the absorbance of the mixture was measured at 517 nm against methanol as blank using Pelkin-Elmer Lambda 45 spectrophotometer. Quercetin and butylated hydroxyanisole (BHA) were used as standards. The radical scavenging activities (%) of tested samples were evaluated by comparing with a control (2 mL DPPH and 1 mL of methanol). Each sample was then measured in triplicate and averaged. The radical scavenging activity (RSA) was calculated using the formula:

\[
RSA = \frac{([Ac-As] / Ac) \times 100}
\]

Where

Ac = Absorbance of the control and Ac is the absorbance of the tested sample after 40 min.

Antioxidant activity of extracts using β-carotene/linoleic acid system

The procedure for evaluating the antioxidant activity was modified from a method described by Taga *et al.* (1984). One mL of β-carotene (2 mg in 20 mL of chloroform) was added to a conical flask with 40 mg of linoleic acid and 400 mg of Tween-20. Chloroform was removed with a rotary evaporator at 40°C and the mixture was dried with nitrogen. To the resulting residue 100 mL of oxygenated distilled water was added and mixed and aliquots (3 mL) of the oxygenated β-carotene emulsion were placed in a tube containing 0.2 mL of the extracts (0.2 mg/mL) and the absorbance activity measured at 470 nm immediately, against a blank, consisting of the emulsion without the β-carotene. The solution was incubated on water bath at 50°C to induce autoxidation. The absorbance was recorded at every 15 minutes interval at 470 nm for 120 min monitored oxidation of the β-

carotene emulsion. A control consisted of 0.2 mL of distilled water instead of the extract. The bleaching rate (R) of β-carotene was calculated using the equation:

\[ R = \ln(a/b) \times \frac{1}{t} \]

Where,
\( \ln \) = natural log,
\( a \) = initial absorbance (470 nm),
\( b \) = absorbance at 120 min interval
\( t \) = time (min).

The antioxidant activity (AA) was determined as percentage of inhibition relative to the control sample using the equation:

\[ AA(\%) = \left( \frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \right) \times 100 \]

**Results**

**Total phenolic contents**

For total phenolics, sample from BLPM contained the highest total phenolics contents (10.05 mg/g dry weight), followed by SMSM (9.77 mg/g dry weight). The result of total phenolics contents of the methanol extracts from different locations was illustrated in Table 2. The total phenolic content of the methanol extracts varied from 6.69 mg caffeic acid/g dry weight to 10.05 mg caffeic acid/g dry weight.

**Free radical scavenging activity of extracts using DPPH assay**

The result of free radical scavenging activity of *O. stamineus* methanol extracts of samples from different locations by DPPH was shown in Fig. 1. The methanol extracts demonstrated a significant inhibitory activity against DPPH radical at 0.2 mg/mL and at the same dosage there was a similar radical scavenging activity for the reference standards (quercetin and BHA). The data obtained showed that the DPPH scavenging effect and standards decreased in the following order of BTRM > quercetin > BLPM > SMSM > PJI > PPDM > KBPM > RWBM > BKAM > KCSM > BHA > JGCM.

**β-carotene/linoleic acid system**

A variation in antioxidant activities ranging from 51.45% in samples from RWBM to 84.16% in samples from BTRM was observed. The antioxidant activity (%) was calculated from data in Fig. 1 and all the extracts tested in relation to one another (Table 2). Antioxidant activities of the *O. stamineus* extracts tested using the β-carotene bleaching method decreased in the order BTRM > PJI > SMSM > KBPM > BLPM > JGCM > PPDM > BKAM > KCSM > RWBM.

**Discussion**

The polyphenolic flavonoids have the diphenylpropane (C₈C₈C₆) skeleton. The family includes monomeric flavans, flavones, anthocyanidins, flavones and flavanols (Rice-Evans, 1996). The chemical property of polyphenols in terms of the availability of the phenolic hydrogens as hydrogen-donating radical scavengers predicts their antioxidant activity. As plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminator, it was reasonable to determine their total amount in the extracts (Millauskas et al., 2004). In this experiment, the total antioxidation effect was measured and compared with that of
total phenolics. Because of the complex nature of phytochemicals, the antioxidant activities of plant extract cannot be evaluated by a single method. Therefore, commonly accepted assays were employed to evaluate the antioxidant effects of *O. stamineus* extract from different localities.

The *O. stamineus* extracts at all locations showed antioxidant activity comparable to that of standards (BHA and quercetin). Some of the location exhibited significant differences of antioxidant value compared to other locations in both assays. These data suggest potential effects of agronomic practices and growing condition on the antioxidant properties. This observation was similar to the study reported by Zhou & Yu (2004) where they evaluate the bran extracts from various localities in Colorado, USA.

Free radicals scavenging were determined by DPPH assay. A purple-colored DPPH is a stable free radical, which is reduced to α,α-diphenyl-β-picryl hydrazine (yellow colored) by reacting with an antioxidant. Antioxidants interrupt radical chain oxidation by donating hydrogen from hydroxyl groups to form a stable end product, which does not initiate or propagate further oxidation of lipids. Figure 2 shows the mechanism of DPPH free radical scavenging activity. However, at 2mg/mL, there was poor correlation between the phenolic content of *O. stamineus* and the free radical scavenging activity ($r^2 = 0.42, P < 0.05$).

The potency of the extracts as antioxidant also evaluated using well-established model system: based on β-carotene coupled with autoxidized linoleic acid. Similarly, antioxidant activity of the *O. stamineus* extract by using β-carotene bleaching method, showed poor relationship between the antioxidant activity and the total phenolic content ($r^2 = 0.048, P < 0.05$).

**Conclusion**

From the results, it can be concluded that antioxidant activity of methanol extract of *O. stamineus* is not solely caused by phenolics compound. The same results on correlation between antioxidant activity of plant extracts and its phenolic content have been reported by Kahkonen *et al.* (1999) and Hopia & Heinonen (1999). The free radical scavenging activity is not due to the phenolic only. The pentacyclic triterpenes such as betulinic acid, oleanolic acid and ursolic acid which also present in *O. stamineus* leaf also showed free radical scavenging activity (Akokah *et al.*, 2004). Further more, *O. stamineus* also contains many staminane-type diterpenes as reported by Tezuka *et al.* (2000). These types of compounds possess hydroxyl, carboxylate and terminal methylene group which have hydrogen-donating ability and may possibly contribute to the free radical activity. Therefore, there is no simple relationship between the concentration of total phenol and antioxidant activity of *O. stamineus* extract.

**Acknowledgement**

Financial support from the Ministry of Science, Technology and Innovation, Malaysia through IRPA No. 305/PFARMASI/6123002 for this research is acknowledged.

ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF ORTHOSIPHON STAMINEUS

References


Table 1. Samples from different localities used in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Locality</th>
<th>district</th>
<th>state/country</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBPM</td>
<td>Kepala Batas</td>
<td>Penang</td>
<td></td>
</tr>
<tr>
<td>BLPM</td>
<td>Bumbung Lima</td>
<td>Penang</td>
<td></td>
</tr>
<tr>
<td>BKAM</td>
<td>Bota Kanan</td>
<td>Perak</td>
<td></td>
</tr>
<tr>
<td>RWBM</td>
<td>Rawang</td>
<td>Selangor</td>
<td></td>
</tr>
<tr>
<td>BTRM</td>
<td>Bohor Temak</td>
<td>Perlis</td>
<td></td>
</tr>
<tr>
<td>SMSM</td>
<td>Semonggok</td>
<td>Sarawak</td>
<td></td>
</tr>
<tr>
<td>KCSM</td>
<td>Kuching</td>
<td>Sarawak</td>
<td></td>
</tr>
<tr>
<td>PPDM</td>
<td>Pasir Putih</td>
<td>Kelantan</td>
<td></td>
</tr>
<tr>
<td>JGCM</td>
<td>Jengka</td>
<td>Pahang</td>
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</tr>
<tr>
<td>PJI</td>
<td>Java Island</td>
<td>Indonesia</td>
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</table>

Table 2. Total phenolic and antioxidant activity of Orthosiphon stamineus extracts

<table>
<thead>
<tr>
<th></th>
<th>Total phenolics (mg g⁻¹ dry wt)</th>
<th>Antioxidant activity (DPPH) (%)</th>
<th>Antioxidant activity (ß-carotene system) (%)</th>
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<tbody>
<tr>
<td>BTRM</td>
<td>9.46²</td>
<td>92.34²</td>
<td>84.16²</td>
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<tr>
<td>KBPM</td>
<td>7.61⁶</td>
<td>88.68⁶</td>
<td>73.27⁶</td>
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<tr>
<td>BKAM</td>
<td>9.28⁴</td>
<td>87.38⁴</td>
<td>66.30⁴</td>
</tr>
<tr>
<td>KCSM</td>
<td>9.59⁴</td>
<td>83.75⁴</td>
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</tr>
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<td>RWBM</td>
<td>8.86⁴</td>
<td>88.49⁴</td>
<td>51.24⁴</td>
</tr>
<tr>
<td>SMSM</td>
<td>9.77⁴</td>
<td>90.31⁴</td>
<td>73.76⁴</td>
</tr>
<tr>
<td>JGCM</td>
<td>7.36⁴</td>
<td>62.82⁴</td>
<td>71.29⁴</td>
</tr>
<tr>
<td>PJI</td>
<td>9.71⁴</td>
<td>90.21⁴</td>
<td>77.72⁴</td>
</tr>
<tr>
<td>PPDM</td>
<td>6.69⁶</td>
<td>88.83⁶</td>
<td>66.34⁶</td>
</tr>
<tr>
<td>BLPM</td>
<td>10.05⁴</td>
<td>91.26⁴</td>
<td>72.77⁴</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-</td>
<td>91.50⁵</td>
<td>58.42⁵</td>
</tr>
<tr>
<td>BHA</td>
<td>-</td>
<td>66.43⁵</td>
<td>72.27⁵</td>
</tr>
</tbody>
</table>

Means within column with different letters indicate significantly different values (P < 0.05).
Figure 1: Antioxidant activity by DPPH method and β-carotene/linoleic acid system

Figure 2: Mechanisms of DPPH free radical activity of antioxidant