



UNIVERSITI PUTRA MALAYSIA

***PHYTOCHEMICALS ANALYSIS AND ANTIOXIDANT ACTIVITIES FROM
WILD, IN VITRO-DERIVED PLANTLET AND CALLUS OF KACIP
FATIMAH (*Labisia pumila* var. *alata* Benth.)***

NAJHAH BINTI MAT YUNUS

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By

NAJHAH BINTI MAT YUNUS

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
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**PHYTOCHEMICALS ANALYSIS AND ANTIOXIDANT ACTIVITIES FROM
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NAJHAH BINTI MAT YUNUS

July 2021

Chair : Mohd Hakiman Mansor, PhD
Faculty : Agriculture

Labisia pumila or Kacip Fatimah is a herbaceous plant that grows widely in the shade of the tropical forest floor and has high flavonoids, phenolic, and other phytochemicals content. *L. pumila* was sought after and high demand for its raw materials because of its medicinal benefits causing the source for this herb in natural habitat to decrease because of its slow growth rate. Hence, the plant tissue culture technique was used to facilitate large-scale production to increase the supply and to make sure this plant did not face extinction and as a tool to produce plant secondary metabolites.

The present study examines the ability of *L. pumila* plantlets to multiply in *in vitro* system under different plant growth regulators such as 6-benzylaminopurine (BAP), zeatin and kinetin supplemented on Murashige and Skoog (MS) media as well as to analyze bioactive compounds and comparing the phytochemicals analysis between wild plants, *in vitro* plantlets and callus culture of *L. pumila*. After six weeks of propagation, the results showed that there are significant differences in all types of treatment of plant growth regulators towards shoot multiplication of nodal explants. Medium supplemented with 3, 1 and 2 mg/L of zeatin resulted in the optimum mean number of shoot per explant, shoot length and number of leaves with 6.6, 4.3 cm and 3.1, respectively. 3 mg/L and 1 mg/L of zeatin also have been observed to be the most optimum results for the percentage of shooting responses, both with one hundred of percentage.

After four weeks of culture, the results showed significant differences between 2,4-dichlorophenoxyacetic acid (2,4-D) and picloram for the callus induction experiment. MS medium supplemented with 0.5 mg/L of 2,4-D recorded 60% of

callus induction than other concentrations. The MS media supplemented 0.5 mg/L of 2,4-D was then further combined with 0.25 mg/L of cytokinins; BAP, zeatin, kinetin and thidiazuron (TDZ) and the results showed that 0.5 mg/L of 2,4-D in combination with 0.25 mg/L of zeatin recorded 100% of callus induction in only 14 days.

Leaf obtained from *in vitro* culture and wild plant and callus of *L. pumila* var. *alata* were extracted and analyzed for antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant power (FRAP) methods, total flavonoid and total phenolic content. Callus culture of *Labisia pumila* var. *alata* showed substantial antioxidant outcomes in DPPH with 75.88 mg TE/g DW and FRAP techniques with 151.76 mg TE/g DW when compared to *in vitro* plantlet and wild plant. Total phenolic content (1.9 mg GAE/g DW) and total flavonoid content (2.38 mg QE/g DW) of callus culture also were significantly higher than *in vitro* plantlet and wild plant.

In conclusion, the results strongly show that callus of *L. pumila* var. *alata* is a good source of antioxidant compared to *in vitro* plantlets and wild plants since the content of antioxidant in this culture are highly significant compared to others.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

**ANALISIS FITOKIMIA DAN AKTIVITI ANTIOKSIDAN POKOK LIAR,
TANAMAN *IN VITRO* DAN KALUS POKOK KACIP FATIMAH (*Labisia pumila*
var. *alata* Benth.)**

Oleh

NAJHAH BINTI MAT YUNUS

Julai 2021

Pengerusi : Mohd Hakiman Mansor, PhD
Fakulti : Pertanian

Labisia pumila atau Kacip Fatimah adalah pokok herba yang tumbuh meluas dalam kawasan teduhan hutan hujan tropika dan telah terbukti mengandungi flavonoid, fenolik dan kandungan antioksidan yang tinggi. Sejak penemuan ciri perubatan yang dimiliki pokok ini, *L. pumila* menjadi carian ramai dan mendapat permintaan yang tinggi terhadap bahan mentahnya di pasaran menyebabkan sumber pokok herba di habitat semulajadi ini dilihat menurun kerana pertumbuhannya yang lambat. Oleh itu, teknik tisu kultur tumbuhan digunakan untuk memudahkan pertumbuhan berskala besar bagi tujuan menambahkan bekalan dan untuk memastikan tumbuhan ini tidak mengalami kepupusan.

Tujuan kajian ini adalah untuk mengetahui kemampuan anak pokok *L. pumila* untuk tumbuh secara berganda dalam sistem *in vitro* dibawah rawatan hormon tumbuhan yang berbeza iaitu BAP, zeatin dan kinetin yang ditambahkan ke dalam media Murashige dan Skoog (MS) dan juga untuk menganalisis bahan bioaktif dan membandingkan bahan bioaktif diantara tumbuhan liar, tumbuhan kultur tisu dan kalus. Selepas enam minggu tempoh inkubasi, keputusan menunjukkan terdapat perbezaan signifikan pada semua jenis hormon tumbuhan terhadap penggadaan pucuk menggunakan eksplan nod batang. Media mengandungi 2 mg/L, 1 mg/L dan 3 mg/L zeatin masing-masing menghasilkan purata optimum untuk bilangan pucuk setiap pokok, panjang dahan dan bilangan daun masing-masing dengan 6.6, 4.3 cm dan 3.1. 3 mg/L dan 1 mg/L zeatin juga menunjukkan keputusan purata optimum untuk peratusan respon induksi pucuk dengan kedua-duanya merekodkan seratus peratus respon.

Selepas empat minggu kultur, keputusan menunjukkan terdapat perbezaan signifikan di antara 2,4-D dan picloram pertumbuhan kalus *Labisia pumila* var. *alata*. Media MS mengandungi 0.5 mg/L 2,4-D mencatatkan 60% pertumbuhan kalus berbanding kepekatan 2,4-D dan picloram yang lain. 0.5 mg/L 2,4-D seterusnya digabungkan dengan 0.25 mg/L sitokinin seperti BAP, zeatin, kinetin dan TDZ dan keputusan menunjukkan gabungan di antara 0.5 mg/L 2,4-D dan 0.25 mg/L zeatin mencatatkan 100% pertumbuhan kalus hanya selepas dua minggu inkubasi.

Daun yang diperolehi daripada kultur *in vitro* dan tumbuhan liar serta kalus *L. pumila* variasi *alata* telah diekstrak dan dianalisis untuk aktiviti antioksidan menggunakan kaedah DPPH dan FRAP, kandungan flavonoid dan kandungan fenolik. Kultus kalus *L. pumila* var. *alata* menunjukkan penghasilan antioksidan yang banyak melalui DPPH dengan 75.88 mg TE/g DW dan kaedah FRAP dengan 151.76 mg TE/g DW berbanding kultur *in vitro* dan tumbuhan liar. Kandungan fenolik (1.9 mg GAE/g DW) dan kandungan flavonoid (2.38 mg QE/g DW) kultur kalus juga jauh lebih tinggi berbanding kultur *in vitro* dan tumbuhan liar.

Kesimpulannya, keputusan jelas menunjukkan kultur kalus adalah sumber terbaik untuk menghasilkan antioksidan berbanding daun daripada anak pokok dan tumbuhan liar memandangkan kandungan antioksidan dalam kultur ini sangat signifikan berbanding yang lain.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd Hakiman Mansor, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Hawa ZE Jaafar, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Juju Nakasha Jaafar, PhD

Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 9 December 2021

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman
of Supervisory
Committee: Dr. Mohd Hakiman Mansor

Signature: _____
Name of Member of
Supervisory
Committee: Prof. Madya Dr. Hawa ZE Jaafar

Signature: _____
Name of Member of
Supervisory
Committee: Dr. Juju Nakasha Jaafar

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LIST OF ABBREVIATIONS

2,4-D	2,4-dichlorophenoxy acetic acid
AlCl ₃	Aluminium chloride
B5	Gamborg basal media
BAP	6-benzylaminopurine
Dicamba	3,6-dichloro- <i>o</i> -aniscic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric reducing antioxidant potential
GAE	Gallic acid equivalent
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
MS	Murashige and Skoog basal media
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PGR	Plant growth regulator
Picloram	4-amino-3,5,6-trichloropicolinic acid
QE	Quarcetin equivalent
ROS	Reactive oxygen species
rpm	Rotations per minute
TDZ	Thidiazuron
TE	Trolox equivalent

CHAPTER 1

INTRODUCTION

1.1 General

Roughly more than 2,000 species of plants with medicinal and therapeutic properties have been recognized right now in Malaysia (Nik Hussain and Kadir, 2013). Saw *et al.* (2006) announced that 63% of patients utilized natural medication among multi-ethnic patients in Malaysia. As per Nadia *et al.* (2012), *Labisia pumila* (Kacip Fatimah), *Eurycoma longifolia* Jack (Tongkat Ali), *Orthosiphon stamineus* (Misai Kucing), *Quercus infectoria* (Manjakani), and *Piper sarmentosum* (Daun Kaduk) are among the renowned species that have been broadly utilized and concentrated in Malaysia. *L. pumila* recorded as one of the five critical herbal plants that can add to the Gross National Income (GNI) through the National Key Economic Area (NKEA) initiative made to guarantee an adequate supply of crude materials for Research and Development (R&D) and clinical preliminaries before production at the initial phase of the Entry Project Point (EPP) of the NKEA (Pemandu, 2013; Nabilah *et al.*, 2015).

Labisia pumila, referred to generally as Kacip Fatimah, is an herbaceous plant that grows broadly in the shade of the tropical forest floor. Malaysia utilizes this plant as a customary medication to believe in offering advantages to ladies' wellbeing. Concentrates of *L. pumila* were found to have high flavonoids and phenolic substances and different phytochemicals that can be separated and few investigations additionally revealed that *L. pumila* has potential pharmacological properties like antioxidant, antibacterial and anticarcinogenic (Jualang *et al.*, 2015; Hasan *et al.*, 2015). It is likewise believed to be used as a potential remedy for dysentery, flatulence, dysmenorrhoea, and gonorrhea; subsequently, the interest is further expanding (Hasan *et al.*, 2014). Lee *et al.* (2012) announced that because of its phytoestrogenic activity that is significant for protection against hormonal dependent cancer such as breast, uterine, prostate cancers, *L. pumila* is in entirely good necessities. The plants are usually boiled, and the decoction drink of *L. pumila* has generally been utilized by ages of Malay ladies believed to prompt and ease labor, and as wellbeing postnatal prescription to help contract the birth channel, to condition the abdominal muscle, and to recover body strength (Chua *et al.*, 2012; Nik Hussain and Kadir, 2013).

Despite the medicinal benefits of *L. pumila*, if used without proper handling and dosage, the usage of this plant might lead to toxicity. Past studies demonstrate that *L. pumila* has been utilized by Malay ladies to ease labor during pregnancy and as postpartum medication, however, the majority of them utilize this herbal medication without stressing over its safety. Reports by Sooi and Keng (2013)

on the use of medicinal herbs by Malay ladies has discovered that 77.2% of ladies accepted herbal medication has natural substances and not have chemical elements and has been a practice for many generations, causing the thoughts of herbal medications as safe and effective medicines. They also noted the inappropriate dosages and dosages form in their study (Sooi and Keng, 2013) because of the diverse social convictions and practices in Malaysia (Law *et al.*, 2009; Soon *et al.*, 2009; Azriani *et al.*, 2008; Mustafa, 2003; Azriani *et al.*, 2007). Utilization of natural medication during pregnancy ought to be practiced with caution since there is possible harm to the fetus because of the pharmacologically active compound in herbal medications (Soon *et al.*, 2009; Azriani *et al.*, 2008; Azriani *et al.*, 2007).

The present study examines the ability of *L. pumila* plantlets to multiply in *in vitro* system under different plant growth regulators as well as to produce and compare antioxidant activities in wild plants, *in vitro* plantlets, and callus. Thus, to characterize it, the formation of callus induction of the plantlets will be determined based on the optimization of parameters using different concentrations of plant growth hormone.

1.2 Justification

Since the discoveries of its medicinal values and its commercial values, *Labisia pumila* has attracted the attention of researchers around the world. Its slow growth rate does not meet the demand for this plant, leading to extinction and severe genetic loss (Ling *et al.*, 2013). There is a lack of studies on micropropagation of *L. pumila* and the potential production of bioactive compounds via callus and the cell suspension is obscure. There are many studies of the bioactive compounds of the wild plant of *L. pumila* through various methods of extractions (Chua *et al.*, 2011; Karimi *et al.*, 2011; Norhaiza *et al.*, 2009; Avula *et al.*, 2011; Ali and Khan, 201; Karimi *et al.*, 2017) with the latest study of the extraction of the phenolic compound of *L. pumila* using supercritical fluid extraction (SFE) by the study by Radzali *et al.* (2020) but none of these studies using extraction from tissue culture of *L. pumila*. To satisfy the increasing demands for *L. pumila*, *in vitro* propagation methods were developed since the conventional propagation of this species is difficult and takes much time for germination to occur (Hasan *et al.*, 2015; Nabilah *et al.*, 2015). Essentially, for the development of large quantity and good quality of plant as far as phytochemical profile and bioactive properties, standardization of *L. pumila* and products derived from extracts can be executed even on a larger scale (Chua *et al.*, 2012). Numerous studies have demonstrated callus culture as an improvement for the production of bioactive compounds in medicinal plants. Hence, to reduce the production cost for future business creation, broad investigations on plant tissue culture optimization, particularly callus culture, are important to increase cell biomass and production of plant secondary metabolites (Hasan *et al.*, 2014).

1.3. Research objective

There are three objectives in this study:

1. To study the effect of plant growth regulators in *in vitro* propagation and multiplication of *Labisia pumila* using nodal segment as explant.
2. To induce and characterize calli using a single of auxin and a combination of auxins and cytokinins.
3. To investigate the antioxidant production in wild plant, *in vitro* plantlet, and callus culture of *Labisia pumila*.



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