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RESEARCH ARTICLE

Influence of light intensity and temperature on antioxidant activity in *Premna serratifolia* L.

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Abstract

Premna serratifolia has been used to treat inflammatory disorders because it was believed to have antioxidant properties. However, scientific research on this species is currently lacking. This work assesses the antioxidant activity of *P. serratifolia* in relation to light intensity and temperature. Harvesting time significantly influenced the antioxidant activities in *P. serratifolia* leaves; the highest 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity and lowest flavonoid and phenolic contents were observed at 9 am. The opposite trend was observed at 12 noon. Temperature had a significant influence on the DPPH free radical scavenging activity and phenolic content. The DPPH antioxidant activity was negatively correlated with light intensity. The results of this study indicated that harvesting time of *P. serratifolia* leaves should be tailored according to the preferred antioxidant activity.

Keywords: Antioxidant activity, flavonoids, light intensity, phenolics, Premna serratifolia, temperature

1. Introduction

Premna serratifolia L. is a shrub that belongs to the family Lamiaceae. This plant has a broad distribution range and can be found along the coasts and islands of tropical and subtropical Asia, Australia, Africa and the Pacific (de Kok, 2013). The leaves of *P. serratifolia* measure 4–21 × 3–16 cm, are opposite, ovate, acuminate at the apex, cordate at the base, and glabrous, have entire margins, and produce a fetid smell when crushed. The petioles are 0.5–7 mm long and glabrous. The twigs are hairy when young and turn glabrous as they mature. There are no bracts at the base of new shoots. The flowers are small, two-lipped, yellow-white, glabrous on the outer surface, with lobes rounded at the apex, and yellow glands.

The fruit is globose, 3–8 × 3–5 mm, and glabrous, with a smooth and glossy outer surface that turns from green to black when mature. The fruit contains four seeds.

Karthikeyan and Deepa (2011) reported that all the plant parts of *Premna corymbosa*, a synonym of *P. serratifolia*, are used in Ayurveda, siddha and other traditional medicine systems in India. The roots have astringent, alexeteric, thermogenic, anti-inflammatory, deputative, carminative, expectorant, alterant, cardiotonic, stomachic, antibacterial, laxative, febrifuge and tonic properties. The leaves are carminative, galactagogue, and stomachic and are found to be useful in the treatment of agalactia, flatulence, cough, fever, dyspepsia, colic, hemorrhoids, neuralgia, rheumatalgia and tumors.

In Peninsular Malaysia and Indonesia, young leaves of *P. serratifolia* are eaten as a vegetable after boiling (de Kok, 2013). A mixture of leaves and roots is used to treat fevers and breathing difficulty, while leaves are consumed by women to promote breast milk production in Indonesia. The plant is used for hedges as it is easy to propagate through cuttings (de Kok, 2013). The Kenyah people in Sarawak, Malaysia, use water boiled with the plant roots to treat stomachache and diarrhea (Bramley *et al*., 2011).

Plants are beneficial to human health when orally consumed; the beneficial effect is mainly attributed to the phytochemical compounds that are synthesized as secondary metabolites to defend against predators such as insects, fungi and attack by herbivorous mammals. Rodrigo and Bosco (2006) and Ferreira *et al*. (2007) reported that phytochemical compounds such as flavonoids are major contributors to the antioxidant activity of medicinal plants and herbs. These compounds are important in plant biochemistry and play an important role in plant physiology by acting as antioxidants, enzyme inhibitors, pigments and light screens (Ghasemzadeh *et al*., 2010).

The content of many of these phytochemicals varies with plant species, time of herbal part collection and others factors. According to Hemm *et al*. (2004), environmental factors such as light intensity and CO_2 concentration, leaf maturity, and plant age influence the synthesis of flavonoids and phenolics in plants. Light is known to affect plant growth and development. In addition, light regulates the biosynthesis of both primary and secondary metabolites (Ghasemzadeh *et al*., 2010). A previous study conducted by Graham (1998) showed that changes in light intensity are capable of changing flavonoid and phenolic production in herbs. There are many reports of increases in secondary metabolites under high light intensities. For example, this has been reported to occur in tea (Wang *et al*., 2012). Ghasemzadeh *et al*. (2010) reported that light intensity, with subsequent changes in plant morphology and physiological characteristics, affected the synthesis of the medicinal component of herbs.

Premna serratifolia is one of four species found in Sarawak, Malaysia (Bramley *et al*., 2011). Information on the antioxidant contents of plant parts and plant food commonly consumed in Malaysia is lacking. There is no information on the effects of different light intensities on total flavonoid and phenolic biosynthesis or the antioxidant properties of leaves of *P. serratifolia*. Therefore, the objective of this study was to investigate the effects of light and temperature on the antioxidant activity of *P. serratifolia*.

2. Materials and Methods

2.1. Plant materials and environmental parameters

After two consecutive sunny days, fresh *P. serratifolia* leaves were collected from the Universiti Putra Malaysia Bintulu Sarawak Campus at different times (6 am, 9 am, 12 pm, 3 pm and 6 pm). Approximately 30 leaves were collected during each collection period.

2.2. Measurement of environmental parameters

Light intensities were measured using a digital light meter (Model 401025, Extech Instruments Corp., USA). Air temperature was measured using a Hobo® data logger (H08-032-08, Onset Computer Corp., USA).

2.3. Extraction procedure

The extraction method previously described by Koh (2009) was used with some modifications. The leaves were washed thoroughly using distilled water and dried in an oven at 50 °C for two days. The dried leaves were boiled in a water bath at 60 °C for an hour. Then, the extracts were filtered through Whatman filter paper and concentrated using a rotary evaporator below 40 °C.

2.4. Measurement of antioxidant activity

The free radical scavenging activity of the leaf extracts was measured using 1,1-diphenyl-2-picryl-hydrazil (DPPH). This method is similar to the method previously used by Ranković *et al*. (2011). Two milliliters of methanol solution of DPPH radical at a concentration of 0.05 mg mL^{-1} and one milliliter of plant extract were placed in a microcentrifuge tube. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at 517 nm using an ELISA microplate reader (Sunrise™, Tecan Group Ltd., Switzerland). Ascorbic acid, butylated hydroxyanisole (BHA) and α-tocopherol were used as positive controls. The concentration of the DPPH radical was calculated using Equation 1:

$$\text{DPPH scavenging effect (\%)} = [(A0 - A1)/A0] \times 100$$

where A0 is the absorbance of the negative control and A1 is the absorbance of the reaction mixture or standards.

2.5. Determination of total phenolic content

The total phenolic content was determined using a method similar to that used by Ghasemzadeh *et al*. (2010). Briefly, 1 mL of leaf extract was added to deionized water (10 mL) and Folin-Ciocalteu reagents (1.0 mL). After 5 minutes, 2.0 mL of 20% sodium carbonate was added to the mixture. The absorbance was measured at 750 nm using a PicoDrop spectrophotometer (Pico200, Picodrop Limited, UK) after being kept in total darkness for 1 hour. The total phenolic content is expressed as µg of pyrocatechol equivalent per mg of extract.

2.6. Determination of total flavonoid content

The total flavonoid content was determined using a method similar to that used by Ranković *et al*. (2011). Briefly, 0.2 µL of 2% aluminum chloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution. The mixture was incubated for 10 minutes at room temperature, and the absorbance was measured at 415 nm using a Picodrop spectrophotometer (Pico200, Picodrop Limited, UK). The total flavonoid content is expressed as µg of quercetin equivalent per mg of extract.

2.7. Data analysis

All bioassays were carried out in triplicate. The antioxidant values for the leaf extracts were evaluated by analysis of variance using Statistical Analysis System Version 9.0 (SAS Institute, Cary, NC, USA). A mean separation test between treatments was performed using Tukey's multiple range test and a P-value of ≤ 0.05 was regarded as significant. Pearson's correlation test was also carried out to determine the relationship between the antioxidant activity (DPPH radical scavenging activity, total flavonoid and total

phenolic content) and environmental (temperature and light intensity) parameters.

3. Results

3.1. Environmental changes

The diurnal fluctuation of light intensity is shown in Figure 1. The light intensity was 21 lx in the early morning (6 am), which increased to 61633 lx at 12 noon and decreased to 2380 lx by 6 pm. The temperature at 6 am was 26.2 °C, which continued to increase to 34.3 °C at 3 pm and then dropped to 29.9 °C by 6 pm (Figure 1).

3.2. Antioxidant activity

The highest DPPH antioxidant activity was measured in the leaf extracts collected at 9 am (73.5%), followed by those collected at 6 am, 3 pm, 6 pm and 12 noon (47.7%). However, the DPPH radical scavenging abilities of the plant extracts were significantly lower than ascorbic acid, BHA and α-tocopherol (Figure 2).

3.3. Total phenolic and flavonoid contents

Leaves that were collected at different times had a significant ($P \leq 0.05$) impact on the total phenolic production (Table 1). Specifically, the leaves collected at 12 noon contained the highest total phenolic content of 590 µg of pyrocatechol equivalent per mg of extract, whereas the lowest total phenolic content was measured in leaves collected at 9 am. Similarly, leaves collected at 12 noon contained the highest total flavonoid content whereas the lowest total flavonoid content was measured in leaves collected at 9 am (Table 1).

3.4. Relationship between light intensity and antioxidant activity

The DPPH antioxidant activity was negatively correlated with the total phenolic and flavonoid contents, temperature and light intensity (Table 2). There was a positive correlation between the total phenolic and flavonoid contents and temperature (Table 2).

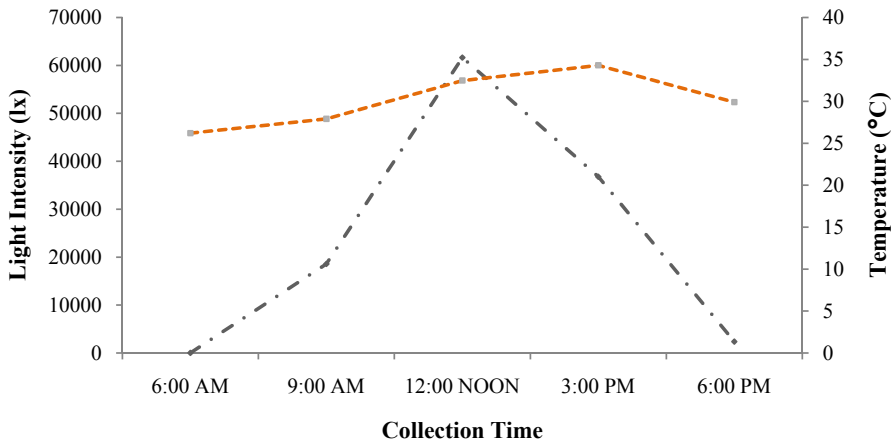

Figure 1. Diurnal fluctuations of light intensity and temperature

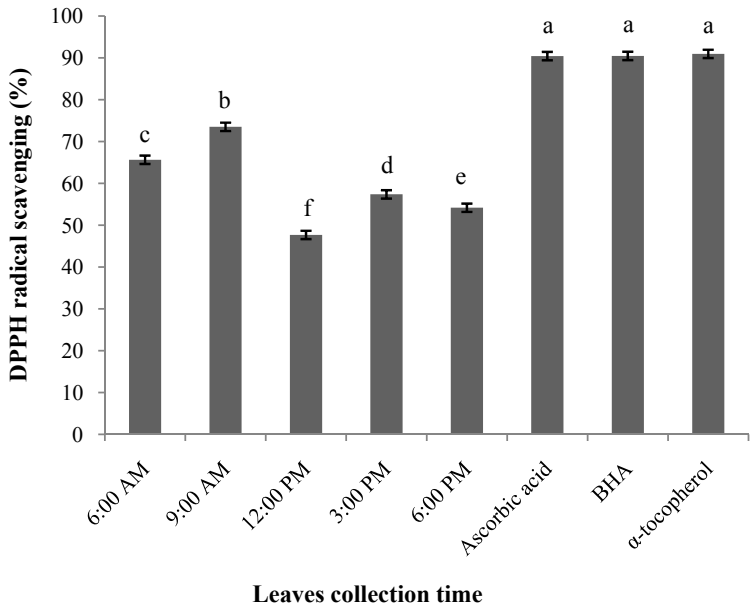

Figure 2. DPPH radical scavenging activity of the leaf extracts of *Premna serratifolia.* All analyses are the means of triplicate measurements ± standard deviation. Means not sharing the sameletter are significantly different at $P \leq 0.05$.

Table 1.Contents of phenolics and flavonoids in *Premna serratifolia* extracts at different collection times

Collection Time	Total phenolics (µg of pyrocatechol equivalent per mg of extract)	Total flavonoids (µg of quercetin equivalent per mg of extract)
6 am	442^{c}±7	760^{b}±1
9 am	265^{d}±4	126^{e}±1
12 pm	590^{a}±4	883^{a}±4
3 pm	556^{b}±1	641^{d}±1
6 pm	583^{a}±2	684^{c}±2

All analyses are the means of triplicate measurements ± standard deviation. Means not sharing the same letter are significantly different at $P \leq 0.05$.

Table 2. Correlation coefficients between various traits

Variables	Total phenolics	Total flavonoids	Temperature	Light intensity
DPPH	-0.944**	-0.817**	-0.680**	-0.557*
Total phenolics		0.860**	0.654**	0.350
Total flavonoids			0.322	0.269

*, ** Significant at 5% and 1% probability levels, respectively

4. Discussion

4.1. Antioxidant activity

DPPH is a stable free radical that is used to determine the radical scavenging activity of an extract. The ability of antioxidants to donate hydrogen is believed to result in free radical inhibitors of DPPH (Liu *et al*., 2008). One of the known free radical scavenging activities that occurs exogenously in the human body is the inhibition mechanism of lipid oxidation by antioxidants (Seow *et al*., 2012).

The leaves collected at 9 am had significantly higher DPPH antioxidant activities compared with those from the other collection times (Figure 2). These values, however, were significantly lower than ascorbic acid, BHA and α-tocopherol levels (Figure 2). This is because ascorbic acid, BHA and α-tocopherol are in a pure form and may actually have higher antioxidant activity compared with crude extracts obtained from *P. serratifolia* in this study. Although the DPPH radical scavenging activities of the extracts were significantly lower than the positive controls, this result showed that *P. serratifolia* extracts have the proton-donating ability to inhibit the activity of DPPH free radicals. Therefore, these extracts could be used as radical scavenging agents, acting as primary antioxidants. The study revealed that *P. serratifolia* extracts have high potential to treat radical-related damage. Xu *et al*. (2010) reported that *Premna microphylla* Turcz. also has similar therapeutic properties.

High light intensity and an increase in temperature reduced the DPPH antioxidant activity (Table 2). A similar relationship between DPPH antioxidant activity and temperature was reported by Boo *et al*. (2011) and Li *et al*. (2010) for *Lactuca sativa* L. Ghasemzadeh *et al*. (2010) found a negative relationship between temperature and DPPH antioxidant activity for two varieties of *Zingiber officinale* Roscoe (ginger), and Islam *et al*. (2003) reported similar observations for *Ipomoea batatas* L. (sweetpotato). The lower DPPH activity at higher temperatures may be related to a reduction in the amount of anthocyanins (He *et al*., 2010; Boo *et al*., 2011).

4.2. Total phenolic and flavonoid contents

Natural antioxidants are present in medicinal plants. They are responsible for inhibiting the harmful consequences of oxidative stress. Many plant extracts exhibit efficient antioxidant properties due to the presence of phytoconstituents, which include phenolics.

Phenolics are secondary plant metabolites that are present in all plants and plant products (Subramanian *et al*., 2013). Ibrahim and Jaafar (2012) stated that plant phenolics have potential health benefits that are mainly due to their antioxidant properties, namely reactive oxygen species scavenging and inhibition, metal chelation, and electrophilic scavenging.

Flavonoids, namely catechins, flavonols, anthocyanins, and proanthocyanidins, are the major polyphenolic components found in food (Mervat and Hanan, 2009). They are effective antioxidants and have recently received attention due to their potential effects on human health in fighting diseases.

Generally, the Lamiaceae species, including *P. serratifolia* (Majumder *et al*., 2014; Radhika *et al*., 2014), show good antioxidant potential (Armatu *et al*., 2010). High levels of phenolic and flavonoid compounds were found in the leaves of *P. serratifolia* in the current study. The collection time had a significant impact on the total phenolic and flavonoid production (Table 1). The highest total phenolic and flavonoid contents were measured in extracts of leaves collected at 12 noon (Table 1).

Phenolic compounds are commonly accepted as the most vital antioxidative plant component (Ranković *et al*., 2011; Majumder *et al*., 2014). This is because plant antioxidant activity is well correlated with the phenolic compound content. Interestingly, there was a negative correlation between DPPH activity and total phenolics and total flavonoids in this study (Table 2). These results indicate that plant antioxidant activity is not only influenced by phenolics (including flavonoids) but also by secondary metabolites such as volatile oils, diterpenes, carotenoids and vitamins (Javanmardi *et al*., 2003; Matkowski *et al*., 2008). Gheldof *et al*. (2002) suggested that the antioxidant capacity is influenced by the collective activity of compounds such as phenolics, peptides, organic acids, enzymes, and other minor components. Miliauskas *et al*. (2004) reported that the antioxidant properties are determined by flavonoids with a certain hydroxyl position.

There was a positive correlation between total phenolics and total flavonoids (Table 2). Similar relationships were reported by Bouba *et al*. (2010) for extracts of 20 Cameroonian plant species, Olajire and Azeez (2011) for Nigerian vegetables, Oloyede (2012) for *Cucurbita pepo* L. (pumpkin) and Farasat *et al*. (2013) for two edible green seaweeds.

In this study, the total phenolic content of *P. serratifolia* leaf extracts increased with an increase in temperature. This result is in accordance with a previous report by Fernandez-Orozco *et al*. (2010) for *Triticum aestivum* L. (wheat). The highest concentration of total phenolics, measured at 12 noon, is possibly due to photoprotective mechanisms utilized by the plant for protectionagainst surplus radiation. Plant leaves regulate the antioxidant system by synthesizing phenolic compounds to act as absorbers of radiation in the epidermal layers (Agati *et al*., 2013). Evans (2009) suggested that temperature has a huge influence on plant phenolic production. The phenolic content (anthocyanins and p-coumaroyl glucose) of strawberry fruit was reported to improve when subjected to high temperatures during planting (Wang and Zheng, 2001).

The increase in phenylpropanoid accumulation is related to carbohydrate availability (Treutter, 2010). Bauer *et al*. (1989) reported that accumulation of starch in *Prunus avium* L. leaves was associated with a buildup of chlorogenic acid, catechin and quercetin 3-glucoside. The highest rate of photosynthesis of *P. serratifolia* was recorded at 12 noon (data not shown), which indicates that accumulation of glucose may contribute to the total phenolic content.

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