

**UNIVERSITI TEKNOLOGI MARA**

**MULTIPLICATION OF  
*Labisia pumila* var. *alata*  
(CLONE KF08)  
THROUGH LEAF CUTTINGS AND  
TISSUE CULTURE**

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## ABSTRACT

*Labisia pumila* is one of the high value Malaysia's herbal plants which are in great demand by the herbal industry. To date, there is a lack of cultivation practice being conducted on *L. pumila* as most of the raw materials supplies to the industry were collected from the wild. Therefore, this study was conducted to identify the suitable propagation method which can mass produce the raw materials of *L. pumila*. Two vegetative propagation techniques which were leaf cuttings and tissue culture have been tested on *L. pumila* var. *alata* (clone KF08). Clone KF08 was selected as the planting material as it has good traits for commercialization in terms of growth and high in total phenolic content (TPC). In leaf cuttings experiment, the leaves were cut into three different parts (upper, middle and lower) in standard size of 30 cm<sup>2</sup> and grown in a mist propagation chamber. Subsequently, the successful rooted cuttings were potted in planting media to enhance shoot production and acclimatized before being transferred to nursery. In propagation by tissue culture, matured leaves were used as the explant for culture initiation. Direct shoot regeneration procedure was applied in this experiment. MS media with combination of BAP (0.1, 0.5, 1.0 mg/L) and NAA (0.1, 0.5, 1.0 mg/L) were prepared as the treatment media to initiate the culture. At multiplication stage, *in vitro* leaves explants were subcultured in similar treatment media as in culture initiation while the *in vitro* nodal explants were subcultured in different MS strength (1/4 MS, 1/2 MS and full MS) in temporary immersion system (TIS) (RITA™). Only plantlets produced through TIS were acclimatized and evaluated. Among the variables observed for growth assessment were stem height, number of leaves, leaf length, leaf width and collar region. The growth assessments were conducted once per month until nine months. While the biomass and total phenolic content (TPC) were evaluated once at nine months period. The results from this study revealed that there were statistically significant difference ( $p < 0.05$ ) between plants produced from leaf cuttings and tissue culture in terms of their growth performances, except for stem height, biomass and TPC. Plants produced from leaf cuttings have greater leaf length ( $8.56 \pm 0.37$  cm), leaf width ( $4.10 \pm 0.16$  cm) and collar region ( $3.31 \pm 0.1$  mm) compared to tissue culture plants (leaf length:  $7.31 \pm 0.39$  cm, leaf width:  $4.10 \pm 0.16$  cm and collar region:  $3.31 \pm 0.11$  mm). However, in terms of leaves production, tissue culture ( $7.80 \pm 0.22$ ) methods able to produce high number of leaves than leaf cuttings ( $4.98 \pm 0.18$ ). For stem height, leaf cuttings plants recorded  $5.15 \pm 0.21$  cm and tissue culture plants recorded  $5.13 \pm 0.21$  cm. The total biomass for nine month old plants from leaf cuttings (fresh weight:  $3.42 \pm 0.40$ g; dry weight:  $0.68 \pm 0.08$ g) and tissue culture (fresh weight:  $2.83 \pm 0.67$ g; dry weight:  $0.57 \pm 0.13$ g) were quantified as followed. For TPC analysis, both leaf cuttings and tissue culture plants recorded  $320.78 \pm 17.19$  mg/100g GAE and  $326.21 \pm 13.37$  mg/100g GAE, respectively. From the overall assessment, leaf cuttings technique able to produce plants with good growth performance in 30 weeks interval. However, a lot of mother plants are required to conduct the cuttings since a single cutting can produced only  $1.50 \pm 0.10$  shoots per cutting. On the other hand, tissue cultured plants can produce  $6.14 \pm 0.40$  shoots per explant although longer period is required for plantlet establishment. Therefore, tissue culture was selected as the suitable propagation technique for mass production of high quality *L. pumila*.

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# CHAPTER ONE

## INTRODUCTION

### 1.1 Research Background

#### 1.1.1 Overview of Supply and Demand of Herbal Plants in Malaysia

Malaysia is a recognize country rich in bio-resources. About 1200 of plants species in our forest have medicinal values with great potential for commercialization (Rasadah, 2005). Today, the herbal industry has become one of the economic engines to the nation. In Malaysia, the local herbal industry had a market value of RM 7.97 billion in 2005 and is growing at a rate of 10% per annum (Zainal Azman, 2007). The growth of the herbal industry is affected by changes in household economic condition, lifestyle, emphasis on health and increasing cost of synthetic medicine (Norani et al., 2008).

The development and sustainability of the herbal industry depends on the source of materials. In a detailed survey conducted by Forest Research Institute Malaysia (FRIM), the forest was a main source of raw materials for planting material suppliers (26%) and cultivators (19%), while the other source was obtained from nurseries, planted by neighbors and government agencies (Ariff Fahmi, 2017). The supply of raw material might not be sustainable in the long run, thus Malaysia still depends on imported herbs and herbal products mainly from China to meet the high demand (Rohana et al., 2017).

Uncertainty in raw material supply and lower price of the imported raw materials than the local one would be the ultimate concern under the production of raw materials. The price of herbs are varies depending on its origins, sources, volumes and form of the herbs (Mohd Azmi and Norini, 2000). In a report prepared by Zurinawati (2004) on medicinal plants and products Malaysian industry status, the various price differences indicated that there is no standard market price either it is collected from the wild or cultivated. As an example, the estimated price for fresh *Labisia pumila* and *Eurycoma longifolia* is in the range of RM 2.50 – RM 10.00/kg while the dried can be up to RM 25 – RM 100/kg. Whereas the fresh price of *Pyllanthus niruri* and *Centella asiatica* is around RM 2.25 – RM 3.00/kg and the dried raw material roughly about