INTRODUCTION

Orthosiphon stamineus is a medicinal plant species that has been widely used by the people in Malaysia and other Asian countries to prevent and cure several diseases and disorders. It is locally known as misaikucing due to its flowers that consisted of long filaments similar to cat’s whiskers. Traditionally, O. stamineus plant extract is used to treat gout, hypertension and kidney problems [1]. It was also reported that the extract exhibited an anti-inflammatory, anti-cholesteric, analeptic, anti-rheumatic and anti-diabetic properties[2]. It is established that O. stamineus therapeutic medicinal properties are due to its secondary metabolites or known as bioactive compounds. The scientific research reports over the last two decades confirmed that most of its bioactive compounds are members of polyphenol groups such as polymethoxylated flavonoids and the caffeic acid derivatives [3]. The medicinal bioactive compounds commonly found in O. stamineus extracts are sinensetin, rosmarinic acid, eupatorin, and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone [4].

Plant secondary metabolites play a very important role in its defense mechanism. The production of secondary metabolites is very essential for a plant’s survival during stress conditions[5]. It has been shown that the biosynthesis of secondary metabolites in plants can be affected by biotic and abiotic stresses. One of the most influential abiotic factor is water deficiency or also known as water stress. Water stress in plant usually occurs due to soil water deficit during intense transpiration rate. Plant can be classified under water stress condition when the plant’s cell water potential and turgor decreases to critical level[6]. Therefore, water stress condition can hinder plant’s growth and development processes that will result in low yield and crop failure. On the other hand, plant secondary metabolites production increase during water stress condition. Water stress (waterlog and drought) increase betulenic acid and phenolic compounds in Hypericum brasiliense[7]. Except rosmarinic acid, the other active constituents content in Salvia miltiorrhiza Bunge increased under water-stress conditions[8].

MATERIALS AND METHOD

Plant Material:

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The experiment was conducted at Institute of Sustainable Agro-technology Uni MAP, Sungai Chuchuh, Perlis in March 2013. Stem cuttings of *O. stamineus* were sown in trays for four weeks before transplanting into the experimental pots. The experiment was design as a Randomized Complete Block Design (CRBD) with three soil moisture levels and three plant growth stages. The soil moisture levels treatment consisted of high, medium and low soil moisture levels ranged between 85% - 75%, 55% - 45%, 35%-25% respectively. The treatments were imposed at three different growth stages; 4, 6, and 8 weeks after transplanting. The treatments were replicated four times with plot size of ten plants.

**Sample preparation:**
The whole *O. stamineus* plants were harvested two weeks after imposition of water stress treatments. The soil was removed from the roots portion and rinsed with water before drying under the shade for about 7 days. The roots, stems and leaves were separated and further dried in the oven at 40°C until attaining about 10% moisture content. The dry weight of leaves and stems sample were recorded. The dried leaves samples were ground using Waring blender and stored in the air-tight plastic containers at room temperature for further analysis.

**Quantification of total phenolic content:**
The quantification of total phenolics content of *O. stamineus* leaves samples was conducted according to the Folin-Ciocalteu technique developed by Singleton and Rossi with some modification. 10g of ground dried *O. stamineus* leaves samples were mixed with 100ml of methanol (99.8%, AR Grade) and incubated at 40°C for 4 hours in a shaking water bath. The extract was filtered using Whatman no.1 filter paper and the filtrate was stored at -20°C for determination of phenolic compound and antioxidant assay.

0.2ml aliquot of *O. stamineus* extract was mixed with 0.2ml of Folin-Ciocalteu reagent and shook rigorously for 5 minutes before adding 1 ml of 15 % Na₂CO₃. After that, 1.5ml distilled water was added to the solution mixture before incubating it for 2 hours at room temperature. The concentration of the total phenolic was quantified using UV-Vis Shimadzu spectrophotometer (UV-1800) and the absorbance was read at 760 nm. The caffeic acid was used as a standard and the concentration of total phenolic compound was expressed as caffeic acid equivalent.

**Free radical-scavenging activity of extracts:**
The antioxidant capacity of the extracts was determined using the modified DPPH Free radical scavenging method as described by Akowuah[4]200μl aliquot of *O. stamineus* extracts prepared earlier was mixed with 2ml of DPPH solution (0.1mM)and then methanol was added to make up a final volume of 3ml. The solution was rigorously mixed and incubated at room temperature for 60 minutes. The absorbance of this extract was measured at 517 nm against methanol blank using Shimadzu (UV-1800) spectrophotometer. The free radical scavenging activity (FRSA) of *O. Stamineus* extract is expressed as percentage of DPPH inhibition. The FRSA was calculated using the following equation:

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FRS = \frac{[(A_c - A_i)/A_c] \times 100}{100}
\]

Where A<sub>c</sub> is the absorbance of the control and A<sub>i</sub> is the absorbance of the tested sample.

**RESULT AND DISCUSSION**

The biomass yield of *O. stamineus* plant two weeks after water stress treatment is shown in figure 1a and 1b. Imposition of water stress treatments had not significantly affecting the total biomass accumulation of the species. The result showed that plants exposed to different level of soil moisture deficit four, six and eight weeks old stages had not significantly influenced the dry weight of leaves and stems. This result indicates that *O. stamineus* plants may have the ability to tolerate to short-term moderate water deficit (25% - 85% of field capacity), irrespective of growth stages. The stress period of two weeks imposed on treatment plant may be too brief to affect plant growth and development. It had been observed that certain plant species overcome water stress effects through tolerance, avoidance, and escape mechanism [9].

Figure 2a and 2b show the effects of imposition water deficit at different growth stages on the total phenolic content of *O. stamineus* leaves and stems. The results revealed that water stress treatment had a profound effect on the total leaves and stems phenolic content. Six weeks old plants treated with high, medium, and low soil moisture level showed a significantly higher phenolic compound content as compared to four and eight weeks old plant. However, water stress treatment imposed at four and eight weeks after transplanting had not significantly affects the concentration of phenolic compound in the leaves. The similar trend was also observed in the total phenolic content of the stem. Water deficit at six weeks after transplanting resulted in significantly higher total phenolic concentration as compare to four and eight weeks plant. It was observed that the stem of six weeks old plants treated with medium soil moisture level exhibited significantly higher phenolic compound content (255mg/g.dry weight) as compared to high and low soil moisture level.
Effects of water deficit at different growth stages on free radical scavenging activity (FRSA) of *O. stamineus* leaves and stems are as in figure 3a and 3b. The FRSA of treated plant ranged from 68% - 84% for both leaves and stems. In general, plants treated after eight weeks of transplanting showed significantly higher inhibition. However, the inhibition is not clear between soil moisture deficit treatments. The same thing happened when the treatments were imposed at four and six weeks old plant. However, the highest inhibition activity was plant treated with medium soil moisture level of six weeks old plants.

![Figure 1](image1.png)  
(a) Effects of water deficit at different growth stages on yield of *O. stamineus* leaves (b) Effects of water deficit at different growth stages on yield of *O. stamineus* stem. Treatments with different code letters are significantly different at P=0.05.

![Figure 2](image2.png)  
(a) Effects of water deficit at different growth stages on total phenolic content of *O. stamineus* leaves. (b) Effects of water deficit at different growth stages on total phenolic content of *O. stamineus* stem. Treatments with different code letters are significantly different at P=0.05.

Plant that suffers from stress is reported to produce large amount of reactive oxygen species (ROS). Production of ROS in normal condition is important for plant cell signaling. However, the amount of ROS will increase significantly under stress condition such as water stress. Large amount of ROS can harm plant because it is very reactive [10]. Therefore, plant will produce its secondary metabolites to encounter the harmful ROS. Most of the plant secondary metabolites belong to phenolic groups and can react as antioxidant. Consequently, higher concentration of total phenolic content and percent of inhibition can indicate plants were stressed and the production of ROS may be increased tremendously. Therefore, we can generally said that *O. stamineus* plant suffer from short-term water stress the most when the soil moisture level range from 45%–55% of field capacity and six weeks treatment imposition time.

Plant secondary metabolites are very beneficial for human health. Therefore, manipulating the production of secondary metabolites by plant is really essential. Water deficit showed positive result on the production bioactive compounds of various plant species especially medicinal and herbal plants. Drought stress was found to affect the production of secondary metabolites in cumin (*Cuminum cyminum*) seeds [11]. Artemisinin, the bioactive compounds of *Artemisia annua* significantly increased under water stress treatment [12].
bioactive compounds of *Tribulus terrestris* L. and *Tribulus pentandrus* namely saponins can be increased by water deficit treatment.

![Graph](image1)

![Graph](image2)

**Fig. 3:** (a) Effects of water deficit at different growth stages on free radical scavenging activity (FRSA) of *O. stamineus* leaves. (b) Effects of water deficit at different growth stages on free radical scavenging activity (FRSA) of *O. stamineus* stem. Treatments with different code letters are significantly different at *P*=0.05.

**Conclusion:**

It can be generally concluded that, irrespective of plant growth stage, *O. stamineus* leaves and stem biomass yield may not be affected by water deficit treatments over the two weeks period. Six weeks old plants exposed to medium water deficit had significantly produced higher content of total phenolic and free radical scavenging activity (antioxidant capacity) as compared to two and eight weeks old plants.

**ACKNOWLEDGMENT**

The researchers wish to express their gratitude to the Ministry of Higher Education (MOHE) for the financial support given under FRGS (9003-00309) and Institute of Sustainable Agrotechnology (INSAT), UniMAP for the realization of this work.

**REFERENCES**


