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Effect of hormone on proliferation of labisia pumila / by  
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**PERPUSTAKAAN SULTANAH NUR ZAHIRAH UMT**

EFFECT OF HORMONE ON PROLIFERATION OF *LABISIA PUMILA*

By

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the requirements for the award of degree of  
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## DECLARATION

I hereby declare that this thesis entitled Effect of Hormone on Proliferation of *Labisia pumila* is the result of my own research except as cited in the reference.

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## EFFECT OF HORMONE ON PROLIFERATION OF *LABISIA PUMILA*

### ABSTRACT

*L. pumila* is a common traditional herb in Malaysia which is more specific with woman health. It claimed to enhance sexual health, as anti aging and faster the delivery during labor and immediately regain strength after birth. The aim of this experiment is to determine the effect of hormone on proliferation of *L. pumila*. Stem was used as explants in this experiment. Explants were cultured in B5 media with BAP, kinetin and zeatin at concentration of 0, 0.5, 1, 2 and 3 mg/L. All cultured media were left to growth respectively for two month and the data is analyzed. Results show that kinetin and BAP does not show any significant difference on shoot tips and leaves productions. Three mg/L zeatin produce the highest shoot tips and explants that produce highest of leaves was in 0.5 mg/L zeatin



## KESAN HORMON TERHADAP PEMBIAKAN *LABISIA PUMILA*

### ABSTRAK

*L. pumila* merupakan tumbuhan herba yang terdapat di Malaysia yang lazimnya adalah spesifik untuk kegunaan kaum wanita. Tumbuhan ini dikatakan dapat meningkatkan tenaga batin, melambatkan proses penuaan dan mempercepatkan proses melahirkan anak serta mengembalikan tenaga selepas bersalin. Tujuan kajian ini dijalankan adalah untuk mengkaji kesan hormon terhadap pembiakan *L. pumila*. Keratan batang daripada tumbuhan ini digunakan sebagai eksplan. Eksplan dikultur dalam media B5 bersama-sama hormon BAP, kinetin dan zeatin pada kepekatan 0, 0.5, 1, 2, dan 3 mg/L. Kesemua kultur dibiarkan selama dua bulan untuk membiak dan kemudian data di analisis. Keputusan menunjukkan kinetin dan BAP tidak memberikan sebarang kelebihan dalam penghasilan pucuk dan daun. Tiga mg/L zeatin menghasilkan kadar pertumbuhan yang paling tinggi manakala eksplant yang menghasilkan bilangan daun yg paling tinggi adalah pada medium yg dikultur pada medium 0.5 mg/L zeatin.

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**LIST OF ABBREVIATIONS**

BAP	Benzylaminopurine
NAA	2,4-dichlorophenoxyacetic acid
2-iP	N <sup>6</sup> - (2-isopentyl) adenine
L	Liter
ml	milliliter
mm	millimeter
g	gram
mg	milligram
mg/L	milligram per liter
°C	degree celcius

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Medicinal Herb

*Labisia pumila* is a common traditional herb in Malaysia along with Tongkat Ali (*Eurycoma longifolia*). Compared to Tongkat Ali, Kacip Fatimah the usage is more specific for woman health. In Malaysia, *L. pumila* is popularly known as Selusuh Fatimah (literally Fatimah's childbirth medicine) and Kacip Fatimah (Fatimah's betel scissors). Kacip Fatimah is used as traditional medicine to maintain healthy female reproductive system (Griuen, 2008).

Even though both Kacip Fatimah and Tongkat Ali play same role as a medicinal herbs, there is here is one fact that strongly sets Kacip Fatimah different from Tongkat Ali. The taste of Tongkat Ali extract is so bitter compare to Kacip Fatimah which has a pleasant taste. Actually, comparatively high dosages of kacip fatima, boiled as tea, have a taste that is so similar to the taste of green tea that the two are indeed very hard to distinguish, taste-wise. Kacip Fatimah does not contain caffeine or theobromine like green tea. It doesn't keep awake and also not a diuretic. Kacip Fatimah also not vasoconstrictive ( Griuen, 2004)

## 1.2 Benefits of *L. pumila*

Usually, peoples mainly in Southeast Asia mainly in Malaysia and Indonesia used Kacip Fatimah to enhance sexual health. Aside from that, there are other benefits that can be getting from this plant. This plant can act as anti aging factor. Recent collaboration research by the University Technology of Malaysia (UTM) and Dongguk University of Korea in 2010 has proved has proved that it helps to stimulate collagen production that keeps connective skin tissue together. It is also proven that it can reduce melanin production and act as anti-oxidant that can make skin fairer and reduce face spots. All of these can result in every woman's desired ageless appearance. Kacip Fatimah also used as a supplement to maintain woman body shape. With frequent intake of Kacip Fatimah, women's abdominal muscle can be toned and firmed. It also helps to increase metabolic functions thus helping women to accomplish a slimmer figure. Finally intake of this medicinal herb can ease during Pre- and post-labor. For pregnant women, it can help reduced the pain of pre and post-labor. They have to make it a routine to drink a braised dried Kacip Fatimah roots. The plant mixture can help women fasten the delivery during labor and immediately regain strength after birth (Lim, 2010).

## 1.3 *L. pumila* as a commercial product

In natural habitat, the growth rate of *L. pumila* is very slow (Mohd Noh et al., 2002) and normally it was propagated through seeds and stem cuttings but seeds are difficult to obtain due to depletion of mother plants. Furthermore, there has so far very little attempt to cultivate this plant (Indu Bala & Ng, 2000; Rozihawati et al., 2003). Therefore, the development of an *in vitro* will be of a great importance for production of planting material to decrease the pressure on the natural populations.



Therefore, this species was broadly used in preparation of herbal product and highly demanded for the manufacturing of commercial product (Jamia et al., 2003). *L. pumila* has been widely used in the herbal and pharmaceutical industries as a health tonic especially for women (Latiff, 1997; Houghton et al., 1999). As Malaysia is a one of the larger supplier for the herbal product, Malaysian market for herbal and natural product has been estimated to be worth RM4.55 billion of which 90% of the raw material used was imported. Besides that, harvesting and cultivation are critical aspects of herbal production with only a small percentage is cultivated, with some still on trial basis (Drewe, 1998; Chang & Rasadah, 2004; Ramlan & Sivakumar, 2006) .So to encounter this problem, biotechnology is used. Burkill (1966) stated that using biotechnology to increase the population of *L. pumila* is the best ways. Biotechnology not just can increase the quantity of this plant, but also can conserve nature.

#### **1.4 Objective**

This experiment was conduct to determine effect of hormones on proliferation of *L. pumila*.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 *Labisia pumila* (Kacip Fatimah)

*Labisia pumila* is a very popular herb among peoples in Malaysia. This herb is from family of Myriaceae. This subherbaceous plant with creeping stems found usually in the lowland and hill forest of Peninsular Malaysia at an altitude of 300-700 m. They also can be found in Thailand, Indochina, Philippines and New Guinea (Stone, 1988; Jamia & Houghton, 2000; Wiart and Wong, 2002; Ong, 2004).

*Labisia pumila* have three varieties which is known as Kacip Fatimah: *Labisia pumila* var. *alata* (LPva), *Labisia pumila* var. *pumila* (LPvp) and *Labisia pumila* var. *lanceolata* (LPvl) (Stone, 1998). Among all these varieties, LPva is usually used for treatment (Burkill, 1935; Zakaria and Mohammed, 1994). According to Stone (1998), this traditional herb was differentiating according to its petioles which are attached at the leaves structure. LPva has wide petioles. LPvp is a little margin and LPvl has leaves and petioles with tapering in shape but its leaves does not wide, only 5-13 cm. In Malaysia, there are five types of Kacip Fatimah, differentiate by its distinctive leaves. This research discovered by Malaysian Agricultural Research and Development Institute (MARDI). The types of this plants are white, green, red keriting and light green (Raihanah, 2002).

To consumed this plant, usually it will be boiled and the water decoction taken as a drink. The water extract from *L. pumila* inhibits estradiol binding to antibodies against estradiol, suggesting the presence of estrogen-like compounds (Husniza, 2002). To assured this herb is good for woman, an experiment were conducted into rat. In ovariectomized rats, orally administered *L. pumila* decreases body weight and plasma concentrations of resistin, increases plasma and adipose tissue mRNA levels of leptin and induces a dose-dependent increase in uterine weight (Fazliana et al., 2009). So the *L. pumila* appears to have estrogenic properties exert uterotrophic effects, and regulate body weight possibly by modulating mRNA expression and secretion of leptin (Manneras et al., 2007).

According to Griuen (2008), Kacip Fatimah is traditionally used to maintain a healthy female reproductive system, to help tighten and lubricate, and to enhance sexual function. Kacip Fatimah consists and rich in phytoestrogen and isoflavones, that may ease menopausal symptoms. The *L. pumila* roots were found to contain a benzoquinone derivative and mixture of resorcinol derivatives. Based on partial characterizations of some isolated compounds, a pelagonidin derivative and long alkenyl chain were suggested to be present.

## **2.2 In vitro process of *L. pumila***

According to Leonard and Kil (1990), tissue culture technique was developed to rapidly obtain large numbers of plants from selected individual plants. It is started when part of the plants (explants) like leaf, stem or cell were cultured in a aseptic and sterile media.

Totipotency is a very important concept to understand tissue culture. This concept defines as the capacity, exhibited by certain types of isolated differentiated plant cell, to regenerate whole plants. The phenomenon is seen as evidence for the theory that all nucleated plant cells possess all the genes necessary to direct the formation of a complete plant. To realize this potential the cell must be removed from the inhibiting influence of

the rest of the plant body and given the appropriate stimuli, namely the correct balance of nutrients and growth substances (Walden, 1999).

*L. pumila* and *Eurycoma longifolia* can be produced by cloning technique where the cells or organs of the plant were put into a media supply with all nutrients needed by the plant and in addition of specialized hormone for one month. Within the process, there will be many of buds will grown from the cells or the organs then transferred into other media for isolation and the next steps of growth. After 2 month growth in the second media, the plants are ready to be planted to natural environment.

During tissue culture, selections of explants are very important. Larger explants will produce more calluses because large callus consist large number of cells (Torres, 1989). Opposite with Langhans (1977), bigger size sometimes causing explants more easily infected by bacteria and fungus. The more optimum size of explants that that can faster the production of callus is 0.5-1.0 cm (Jone, 1979).

### **2.3 Treatment using plant growth regulator (PGR)**

Organic compound, either natural or synthetic, that modifies or controls one or more specific physiological processes within a plant is called plant growth regulator (PGR). Other definitions that define PGR are by Liub and Schott (1990). They said, for practical purposes, PGRs can be defined as either natural or synthetic compounds that are applied directly to a plant to alter its growth processes or structure in some beneficial way. The term plant hormone used if the compound is produced within the plant. Environmental Protection Agency defined plant regulator as any substance or mixture of substances intended, through physiological action, to faster or slower the growth or maturation or otherwise alter the behavior of plants or their produce. Plant growth regulator had been characterize by their low rates of application, which is high application rates of the same compounds often are considered herbicidal (Peggy, 1999).

Cytokinins are compounds with a structure derivative from adenine which promote cell division and have other similar function like kinetin. The first cytokinin was discovered is kinetin and it is named as cytokinin because the compound have an ability to promote cytokinesis (cell division). Even though it is a natural compound, cytokinin is not made in plants and so usually considered as “synthetic” cytokinin which mean that the hormone is synthesized somewhere other than in a plant. Zeatin is the common cytokinin hormone that forms naturally in plant today which was isolated from corn (*Zea mays*) (Mauseth, 1991; Salisbury and Ross, 1992).

There are six general functions of cytokinin. But all these function depends on types of cytokinin and also types of the plant itself. The functions are; stimulates cell division, stimulates morphogenies (shoot initiations/bud formation) in tissue culture, stimulates the growth of lateral buds-release of apical dominance, stimulates leaf expansion resulting from cell enlargement, may enhance stomatal opening in some species, and promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis ( Mauseth, 1991; Salisbury and Ross, 1992; Davies, 1995).

Cytokinin is a essential requirement for induction of multiple shoots from cotyledon (Yang et al., 2001) and epicotyls or hypocotyls explant in different plant species (Figueiredo et al., 2001; De Paiva Neto et al., 2003). These is because cytokinins are made and synthesized in root tips and are active in the maintenance of ongoing processes and nutrient mobilization in the shoot (Hearn and Constable, 1984). According to Moore (1989), cytokinins might be rapidly incorporated into micromolecules such as RNA. From the finding, it could be stated that cytokinins promote RNA and protein synthesis and that exogenous cytokinins often have highly localized effects when applied to whole plants and plants organs.

Since most plant development and growth are depending by natural plant hormone, many of these processes have been manipulated either by altering the plant hormone level or increasing the capacity of the plant to it natural hormone. By having the

technology of altering the development system of plants, many characteristic of the plant can be produce according on what it desire. Indirectly, it may increase the agriculture productivity or other sector generally (Sawan et al., 2000).

## CHAPTER 3

### METHODOLOGY

#### 3.1 Selection of plants

Plant that was used in this experiment is *Labisia. pumila*. This medicinal herb was obtained from Biotechnology Laboratory of University Malaysia Terengganu (UMT). Explants that used in this experiment are stem.

#### 3.2 Media and treatment

B5 media were used in this experiment. As for the treatment the media were supplemented with hormones BAP, kinetin, and zeatin are used. Each treatment hormones are then divided into for concentration; control (0 mg/L), 0.5 mg/L, 1 mg/L, 2 mg/L and 3 mg/L. Hormones BAP and kinetin were mix with the media before autoclave because this hormones are not heat labile constituent. For heat labile constituent like zeatin, it should not be autoclaved but filter sterilized before adding to the autoclaved culture medium after the medium has cooled to 40-50 °C. PP/PE syringe and Acrodisc syringe membrane filter are used. The PP/PP syringe was filled with zeatin. After that, the Acrodisc syringe membrane filter was mounted on the filter. The hormone in the syringe is then filtered into the sterile culture tube. This operation should be performing in the laminar flow cabinet to avoid any contamination to the media supplemented (Michael & Paul, 2010).

### 3.3 Proliferation process

Each hormone that already prepared was put into test tubes. Each test tube then was filled with five ml of media supplemented hormones. To get the mean number of leaves and shoot tips, each concentration of hormones is divided into five replicates. After cultured the explants, all test tubes were kept in culture room with the temperature of 27 °C (room temperature). two months or eight weeks are taken to let the explants grow. After the time, data were recorded.

### 3.4 Data Analysis

Data was analyzed using statistical software, SPSS version 16.0 for Windows. Analyze of growth of *L. pumila* by ANOVA. The level significant difference is at  $P < 0.05$ . Besides that, Duncan's Multiple Range Test (DMRT) was used in order to compare treatment that are factorial in nature or that correspond to several levels of a quantitative or continuous variable.



## CHAPTER 4

### RESULTS

Figure 1 show the effect of BAP, kinetin and zeatin on number of shoot tips produced by *Labisia pumila*. The highest number of shoots (3.6) was observed on the medium supplemented with 3 mg/L zeatin. Medium supplemented with BAP and kinetin does not show any significant on shoot productions.

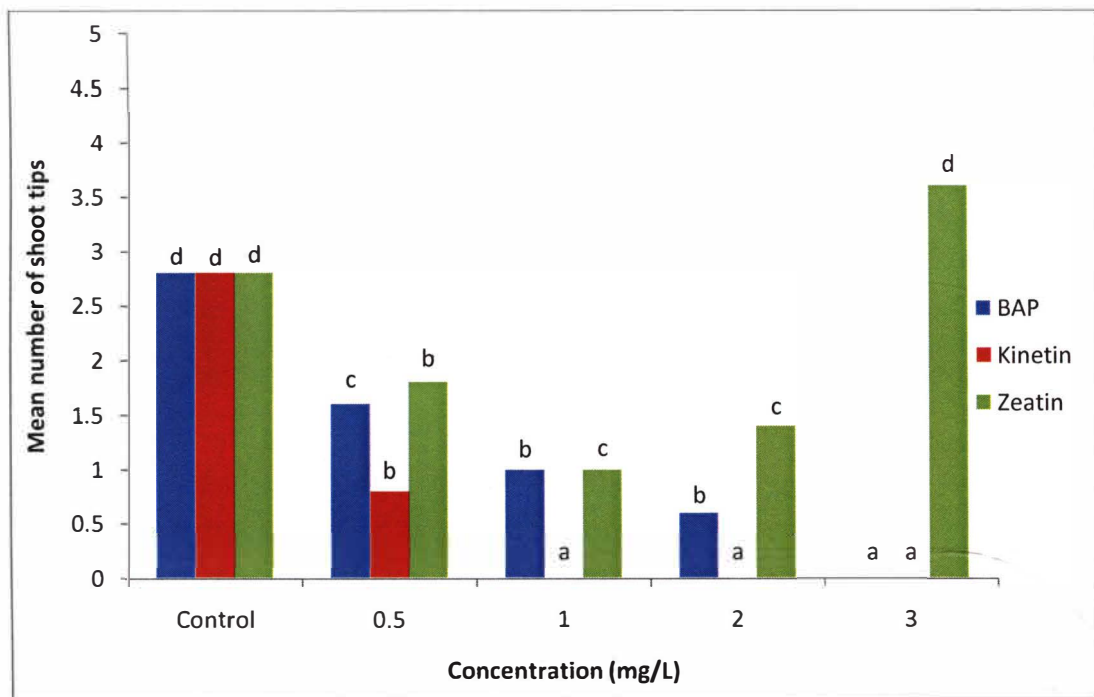


Figure 1: Effect of cytokinin on proliferation of shoot tips of *L. pumila* after six month on B5 media

Figure 2 show the effect of BAP, kinetin and zeatin on number of leaves produced by *L. pumila* after 2 month. Similarities with the test on production of shoot tips, kinetin and BAP also does not exhibit on producing the high mean number of leaves. Among the three ptohormones, 3 mg/L zeatin was most significantly higher (4) followed by zeatin with concentration of 1 (3.6). It is also found that no significant between it concentrations.

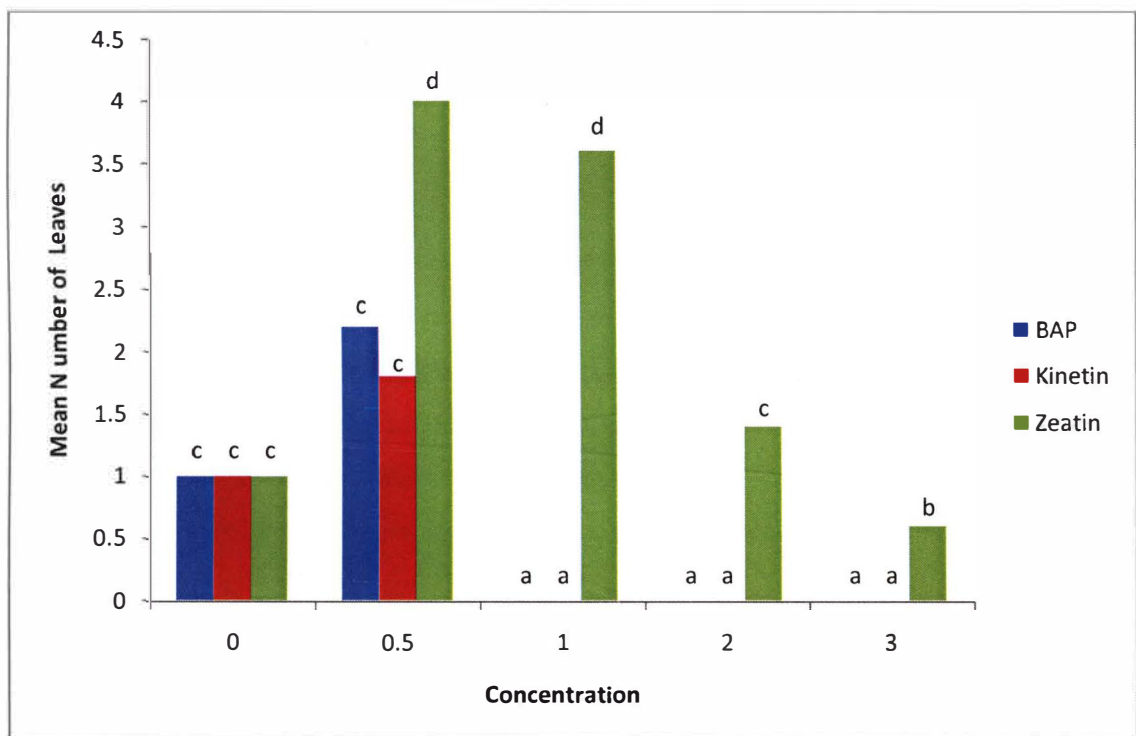


Figure 2: Effect of cytokinin on proliferation of leaves of *L. pumila* after six month on B5 media

## CHAPTER 5

### DISCUSSIONS

From the experiment, kinetin and BAP do not show any significant difference in producing a higher number of shoot tips or number of leaves. Mackay et al. (1995) report that the poor effect of kinetin on shoot proliferation of *Cercis canadensis*. Differ to the present observation regarding the superiority of zeatin in proliferation of *L. pumila*, earlier report indicated that BAP was the most effective for shoot formation among other cytokinins in the culture of *Polygonatum odoratum* seedling explants (Yoon and Choi, 2002). In other plant species such as *Myrica esculenta*, kinetin showed the best performance with regards to shoot proliferation (Bhatt and Dhar, 2004).

Fonnesbech et al. (1979) discovered that the natural cytokinin zeatin were better able to promote the growth of shoot cultures of *Asparagus plumosus* than kinetin. Same situation is found in plants of the family Ericaceae, where the natural compound zeatin is more effective than other cytokinins for shoot proliferation.

Differences in the activity of cytokinins can be caused by the different uptake rate in different genomes, according to Kaminek, (1992), various translocation rates to meristematic regions and metabolic processes, in which the cytokinin may be lowered or conjugated with sugars or amino acids to form biologically inert compounds.

Most experiment of requirement for a particular cytokinin, have been made with a shoot cultures and the test had been made over for many species (Geert & Michael, 2007). In experiment by Vieitez and Vieitez (1980), BA promoted axillary bud proliferation of *Castanea* while kinetin was show no effect. Zeatin tended to promote the growth of main shoots and gave only a slight increase in the propotion of lateral buds sprouting. Similarly, 2-iP and kinetin produced only single shoots, and to obtain multiple shoots, it was necessary to use BA. Kinetin to be capable of promoting the growth of rose shoot tips. On the other hand, only 0.5-5 mg/L kinetin induced the proliferation of potato shoots and BA and 2- iP were not effective.

Many authors cited that cytokinins play in the regulation of primary metabolism, in particular sink formation. According to Samuelson et al. (1995), assimilation of inorganic nitric into organic form, which is essential for plant growth is stimulated by cytokinin. Nitrate reductase is co-regulated by nitrate, light and cytokinins. An increase in efficiency with which nitrogen is incorporated into organic form has marked effect on plant productivity, biomass and crop yield (Oliveira et al., 1997). The link between cytokinins and N-metabolism is underlined by the fact that response regulator genes are primary regulator targets for both cytokinins and nitrate (Taniguchi et al., 1998). Perhaps, cytokinins are signal that are co-transported with nitrate from the roots to the shoots, informing the shoot, informing the shoot about the N-status of the root.

The significant of zeatin on promoting leaves growth also supported by Hartinie and Azlan (2007). The result also consistent with others reports which indicate the substantial role of zeatin on promoting number of leaves on culture of *Bixa orellana*.

The differences of leaf sizes between explants cultured on control medium and media supplemented with various cytokinins revealed the absolute requirement of cytokinins during leaf formation. During this stage, cytokinins are required to drive the cell division cycle at a normal speed and to obtain the required number of cell divisions to

reach a normal leaf size. Therefore, in the absence or deficient of cytokinins, a reduced leaf size may cause mainly by a reduced rate of cell division (Werner et al., 2001).

Cytokinins are needed to drive the cell division cycle at a normal speed and to obtain the required number of cell divisions to reach a normal leaf size. That is why when the absence or deficient of cytokinins, a reduced leaf size may cause mainly by a reduced rate of cell division. Growths of plants are depending to specific plant growth regulators (Werner et al., 2001).

To increase the number of proliferation, combination of phytohormone such kinetin and BAP, or other combination should be done. This was approved had been reported on other plants. Talukder et. al. (2003), show that BAP and NAA improve the proliferation of *Dendrobium* orchid compared with supplemented media with BAP without combination. Another experiment also shows the same result when using combination of hormone.

## CHAPTER 6

### CONCLUSION AND SUGGESTION

This study indicates that addition of phytohormone show significant in proliferate the shoot tips and leaves of *L. pumila*. B5 media supplemented with 3 mg/L zeatin shows higher significant in producing shoot tips while zeatin with 0.5 mg/L zeatin shows higher significant in producing leaves of *L. pumila*.

After all this experiment was done, there is some suggestion on how to increase the growth of this precious medicinal plant. The suggestion is, aside of using single hormone, using combination hormone will be more gives great production to the plants itself. In their experiment, using single hormone does not yield higher growth if compared by using combination of hormones.

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## APPENDIX A

### List of materials

1. Macro media
  - 1.1.  $\text{KNO}_3$
  - 1.2.  $(\text{NH}_4)_2\text{SO}_4$
  - 1.3.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$
  - 1.4.  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
  - 1.5.  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$
  
2. Micro media
  - 2.1.  $\text{H}_3\text{BO}_3$
  - 2.2.  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$
  - 2.3.  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
  - 2.4.  $\text{NaM}_0\text{O}_4 \cdot 2\text{H}_2\text{O}$
  - 2.5.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
  - 2.6.  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
  - 2.7. KI
  
3. FEDTA
  
4. Vitamin B5
  
5. Sucroce
  
6. Phyta Gel
  
7. Phtohormones
  - 7.1. Zeatin
  - 7.2. Kinetin
  - 7.3. BAP

### List of apparatus

1. Petri Dish
2. Scalpel
3. Spatula
4. Culture tube

## APPENDIX B

Table 1: Effect of cytokinin on proliferation of shoot tips of *L. pumila* after six month on B5 media

Phytohormones	Concentration (mg/L)	Mean $\pm$ S.E
<b>Control</b>	0	1.0 $\pm$ 0.000
<b>BAP</b>	0.5	2.20 $\pm$ 0.583
	1	0.00 $\pm$ 0.000
	2	0.00 $\pm$ 0.000
	3	0.00 $\pm$ 0.000
<b>Kinetin</b>	0.5	1.80 $\pm$ 0.374
	1	0.00 $\pm$ 0.000
	2	0.00 $\pm$ 0.000
	3	0.00 $\pm$ 0.000
<b>Zeatin</b>	0.5	4.00 $\pm$ 1.304
	1	3.60 $\pm$ 0.812
	2	1.40 $\pm$ 0.400
	3	0.60 $\pm$ 0.245

Values represent mean  $\pm$  standard error of 5 replicates per treatment. Mean followed by the same letter did not differ significantly at ( $p < 0.05$ ) according to Duncan multiple range tests.

Table 2: ANOVA for shoot tips proliferation

	Sum of square	df	Mean square	F	Sig.
<b>Between groups</b>	75.815	12	6.813	5.198	0
<b>Within groups</b>	63.200	52	1.215		
<b>Total</b>	139.015	64			

Table 3: Effect of cytokinin on proliferation of leaves of *L. pumila* after six month on B5 media

Phytohormones	Concentration (mg/L)	Mean $\pm$ S.E
<b>Control</b>	0	2.0 $\pm$ 0.000
<b>BAP</b>	0.5	2.20 $\pm$ 0.583
	1	0.00 $\pm$ 0.000
	2	0.00 $\pm$ 0.000
	3	0.00 $\pm$ 0.000
<b>Kinetin</b>	0.5	1.80 $\pm$ 0.374
	1	0.00 $\pm$ 0.000
	2	0.00 $\pm$ 0.000
	3	0.00 $\pm$ 0.000
<b>Zeatin</b>	0.5	4.00 $\pm$ 1.304
	1	3.60 $\pm$ 0.812
	2	1.40 $\pm$ 0.400
	3	0.60 $\pm$ 0.245

Values represent mean  $\pm$  standard error of 5 replicates per treatment. Mean followed by the same letter did not differ significantly at ( $p < 0.05$ ) according to Duncan multiple range tests.

Table 4: ANOVA for leaves proliferation

	Sum of square	df	Mean square	F	Sig.
<b>Between groups</b>	119.815	12	9.985	8.4884	0
<b>Within groups</b>	61.200	52	1.177		
<b>Total</b>	181.015	64			

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