

ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF DIFFERENT PARTS OF *ORTHOSIPHON STAMINEUS* GROWN UNDER DIFFERENT LIGHT INTENSITIES

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FARHAN M, ABDUL RAZAK S, PIN KY & CHUAH AL. 2012. Antioxidant activity and phenolic content of different parts of *Orthosiphon stamineus* grown under different light intensities. *Orthosiphon stamineus* is an important medicinal plant in South-East Asia. Although already widely cultivated, the influence of growing conditions, especially light intensity, on the antioxidant property of *O. stamineus* is still unknown. Thus, the objective of this study was to determine the antioxidant activity and phenolic content of different plant parts of *O. stamineus* and to propose the best environment condition for its cultivation. The leaves, stems and roots of *O. stamineus* treated in open environment were extracted with methanol for 4 hours in a shaking water bath at 40 °C. Total phenolic content (TPC) and antioxidant activity were determined using Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods respectively. In the first part of the experiment, only seedlings grown in the open environment were used. TPC was higher in leaves (230 mg gallic acid g⁻¹ dry weight) compared with stems and roots (160 and 140 mg gallic acid g⁻¹ dry weight respectively). However, antioxidant activity was lower in leaves (78.43%) than stems (80.66%) and roots (80.26%). By selecting leaves as the main part to study, *O. stamineus* grown under shaded treatment (50 and 70% shaded) as well as open environment were harvested to determine their TPC and antioxidant activities. It was observed that TPC in *O. stamineus* leaves grown in open environment was highest (230 mg gallic acid g⁻¹ dry weight) compared with shade-grown seedlings. Similarly, antioxidant activity was also highest in seedlings grown in open environment (80.02%) compared with in shade (50% = 77.72%, 70% = 74.37%).

Keywords: DPPH, methanolic extract, shade treatment, open environment

FARHAN M, ABDUL RAZAK S, PIN KY & CHUAH AL. 2012. Aktiviti antioksidan dan kandungan fenol dalam beberapa bahagian pokok *Orthosiphon stamineus* yang ditanam di bawah keamatan cahaya berbeza. *Orthosiphon stamineus* merupakan tumbuhan ubatan yang popular di Asia Tenggara. Walaupun sudah ditanam secara meluas, pengaruh keadaan penanaman, terutama keamatan cahaya, terhadap ciri antioksidan *O. stamineus* masih tidak diketahui. Oleh itu, objektif kajian ini adalah untuk menentukan aktiviti antioksidan dan kandungan fenolik pada bahagian tumbuhan yang berbeza dan mencadangkan keadaan persekitaran yang terbaik untuk penanaman *O. stamineus*. Bahagian berbeza *O. stamineus* iaitu daun, batang dan akar yang ditanam di persekitaran terbuka diekstrak dengan 100% metanol selama empat jam dalam rendaman air pada 40 °C. Kandungan jumlah fenol (TPC) dan aktiviti antioksidan ditentukan masing-masing menggunakan kaedah Folin-Ciocalteu dan 2,2-difenil-1-pikrilhidrazil (DPPH). Dalam bahagian pertama eksperimen, hanya anak benih yang ditanam di persekitaran terbuka digunakan. Keputusan menunjukkan bahawa kandungan TPC paling tinggi dalam daun dengan nilai 230 mg asid galik g⁻¹ berat kering sampel berbanding dengan batang (160 mg asid galik g⁻¹ berat kering sampel) dan akar (140 mg asid galik g⁻¹ berat kering sampel). Bagi ujian aktiviti antioksidan, terdapat perbezaan yang signifikan antara daun (78.43%) dengan batang (80.66%) dan akar (80.26%). Justeru, kami memilih daun untuk kajian selanjutnya. Daun *O. stamineus* yang ditanam di bawah teduhan (50% dan 70% teduhan) serta persekitaran terbuka dituai untuk menentukan TPC dan aktiviti antioksidan. Keputusan menunjukkan daun *O. stamineus* yang tumbuh di persekitaran terbuka mempunyai TPC yang paling tinggi iaitu 230 mg asid galik g⁻¹ berat kering sampel berbanding yang tumbuh dalam teduhan. Kajian turut menunjukkan bahawa aktiviti antioksidan paling tinggi bagi anak benih yang tumbuh di persekitaran terbuka (80.02%) berbanding dengan anak benih yang tumbuh dalam teduhan (50% = 77.72%, 70% = 74.37%).

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INTRODUCTION

Orthosiphon stamineus is an important healing plant grown in South-East Asia and currently cultivated in Malaysia. It is a member of the family Lamiaceae, and has several local names, e.g. 'misai kucing' in Malaysia, 'kumis kucing' in Indonesia and 'rau meo' in Vietnam (Tezuka et al. 2000). Different parts of this plant are commonly used traditionally for treatment of various ailments such as diabetes, hypertension, rheumatism and menstrual disorders (Ahamed-Basheer & Abdul-Majid et al. 2010).

Phytochemical and pharmacological studies of this plant have been conducted since 1930 (Tezuka et al. 2000). Almost 20 phenolic compounds have been isolated so far which include 9 lipophilic flavones, 2 flavonol glycosides and 9 caffeic acid derivatives such as rosmarinic acid. It has been reported that the leaf of *O. stamineus* has the highest antioxidant properties because of its greater phenolic fraction compared with the other parts of the plant (Akowuah et al. 2005).

Although already widely cultivated, the influence of growing conditions, in particular light intensity, on antioxidant property of *O. stamineus* is still unknown. Thus, the aim of this study was to demonstrate the effects of light intensity on total phenolic content and antioxidant activity of *O. stamineus* and also to propose the best environment for its cultivation.

METHODOLOGY

Materials

Orthosiphon stamineus seeds were grown in three different plots, i.e. under 50% shade (57,500 lux), 70% shade (34,500 lux) and open environment. The shade was set up using black tindle net of 50 and 70% light passage facility. The plots had mixture of peat, sand and loam (1:3:1) and seedlings were allowed to grow for about three months. The growing seedlings were watered automatically twice a day.

Sample preparation

To study effects of different parts of *O. stamineus* on total phenolic contents (TPC) and antioxidant activity, the harvested seedlings were washed thoroughly under running tap water and separated into leaves, stems and roots. The samples were dried in an oven for three days at

45 °C. All plant parts were separately ground into fine powder and stored at room temperature (25 °C) for further extraction.

From the different parts of *O. stamineus*, leaves showed the highest concentration of TPC and antioxidant activity. Hence, only leaves were selected to determine the effects of light intensity on TPC and antioxidant activity. Leaves from different treatments (50%, 70% and open environment) were harvested and washed thoroughly under running tap water. The leaves were dried in an oven for three days at 45 °C and ground into fine powder and stored at room temperature (25 °C) for further extraction.

Preparation of plant extract

One gram of each sample was extracted with 100 ml methanol at 40 °C for 4 hours with continuous stirring at 150 rpm using a shaking water bath. The extract was filtered under vacuum and cooled to room temperature before freezing (-20 °C) for further analyses. All extractions were done in triplicates.

Determination of TPC

TPC was determined using Folin-Ciocalteu method (Akowuah et al. 2004). For this, 0.2 ml plant extract was added to a test tube containing 0.2 ml of Folin-Ciocalteu reagent and mixed thoroughly. Then 1.0 ml of sodium carbonate (15%) was added after 4 min and the mixture was allowed to stand at room temperature (25 °C) for 2 hours. The resulting blue complex, indicating the presence of TPC, was measured at 760 nm using a spectrophotometer. Gallic acid monohydrate was used as standard. The analysis was done in triplicate.

Analysis of antioxidant activity

A total of 2.0 ml 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) was mixed with 0.2 ml plant extract and made up to 3.0 ml with methanol. The mixture was allowed to stand for 1 hour at room temperature (25 °C) after which its absorbance was measured at 517 nm using a spectrophotometer, against methanol as blank. The free radical scavenging activity (FRSA) of the tested sample was evaluated by comparing its absorbance with the control. Each measurement

was done in triplicate. FRSA was calculated using the following formula (Akowuah et al. 2004):

$$\text{FRSA} = \left[\frac{A_c - A_s}{A_c} \right] \times 100 \quad (1)$$

where A_c = absorbance of control and A_s = absorbance of tested sample

RESULTS AND DISCUSSION

Effects of different parts of *O. stamineus* on TPC and antioxidant activity

Leaves (230 mg gallic acid g^{-1} dry weight) of *O. stamineus* grown in open environment showed the highest TPC compared with stems (160 mg) and roots (140 mg) (Figure 1). Leaves are exposed to the environment and thus more phenolic compounds are produced to provide protection against pathogens, predators and also high visible light. Therefore, the highest TPC was recorded in the leaf. This result was similar to that reported by Zainol et al. (2003) who observed higher concentration of TPC and antioxidant activity in the leaf of *Centella asiatica* compared with its petiole.

Significant differences in antioxidant activities between leaves and the rest of the plant parts were observed in *O. stamineus* planted in open environment (Table 1). The antioxidant activity of leaves (78.43%) was lowest among the parts studied although it contained the highest phenolic compound as shown in Figure 1. This contradicts finding by Akowuah et al. (2005). This might be due to the presence of a few other compounds in the stems and roots that contributed to the antioxidant property. Higher antioxidant activity was also exhibited by roots of *Rheum ribes* followed by its stems (Öztürk et al. 2007).

Effects of light intensity on TPC and antioxidant activity of leaves of *O. stamineus*

The TPC of *O. stamineus* leaves increased with increasing light intensity (Figure 2). Open environment (230 mg gallic acid g^{-1} dry weight in this study) reflected high TPC because more phenolic compounds were produced by the plants for acquiring protection against the damage caused by high light intensity (Rispaill et al. 2005).

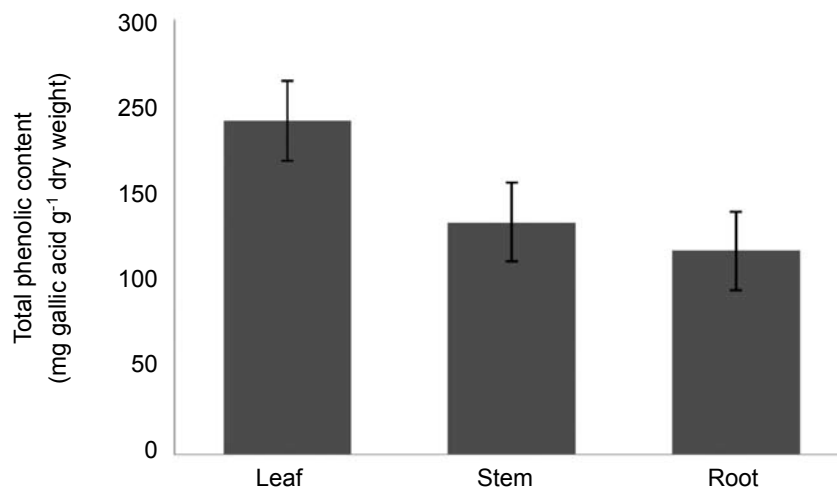


Figure 1 Total phenolic content in different parts of *Orthosiphon stamineus* in open environment

Table 1 Antioxidant activity for different parts of *Orthosiphon stamineus* in open environment

Sample	Inhibition (%)
Leaf	78.43 \pm 0.5074 a
Stem	80.66 \pm 0.7502 b
Root	80.26 \pm 0.9342 b

Values with different letters are significantly different at $p < 0.05$

A study using *Hypericum perforatum* proved that that secondary metabolites, namely, hyperforin and hypericins were directly proportional to light intensity (Odabas et al. 2009). It was also reported that increasing light intensity resulted in higher volumetric contents of fatty and arachinodic acids in *Parietochloris incisa* (Solovchenko et al. 2008).

The effect of light intensity on antioxidant activity is given in Table 2. There were significant differences between open environment (80.02%) and shade treatments (77.72 and 74.37% for 50 and 70% shade respectively), probably due to alteration in TPC inside the plant. When TPC was high in *O. stamineus*, the antioxidant activity was also high (Figure 2, Table 2). This is because phenolic compounds are also effective hydrogen donors, which make them good antioxidants (Zainol et al. 2003). Phenolic compounds are an important group of secondary metabolites which is synthesised by plants during their adaptation towards biotic and abiotic stresses such as infection, water stress, cold stress and high visible light (Hakkim et al. 2008). At the

cellular level, phenolic compounds participate in cell protection against oxygen free radicals, light intensity and mineral nutrients deficiency (Meot-Duros et al. 2008).

CONCLUSIONS

Results of this study showed that leaves had the highest concentration of phenolics as well as antioxidant activity. *Orthosiphon stamineus* grown under open environment exhibited higher TPC and antioxidant activity compared with the plants grown under 50 and 70% shading. Thus, open environment is suggested as an optimum condition for growth of *O. stamineus*.

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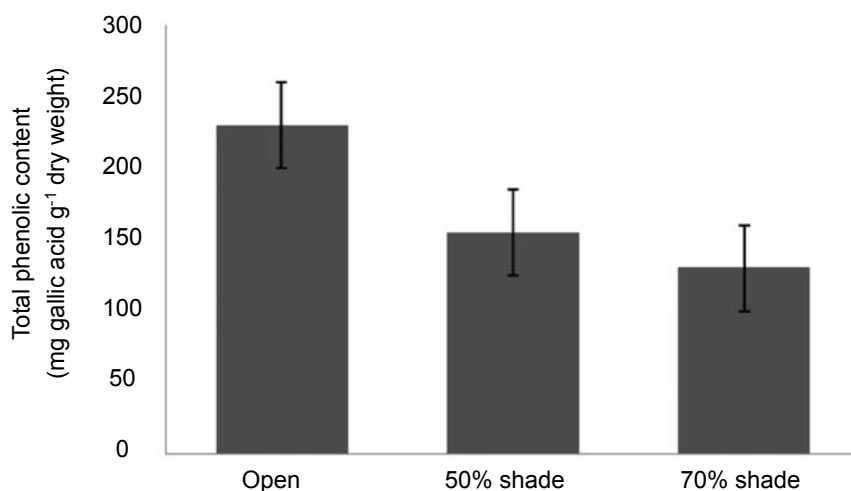


Figure 2 Total phenolic content of *O. stamineus* planted under different light intensities

Table 2 Antioxidant activity of *O. stamineus* leaves at different light intensities

Light intensity	Inhibition (%)
Open environment	80.02 ± 0.87 a
50% shade	77.72 ± 2.79 b
70% shade	74.37 ± 3.35 b

Values with different letters are significantly different at $p < 0.05$

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