

SUSTAINABLE SUPPLY OF HIGH QUALITY RAW MATERIAL *LABISIA PUMILA* (KACIP FATIMAH) AT KAMPUNG SAGIL, LEDANG, JOHOR

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ABSTRACT

Labisia pumila or locally known as Kacip Fatimah is one of the valuable medicinal herb in Malaysia. It have been used for a long time ago as traditional medicine especially to maintain healthy female reproductive function and as postpartum medicine. It was reported that several phytochemicals in this herb such as phenolics and flavonoids have a lots of biological activity that make it effective against various illness. Nowadays, *L. pumila* getting high demands from herbal industries as well as from researchers. However, the supply of raw materials in the country for the purpose of products is insufficient. The main contributing factor is about massive harvesting and logging activities from wild. Another factors are limited cultivation area and high dependency on import raw materials from other countries. This situation eventually lead to the adulteration of raw materials and reduce the quality. Thus researchers from Plant Improvement Programme, Forest Research Institute Malaysia (FRIM) have taken initiatives to mass-produce this herb by leaf cutting and tissue culture techniques. These plants were planted as integrated plantation with rubber trees at Kg. Sagil, Ledang, Johor. They were arranged in randomized complete block design (RCBD) with three replicates and were shaded with 70% of black netting. This project which funded by Bioeconomy Community Development Program is one of the pioneer project to establish a large scale of *L. pumila* plant for future commercial production. The objectives of the study are i) to evaluate *L. pumila* growth performances produced from tissue culture and leaf cuttings after field planting and ii) to determine total phenolic content (TPC) and gallic acid from the two sources of plants after field planting. Data collection on the growth performances were collected for every three months interval (3, 6 and 9 months) after planting. Whereas, analysis of gallic acid was determined at the beginning of 3 months after planting and TPC was determined at 9 months after planting. The findings of this study showed that *L. pumila* plants by leaf cuttings and tissue culture was not significant at 3, 6 and 9 months after planting for the characteristics of leaf length, leaf width, and collar diameter. For plant height, *L. pumila* plants by leaf cuttings and tissue culture were not significant at 6 and 9 months after planting while for leaf number, the significant data was observed at 3 and 6 months after planting. However, both plants showed an increment and have better growth performance at the age of 9 months. At 3 months after planting, gallic acid from tissue culture (0.87 ± 0.01 % w/w) produced higher value compared to leaf cuttings plants (0.77 ± 0.001 % w/w). Both sources of planting materials have met the criteria according to the standard of MS 2540:2013. This study showed *L. pumila* plants either by leaf cuttings or tissue culture can be planted as large scale plantation at Kg Sagil, Ledang, Johor. This project also highlights the best harvesting time for *L. pumila*.

Keywords: integrated plantation, *Labisia pumila*, demand, growth performance

INTRODUCTION

Labisia pumila from Primulaceae family is an herbaceous under shrub plant that native to Malaysia. This herb is locally known as Kacip Fatimah (Fatimah's betel cutter). Malays women often use it as well as in other Southeast Asia regions for numerous purposes especially in promoting women's health (Wan Ezumi, 2009). Three varieties of *L. pumila* have been recognized in Malaysia (Stone, 1988) and it is believed that each of them has its own used. However, only *L. pumila* var. *alata* commonly used and getting attention from many researches as this variety widely used in traditional medicine. It is reported that the species have

important bioactive compounds that make it effective against various illnesses (Karimi et al., 2013). Some studies reported that *L. pumila* extracts contained high phenolic acid and flavonoid (Karimi et al., 2011).

Based on statistic, value of herbal related products is more than RM 4.5 billion a year in 2005 and it is expected to achieve a growth rate of 10 to 15% per annum (Anon, 2009). It shows that the demand on herbal products is keep increasing year by year. However, our herbal industries are facing with insufficient raw materials to cater the demands from the industries. The reasons might due to destructive of natural habitats because of logging activities and over-exploitation from wild. It is reported that 83% of medicinal plant raw materials were harvested from natural forest and only 17% were cultivated (Rohana et al. 2017). Eventually, most of the raw materials are imported from other country such as China, United States and Indonesia (Globinmed, 2018) without knowing the quality of the sources.

Thus, researchers Forest Research Institute Malaysia (FRIM) together with Bioeconomy Community Development Program and community of Kg. Sagil in Ledang, Johor has initiated a pioneer project under Bioeconomy Community Development Programme (BCDP) by establishing a large scale of *L. pumila* plantation for future commercial production. Therefore, this study was conducted with two main objectives; i) to evaluate *L. pumila* growth performances produced from tissue culture and leaf cuttings after field planting and ii) to determine total phenolic content (TPC) and gallic acid from the two sources of plants after field planting. It was expected that the good growth performances and high chemical content of *L. pumila* from tissue culture or leaf cuttings plants will maximize the productivity and plantation yield.

MATERIALS AND METHODS

ESTABLISHMENT OF TRIAL PLOTS

Trial plots were established at Kampung Sagil located in Ledang, Johor. A total of 180 *L. pumila* plants sourced from tissue culture and leaf cuttings were planted in Randomized Complete Block Design (RCBD) within three replicates. The plants were grown under 70% shade and watered twice a day using sprinkler system.

EVALUATION OF GROWTH PERFORMANCES

Data on the growth performances such as plant height (cm), leaf number, leaf length (cm), leaf width (cm) and collar diameter (mm) were collected at the age of 3, 6 and 9 months after planting. All data were analysed with one-way analysis of variance (ANOVA) using Minitab version 11.

DETERMINATION OF TOTAL PHENOLIC CONTENT AND GALLIC ACID

Total Phenolic Content (TPC) from the two sources of planting materials were determined at nine months after planting using Follin-Ciocalteu method by Singleton & Rossi (1965). Gallic acid, as a chemical marker of *L. pumila* was also determined at the early stage of the plantation using High Performance Liquid Chromatography (HPLC).

RESULTS AND DISCUSSIONS

Table 1 showed the analysis of variance (ANOVA) for the growth performances of *L. pumila* sourced from tissue culture and leaf cuttings. The results showed there is no significant differences ($P < 0.05$) for the growth performances of *L. pumila* produced from both propagation method at three, six and nine months after planting.

Table 1: The growth performances of *L. pumila* sourced from tissue culture and leaf cuttings at three, six and nine months after planting

Age	Propagation methods	Plant Growth of <i>L. pumila</i>				
		Height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)
3	Cuttings	6.3 ± 0.3 ^a	6 ± 0.3 ^b	14.3 ± 1.4 ^a	5.9 ± 0.1 ^a	4.8 ± 0.1 ^a
	Tissue culture	6.7 ± 0.3 ^a	7 ± 0.3 ^a	12.1 ± 0.3 ^b	5.7 ± 0.2 ^a	4.6 ± 0.2 ^a
6	Cuttings	7.1 ± 0.3 ^a	6 ± 0.2 ^b	17.1 ± 0.4 ^a	7.3 ± 0.2 ^a	5.6 ± 0.1 ^a
	Tissue culture	9.0 ± 0.3 ^b	7 ± 0.3 ^a	17.1 ± 0.4 ^a	7.4 ± 0.2 ^a	5.5 ± 0.1 ^a
9	Cuttings	8.7 ± 0.3 ^a	7 ± 0.3 ^a	18.5 ± 0.4 ^a	8.1 ± 0.4 ^a	6.2 ± 0.1 ^a
	Tissue culture	9.0 ± 0.4 ^a	6 ± 0.3	17.6 ± 0.5 ^a	7.6 ± 0.2 ^a	6.0 ± 0.1 ^a

Means followed with the same letter are not significantly different at significance level 0.05

For plant height, both plants showed no significant difference ($p < 0.05$) at three months after planting (Figure 1). However, at six months, plants from tissue culture produced greater plant height compared to leaf cuttings plant. At nine months both plants started to shows uniform growth of plant height. The maximum value of plant height for cuttings is 8.7 ± 0.3 cm and for tissue culture is 9.0 ± 0.4 cm at 9 months after planting. This finding, is in line with the study conducted by Sandhu et al. (2009) where higher early yield can be obtained from micro-propagated species compared to the conventional technique.

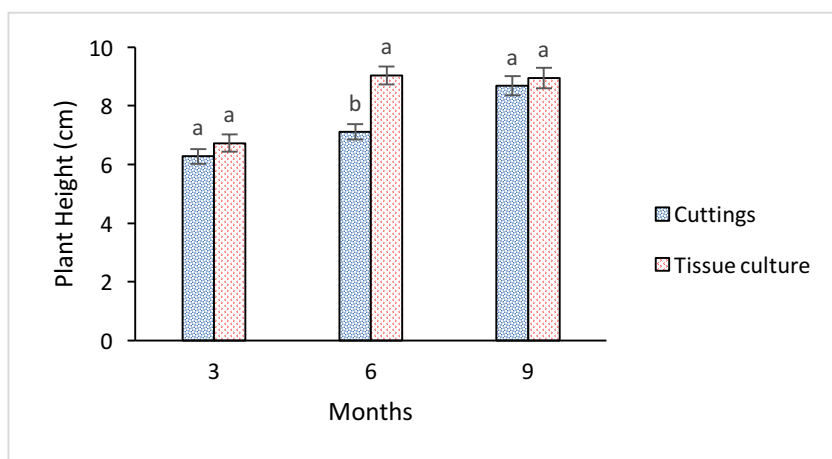


Figure 1: Plant height of *L. pumila* from cuttings and tissue culture technique after planting at field

Based on the results obtained in Figure 2, plants sourced from tissue culture produced greater leaf number compared to cuttings at three and six months after planting. However, the results showed otherwise when cuttings plants have greater leaf number compared to tissue culture at nine months after planting which may influenced by environmental factor. Theoretically, the addition of plant growth hormones during in vitro propagation are possibly influenced the vigorous leaf production and size of tissue culture plants. This is in agreement with the finding of Neeta et al., (2001), that noted micropropagated plant retained more healthy leaves, produced longer shoots and more number of leaves.

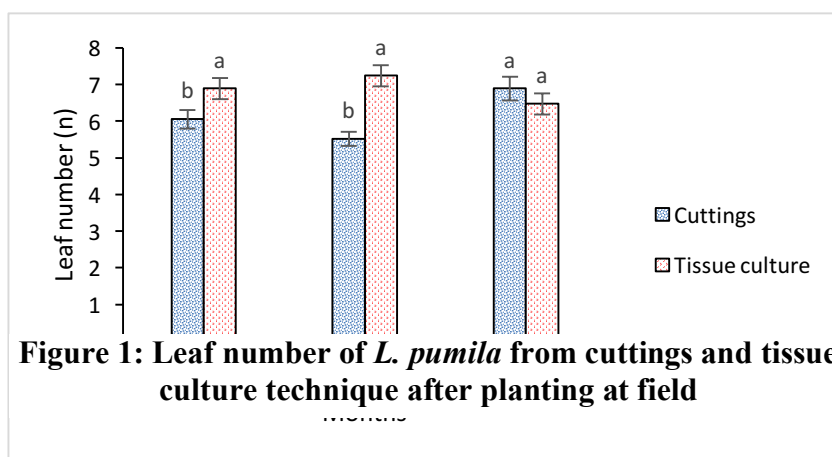


Figure 1: Leaf number of *L. pumila* from cuttings and tissue culture technique after planting at field

For leaf length results in Figure 3, tissue culture produced short leaves (12.1 ± 0.3 cm) compared to cuttings plants (14.3 ± 1.4 cm) at three months after planting. Regardless of how, both plants showed an increment in leaf length from six to nine months after plantings. The maximum leaf length of cuttings plant is 18.5 ± 0.4 cm while for tissue culture plant is 17.0 ± 0.5 cm. Plant productivity for both plants were more equal at this stages. The findings is similar with the study of Salata & Kozak, (2013) which plant productivity of Rhubarb (*Rheum rhaponticum* L.) is more equal in the third year of plantation.

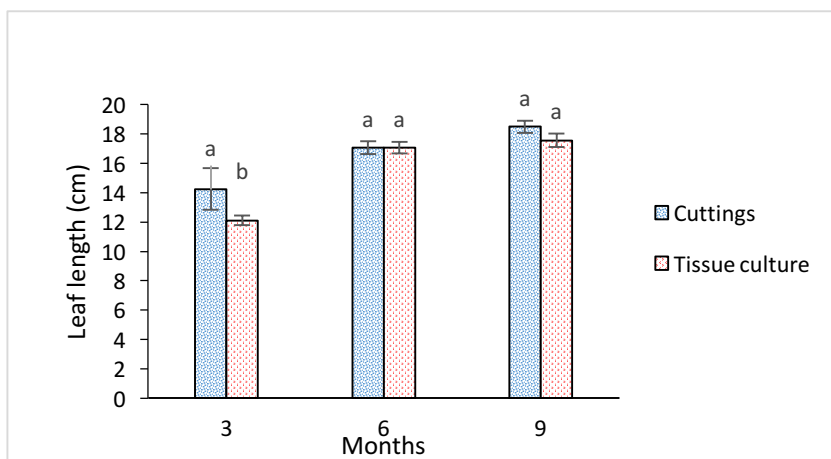


Figure 2: Leaf length of *L. pumila* from cuttings and tissue culture technique after planting at field

There were no significant differences of leaf width between two techniques; cuttings and tissue culture at three, six, and nine months after planting (Figure 4). However, both plants showed increment in leaf width from three to nine months after plantings. The range of leaf width for cutting plants is $5.9 \pm 0.1 - 8.1 \pm 0.4$ cm whereas tissue culture plants in the range of 5.7 ± 0.2 m and 7.6 ± 0.2 cm.

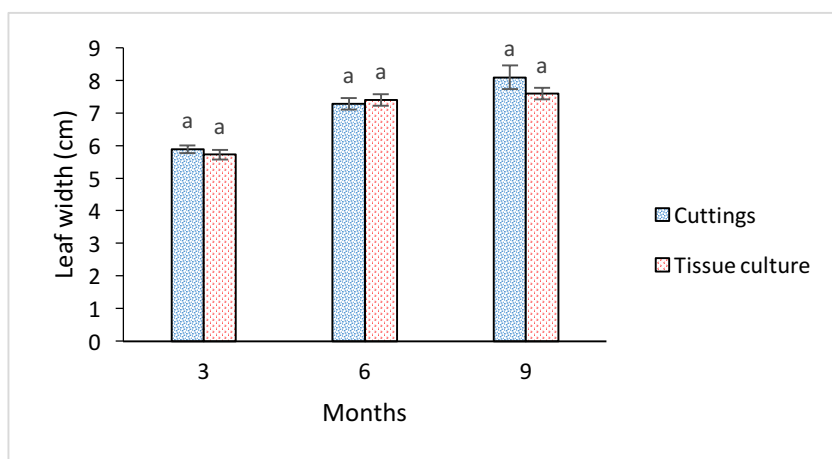


Figure 3: Leaf width of *L. pumila* from cuttings and tissue culture technique after planting at field

The similar growth pattern also were observed for collar diameter (Figure 5). There were no significant difference of collar diameter between these two propagation techniques at $P < 0.05$. The graph in Figure 5 showed an increment in collar diameter for both plants from three to nine months after planting. The range value of collar diameter for cuttings plants is 4.8 ± 0.1 cm to 6.2 ± 0.1 cm and for tissue culture plants is 4.6 ± 0.2 cm to 6.0 ± 0.1 cm.

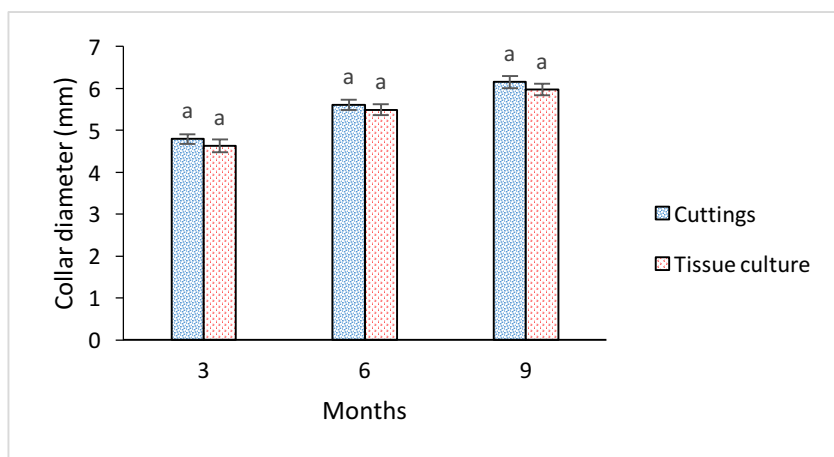


Figure 4: Collar diameter of *L. pumila* from cuttings and tissue culture technique after planting at field

In terms of total phenolic compounds, plants from tissue culture produced higher TPC (431.3 ± 19.4 mg/100g GAE) compared to the plants from cuttings, 366.0 ± 32.9 mg/100g GAE at 9 months after planting at field (Figure 6). It was also found at the age of 3 months, gallic acid in the leaves of *L. pumila* presented at the retention time of $t_r = 2.93$ min (standard, $t_r = 3.00$ min) (Figure 7). The amounts of gallic acid in plants from tissue culture (0.87 ± 0.01 % w/w) is slightly higher compared to the leaf cuttings plants (0.77 ± 0.001 % w/w). Nonetheless, both have meet the criteria according to the standard MS 2540:2013.

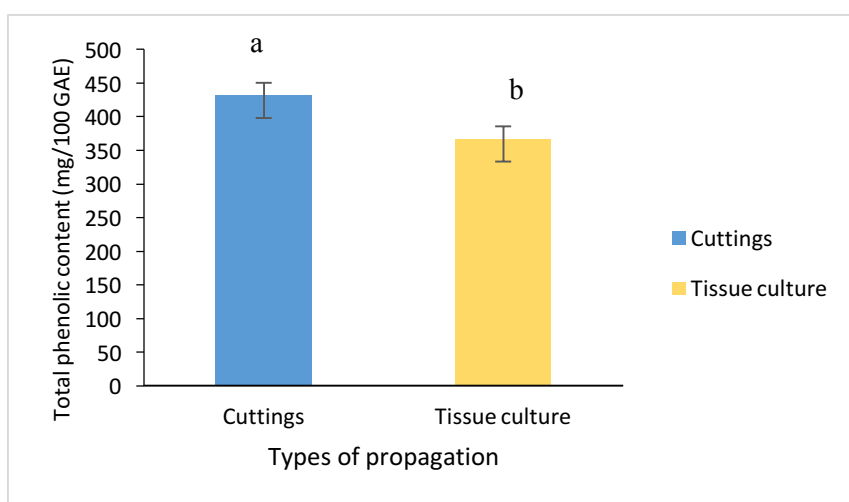
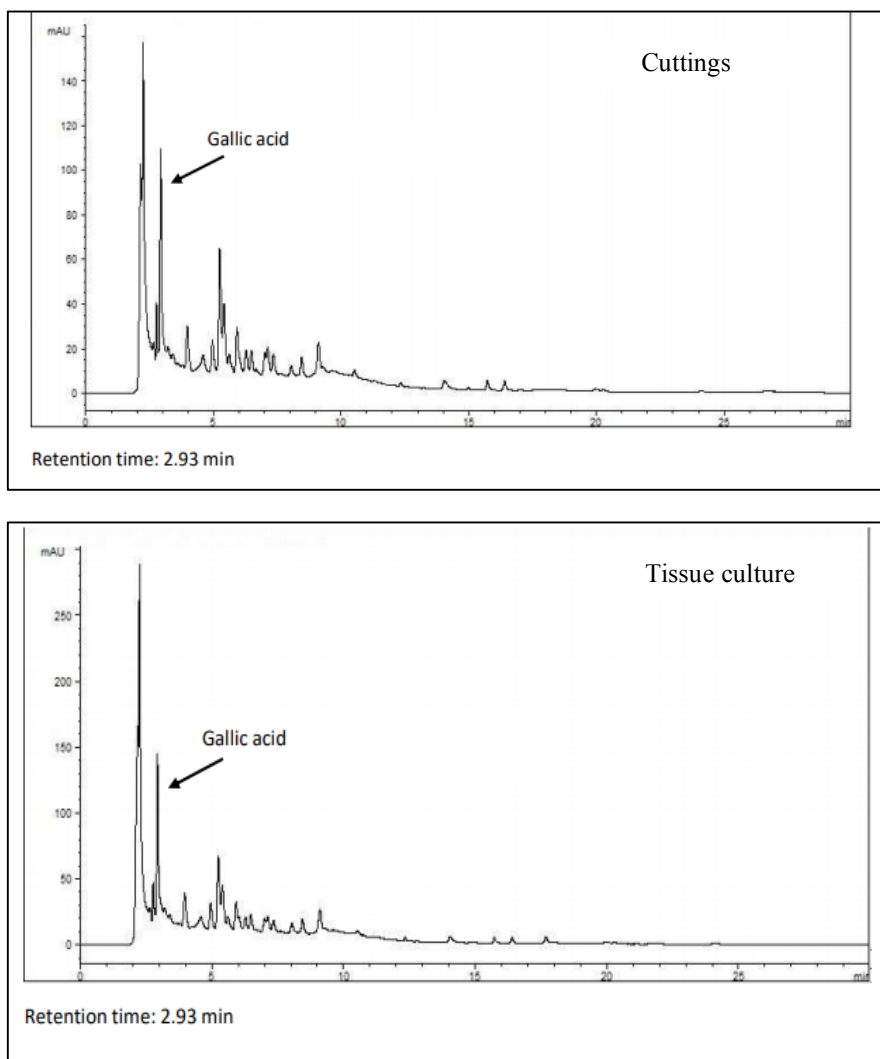


Figure 6: Total phenolic content (TPC) of *L. pumila* from cuttings and tissue culture technique after 9 months planting at field



CONCLUSION

This study concludes that *L. pumila* produced from tissue culture and leaf cuttings is practicable for commercial plantation as both plants showed good growth performances and chemical contents. The trial plot at Kg. Sagil could be one of the integrated plantation models for commercialization of *L. pumila* to local communities and directly supporting our herbal industry by reducing the dependency on imported raw materials as to meet the demands by the key players.

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