EVALUATION OF VEGETATIVE GROWTH, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT STATUS OF *LABISIA LONGISTYLA*

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ABSTRACT

Labisia longistyla is an undershrub herb that grows in highland forests of Peninsular Malaysia. Belonging to Myrsinaceae, L. longistyla has the potential to be used as a medicinal plant like many other plants in this family. However, there is a lack of research on the biological properties and the medicinal potential of L. longistyla. Hence, this study aims to gain a better understanding of L. longistyla. This study evaluated the morphological characteristics, propagation techniques, total phenolic content, antioxidant activities and the soil properties of the habitat of L. longistyla. Inflorescences panicles were observed to differentiate the species within the same genus as the morphological and growth characteristics may look similar to one another. Soil macronutrient analysis were conducted to identify the total nitrogen, available phosphorus and total potassium as well as the cation exchange capacity (CEC) at the study site. The results indicated that the habitat of this species had high organic matter content as the CEC level was more than 40 cmol/kg. Total phenolic content (TPC) was determined using UV-VIS Spectroscopy and the antioxidant activities was measured using the DPPH method. The results showed that L. longistyla possessed high TPC value in the ranges of 7,000 – 16,000 mg GAE/g and this was reflected in the antioxidant activity, which was in the ranges of 69% to 78%. This study found that L. longistyla could be used as a natural antioxidant for medicinal purposes and further study is needed to explore other potential value of the species.

Key words: *Labisia longistyla*, antioxidant activities, highland species, medicinal plant, phenolic compounds

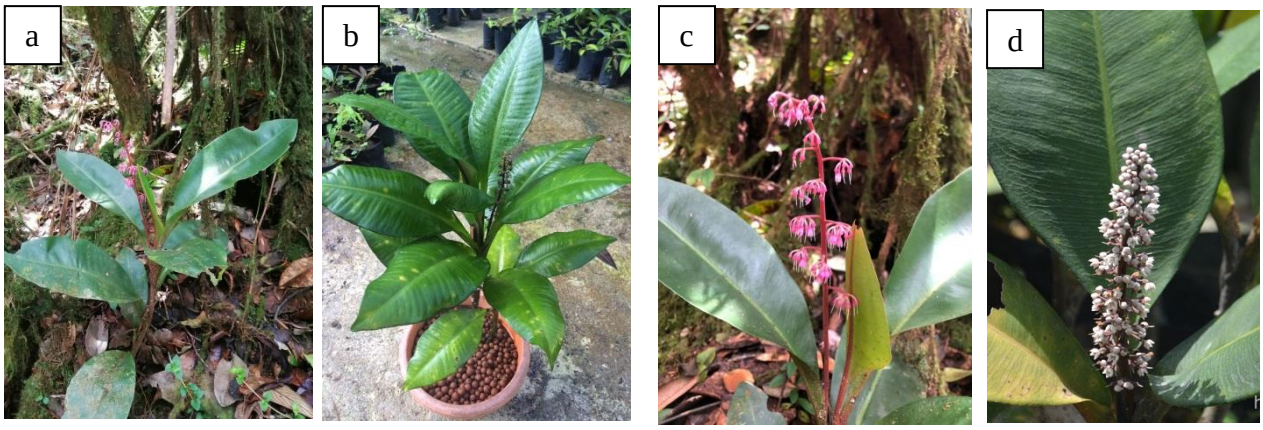
INTRODUCTION

Labisia longistyla belongs to the Myrsinacea family and is found in montane peat forests of Penisular Malaysia and hill forests of west Sumatra, at altitudes of up to 1500m (Sunarno, 2005). It shares the same genus with *Labisia pumila*, a renowned Malaysian medicinal plant which is traditionally used as pre- and post-partum medicine. In natural forests, these two species appear similar (Figure 1 (a) - 1 (b)), thus the inflorescence body (Figure 1 (c) – (d)) is observed to distinguish the two species. The distance between each racemes of *L. longistyla* is longer when compared to *L. pumila*, while the peduncle is reddish and flowers pinkish or purplish. Matured plants of *Labisia longistyla* has broad leaves with elliptic to suborbicular leave shape. It has coriaceous or subcoriaceous leave texture, with glabrous upper and lower surface.

Plant secondary metabolites play a major role in the adaptation of plants to their environment, but also present an important source of active phytochemicals or bioactive compounds. Bioactive compounds are compounds that have health benefits to humans, hence these plants have the potential to be used as herbal medicine (Mattila and Hellstrom, 2007; Sacchetti et al., 2005). For instance, *L. pumila* is widely used in the herbal medicine industry due to its high phenolic compound concentrations. Phenolic compounds are bioactive compounds characterized by hydroxylated aromatic rings and have been reported to have antioxidants, antibacterial, antiviral, anti-cancer and anti-inflammatory properties (Mattila and Hellstrom, 2007; Sacchetti et al., 2005). Both *L. pumila* and *L. longistyla* belong to Myrsinaceae; A widespread family consisting of 30 genera and about 1000 species of tropical plants, of which about 40 species are medicinal in the Asia-Pacific region, particularly for the treatment of inflammation (Shah et al., 2011, Abu Bakar et al., 2018). However, there is a lack of research on the biological properties and the medicinal potential of *L. longistyla*.

Hence, this study aims to gain a better understanding of *L. longistyla*. This study evaluated the morphological characteristics, total phenolic content, antioxidant activities and the soil properties of the habitat of *L. longistyla*. The study was conducted at Batu Gangan Forest Reserve, a mossy type forest located at Cameron Highlands, Pahang, Peninsular Malaysia. This is due to the forest having naturally growing *L. longistyla* plants.

Figure 1: Differentiation of (a) Labisia longistyla and (b) Labisia pumila. Inflorescence body of (c) Labisia longistyla and (d) Labisia pumila.



MATERIALS AND METHODS

Leaf morphological data collection

Three different clumps of *Labisia longistyla* were identified at the elevation of 1982 meters above sea level, located at Batu Gangan Forest Reserve, Cameron Highlands, Pahang. The leaves morphology at each clump were recorded such as number of leaves, leaf length, leaf width, petiole length and petiole width.

Soil sampling and macro element analysis

A standard semi detail soil survey (Soil Survey Division Staff, 1993) was conducted at the clumps of *Labisia longistyla*. Soil samples were collected at each site, at a depth of 0-20 cm. Several soil chemical analyses were carried at the Soil Chemistry Laboratory, FRIM using standard soil analysis methods (USDA, 2014). Total nitrogen was determined using Kjedahl and distillation method. Available Phosphorus was extracted based on the Bray and Kurtz no. 2 procedure and its concentration was determined using UV-Visible Spectrophotometer (Shimadzu UV-Vis 160A). Potassium was extracted using 1 N ammonium acetate and K concentration, and was analyzed using Flame Photometer (Sherwood M410). Cation exchange capacity (CEC) was determined via Flow Analyzer. The soil acidity was measured using pH meter at 1:2.5 ratios of soil and water.

Leaves and stem cuttings

Propagation of plants by cuttings is the most widely used technique to produce clones in many herbaceous, ornamentals and woody plants. There are several studies on the propagation of *L. pumila* through leaves cuttings (Aminah et al., 2008; Rozihawati, 2008; Farah Fazwa et al., 2013; Syafiqah Nabilah et al., 2014) and stem cuttings (Rozihawati et al., 2005). In this study, similar approaches were used to multiply and conserve *L. longistyla*. Selected mother plants of *L. longistyla* at each clump were transported to FRIM for propagation. The mother plants were transferred to polybags and acclimatized in growing chambers for a month prior to cutting. Matured leaves and stem were used as the cutting materials. The leaves were cut into 30cm^2 while the stems were cut into 5 cm length. Commercial rooting hormone (Seradix 1) containing 0.1% Indole Butyric Acid (IBA) were applied on the base of each cutting. The cuttings were grown in an enclosed mist propagation chamber and watered twice daily for one minute per session. The rooting performances were observed weekly.

Quantification of Total Phenolic Content (TPC)

Determination of TPC was performed using Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965), with modifications into high-throughput microplate system. 0.5 mg of leave sample were dissolved in ethanol, distilled water and hydrochloric acid at a ratio of 10:1:1. The mixture was centrifuged at 6000 rpm for 15 minutes and the supernatant decanted into vials. The supernatant was then used for the determination of TPC.

50.0 ml of supernatant extract was mixed with 100.0 ml of Folin-Ciocalteu reagent (0.1 ml/0.9 ml) in a 96 well microtiter plates, in triplicates. The plate is allowed to stand at room temperature for 5 minutes. Then, 100.0 ml of sodium bicarbonate (60.0 mg/ml) solution was added and the mixture was allowed to stand at room temperature for 90 minutes. Absorbance was measured at 725 nm. The samples were expressed as Gallic acid equivalents GAE-TPC mg/g extract.

1, 1- Diphenyl-2-picrylhydrazyl (DPPH) assay

Antioxidant reducing activity on DPPH radical was estimated using the method developed by Blois (1958), with modification into a high-throughput microplate system. Samples (50 µl of 0.5 mg/ml) were added to 50 µl of DPPH (FG: 394.32) (1 mM in ethanolic solution) and 150 µl of ethanol (absolute, AR Grade) in a 96 well microtiter plate, in triplicates. The plate was shaken (15 s, 500 rpm) and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured spectrophotometrically at 520 nm.

Statistical analysis

The leaf morphological data, TPC and DPPH were analyzed using analysis of variance (ANOVA) with IBM SPSS Statistics version 22. Significant differences were determined using Duncan's Multiple Range Test (DMRT).

RESULTS & DISCUSSION

Table 1 shows leaf morphology of *L. longistyla* at three different clumps. There was no significant difference in leaf morphology between each clump. The number of leaves, leaf length and leaf width recorded were in agreement with Sunarno (2005) except for petiole size. The plant could be in the middle stage of growth as the petiole width was smaller.

Table 1: Vegetative growth of *Labisia longistyla* at three different clumps

Sample No.	No. of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole width (cm)
Clump 1	5.00a±0.83	14.5a±0.59	5.79a±0.25	3.08a±0.09	0.78a±0.08
Clump 2	3.80a±0.37	14.3a±1.97	6.58a±0.81	2.52a±0.33	0.82a±0.21
Clump 3	5.40a±0.81	14.1a±0.82	7.37a±0.41	2.72a±0.24	0.75a±0.06

Means sharing a common letter were not significantly different at $p \leq 0.05$.

Cuttings of *L. longistyla* started to root at week-18. The process of root initiation was very slow compared to *L. pumila*, which started to root at week-3 (Aminah et al., 2008; Farah Fazwa et al., 2013; Syafiqah Nabilah et al., 2014). Cuttings from clump 1 recorded the highest survival rate, followed by clump 3 and clump 2 (Table 2). The rooting percentage recorded were below 40%. The rooting performance was recorded until week-30 (Figure 2). Controlling environmental factors is important in ensuring the survivability of the cuttings, as most plants are quite sensitive to environmental changes such as low humidity and high temperatures.

Table 2: Survival rate and rooting percentage.

Sample No.	Survival rate (%)	Rooting percentage (%)
Clump 1	87.6a±6.40	23.8c±13.9
Clump 2	52.4c±5.79	27.9bc±16.3
Clump 3	68.3b±4.52	39.4abc±24.7

Means sharing a common letter were not significantly different at $p \leq 0.05$.

Figure 2: Root growth of *Labisia longistyla* at week 30 a) leaf cuttings b) stem cuttings

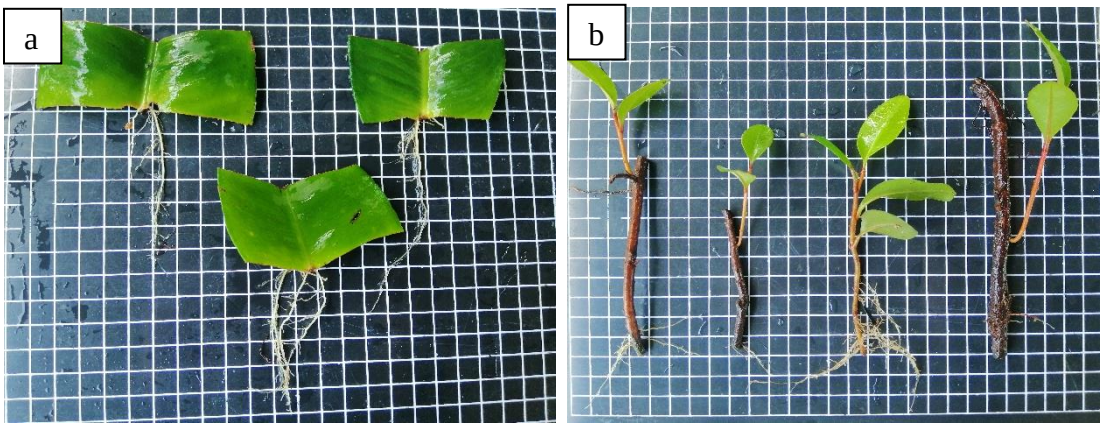


Table 3 presents the results of soil chemical properties at *Labisia longistyla* study sites. All sites had high total N, while the available P varied among the sites. Clump 1 recorded the highest available P concentrations, compared to clump 2 and clump 3. The exchangeable potassium (K) was low at all sites. Cation exchange capacity (CEC) was high at all sites, which indicated high organic matter content. The soil for all sites were very acidic. Based on the NPK and CEC values, it can be concluded that H. S. Batu Gangan had better soil conditions for the growth of *Labisia longistyla*, compared to lowland forests (Farah Fazwa et al., 2012a; Syafiqah Nabilah et al., 2015). It also should be noted that *Labisia longistyla* was able to tolerate very low pH levels, as the soil pH for all three sites ranged between 3.33 to 3.61.

Table 3: Soil chemical properties at *Labisia longistyla* sampling sites

Sample No.	Total N (%)	Avail. P (ppm)	Exc. K (cmol/kg)	CEC (cmol/kg)	Dry pH
Clump 1	1.20	55.25	0.29	46.92	3.37
Clump 2	1.00	22.75	0.34	50.51	3.33
Clump 3	0.95	29.00	0.26	41.03	3.61

Table 4 presents the total phenolic content (TPC) and Table 4 shows the DPPH value of *Labisia longistyla.* Clump 3 had the highest TPC (16,074 mg GAE/g ± 1670) and DPPH (78.7% ± 0.3) among all clumps. High light intensity received by the plants at clump 3 could be a contributing factor that caused the plants to produce high concentrations of secondary metabolites, as a defense mechanism (Isah, 2019). The concentration of TPC in *L. longistyla* from this study were higher compared to two varieties of *Labisia pumila* reported by Farah Fazwa et al. (2012b).

Table 4: Concentration of Total Phenolic Content (TPC) of *Labisia longistyla* at different clumps

Sample No.	Total Phenolic Content (TPC) mg GAE/100g
Clump 1	93.45b ± 26.1
Clump 2	75.97c ± 15.4
Clump 3	160.74a ± 16.7

Means not sharing a common letter were significantly different at $p \leq 0.05$.

Table 4: DPPH scavenging activities of different clump of *Labisia longistyla* at concentration of 100µg/ml

Sample No.	DPPH radical scavenging (%)
Clump 1	69.9c ± 0.3
Clump 2	70.6b ± 0.3
Clump 3	78.7a ± 0.3

Means not sharing a common letter were significantly different at $p \leq 0.05$.

CONCLUSION

The findings of this study could serve as a foundation for future studies on *L. longistyla*. The data proved that *L. longistyla* has the potential to be used as a highland medicinal plant due to its strong antioxidant potential. There also needs to be more research on the isolation and characterization of the active antioxidants, which may serve as a potential source of natural antioxidants. Based on these research findings, *Labisia longistyla* has the potential to be commercialized as an herbal medicine.

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