EFFECT OF NATURAL AUXIN FROM *PORTULACA GRANDIFLORA* HOOK AND *IPOMOEA BATATAS* (L.) POIR ON THE FORMATION ADVENTITIOUS ROOTS *IN VITRO* OF *PLUMBAGO ZEYLANICA* L.

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ABSTRACT

Plumbago zeylanica L. is a traditional herbal that has been reported to treat on skin diseases. Furthermore, some researchers have found plumbagin extracted from roots of this species can prevent cancer cell development. In current study, stems of *Plumbago zeylanica* L. were cultured on MS medium with BA 1.0 mg/L and IAA (0.01-0.15 mg/L) or NAA (0.1-0.15 mg/L). After 8-week cultured, stems were transferred to MS medium with extracted from stems of *Portulaca grandiflora* Hook (2-10 ml/L) or extracted from stems of *Ipomoea batatas* (L.) Poir. The results showed that, the appropriate medium for shoot formation was in MS with BA and IAA 0.1 mg/L or NAA 0.1 mg/L. The adventitious roots *in vitro* were formatted in MS medium supplied with extracted from stems *Portulaca grandiflora* Hook or from stems of *Ipomoea batatas* (L.) Poir 6 ml/L. Simultaneously, after 8-week cultured, the adventitious roots were collected and plumbagin qualitative were analyzed with pure plumbagin of Sigma. As the results, plumbagin presents in adventitious roots cultured.

Keywords: Adventitious roots; *Ipomoea batatas* (L.) Poir; *Plumbago zeylanica* L.; Plumbagin; *Portulaca grandiflora* Hook.

1. Introduction

Plumplings called *Plumbago zeylanica* L., belonging to the *Plumbaginaceae* family, are a medicinal plant originating from Northwest Asia, grown in India, West Bengal, tropical Africa and some countries in Southeast Asia, including Malaysia, Philippines, Thailand, Laos, Cambodia, Indonesia (Dang Quang Chung et al., 2006). *Plumbago zeylanica* L. is found only to cure skin diseases, ulcers, wounds in human (Nguyen Duc Luong and Le Thi Thuy Tien, 2000). The roots contain a crystalline acridine called plumbagin, a yellow naphtoquinone used as a drug in India from 750 BC. It is used for parasitic resistance, cardioprotection, liver protection, and neurodevelopment. In addition, many different active ingredients have been identified in this plant roots, including phenolic acids, tannins, anthocyanins, etc. (Tran Nguyen Bich Tran, 2003). This is a substance that inhibits the growth of Gram-positive bacteria (*Staphylococcus*, *Streptococcus* and *Pneumococcus*), anti-cancer, antioxidant, anti-malaria. It also has anti-inflammatory, anticoagulant effect in white rats, treatment at the beginning of the disease and baldness.

Plumbago zeylanica L. grow slowly in natural conditions, the roots of plants used to acquire plumbagin need many years for high levels of active ingredients (Quach Ngo Diem Phuong, 2012). In Vietnam, it is grown both wild and newly planted in some places (Quach Ngo Diem Phuong, 2012). Therefore, the problem is that if harvesting in the wild, it is necessary to remove the plant so it is very costly and time consuming to regenerate the material. The use of synthetic growth regulators will have an influence on the biological active substances that plants synthesize during growth. Therefore, we use *Portulaca grandiflora* Hook or *Ipomoea batatas* (L.) Poir extracts as natural auxin source which are beneficial for the purpose of producing adventitious roots, contributing to safe source of raw materials, increasing efficiency in pharmaceuticals, cost effective

and environmentally friendly.

2. Material and Method

Material

Plumbago zeylanica L., *Portulaca grandiflora* Hook and *Ipomoea batatas* (L.) Poir were collected from herbal garden in Open University of Ho Chi Minh City.

Method

Stems of *Plumbago zeylanica* L. were asepted with alcohol 70% for 3 minutes, follow by soaking in $Ca(OCl)_2$ 15% for 15 minutes, and washing three times with sterile distilled water. After that, the stems were cut into short sections 1.0-1.5cm with dormant buds and cultured on MS medium supplied with BA 1mg/L and IAA (0.01, 0.05, 0.10, 0.15mg/L) or NAA (0.01, 0.05, 0.10, 0.15mg/L). After 8-week culture, stems *in vitro* in experiments before were transferred to MS medium supplied *Portulaca grandiflora* Hook or *Ipomoea batatas* (L.) Poir extracts at the concentration of 0 - 10ml/L. The pH of medium was adjusted to 5.8, and then autoclave at 121^{o}C, 1 atm pressure for 30 minutes. The cultures were incubated at 22 ± 2^{o}C under a light intensity of 2,000 ~ 3,000 lux, with a photoperiod of 12/12 light/dark.

25 g fresh roots from experiments *in vitro* were sliced and soaked with 50mL diethyl ether supplied 1% H_2SO_4 overnight. The extract was filtered, plus 25 mL H_2SO_4 2 M and incubated at 50^{o}C. The solvent were removed and collected the extract and quality plumbagin by thin layer chromatography (TLC). The extract from experiments was plus with 1 mL chloroform, pointed on TLC silica gel 60 F254 (5 x 12cm), and put the TLC on the solvent including petroleum ether: ethyl acetate (6:3) and compared with pure plumbagin of Sigma under ultra violet light wave 254 nm.

Statistical analysis

All experiments were designed in a completely randomized form. Each experiment was repeated 4 times, data were processed by software Statgraphics Plus 3.0 Duncan multiple Range Test (Duncan, 1995) at $p< 0.05$.

3. Results

Effect of phytohormones on shoot formation

The result on table 1 and figure 1 showed that MS medium with BA 1.0mg/L and IAA 0.10mg/L or BA 1.0mg/L and NAA 0.10mg/L have the best effect on number and height of shoot of *Plumbago zeylanica* L. But if the concentration of IAA or NAA is less or more than 0.10mg/L, the number of shoot will reduced.

Table 1

Effect of phytohormones on shoot formation after 8-week culture

Experiment	Shoot number	Shoot height (cm)
Control	1.50^b	1.57^b
(BA 1.00 and IAA 0.01 mg/L)	1.67^b	1.35^b
(BA 1.00 and IAA 0.05 mg/L)	3.50^a	1.52^b
(BA 1.00 and IAA 0.10 mg/L)	3.50^a	2.25^a
(BA 1.00 and IAA 0.15 mg/L)	1.17^b	1.38^b
(BA 1.00 and NAA 0.01 mg/L)	1.33^c	1.25^{bc}
(BA 1.00 and NAA 0.05 mg/L)	2.33^{bc}	1.45^b
(BA 1.00 and NAA 0.10 mg/L)	3.67^a	2.33^a
(BA 1.00 and NAA 0.15 mg/L)	2.00^{bc}	1.33^{bc}

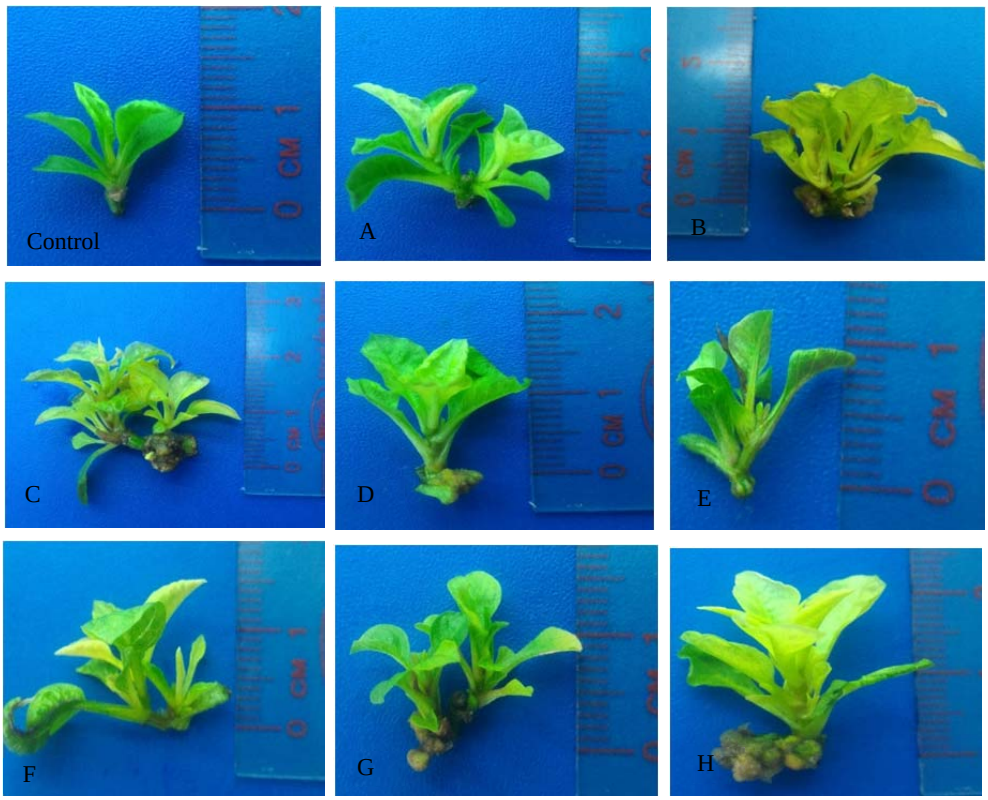

Figure 1. Effect of phytohormones on shoot formation after 8-week culture
A-D: BA 1.00 mg/L and IAA (0.01-0.15 mg/L)
E-H: BA 1.00 mg/L and NAA (0.01-0.15 mg/L)

Effect of the concentration *Concentration of grandiflora* Hook extract on the ability root formation from stem of *zeylanica* L.

The results in table 2 and figure 2 showed that different levels of *Portulaca grandiflora* Hook extract have different effects on root formation and morphological characteristics of roots. After 8 weeks of culture, medium supplemented with *Portulaca grandiflora* Hook extracts 6ml/L had the highest rooting rate was 336.25 roots/stem, the root length was 4.50cm. However, the extract of *Portulaca grandiflora* Hook was higher or less than 6 ml/L had negative effect.

Table 2

Effect of the extracted of *Portulaca grandiflora* Hook he ability root formation from stem *Plumbago zeylanica* L.

Portulaca grandiflora Hook extract concentration (ml/L)	Root length (cm)	Root number
Control	2.35^{bc}	51.00^{c}
2	2.13^{c}	81.50^{bc}
4	2.88^{b}	121.25^{b}
6	4.50^{a}	336.25^{a}
8	2.63^{bc}	115.75^{b}
10	1.40^{d}	30.625^{c}

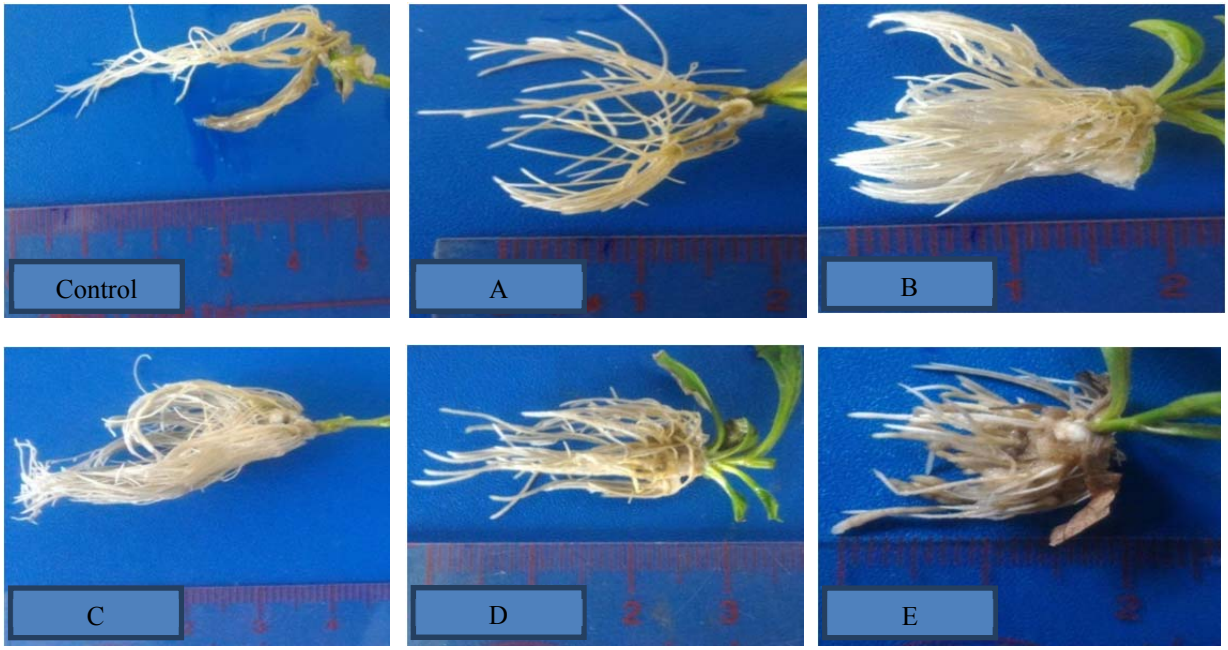

Figure 2. Roots of *Plumbago zeylanica* L. after 8-week culture in MS medium supplied extract from *Portulaca grandiflora* Hook Control
A, B, C, D, E: 2, 4, 6, 8, 10 (ml/L)

The effect of *Ipomoea batatas* (L.) Poir extract on the ability of root formation from stem of *Plumbago zeylanica* L

The results in table 3 and figure 3 showed that different levels of *Ipomoea batatas* (L.) Poir extract have different effects on root formation and morphological characteristics of roots. After 8 weeks of culture, the results showed that in MS medium supplied with *Ipomoea batatas* (L.) Poir extract concentrations of 6ml/L, the highest root rate was 599.75 roots/stem, the root length was 7.25cm.

Table 3

Effect of the extracted concentration of *batatas* (L.) Poir the ability root formation from stem *Plumbago zeylanica* L.

Ipomoea batatas (L.) Poir extract concentration (ml/L)	Root length (cm)	Root number
Control	2.15^{c}	62.75^{c}
2	2.40^{bc}	139.75^{b}
4	3.50^{b}	121.25^{b}
6	7.25^{a}	599.75^{a}
8	2.50^{bc}	115.75^{b}
10	1.75^{c}	68.75^{c}

Figure 3. Roots of *Plumbago zeylanica* L. after 8-week culture in MS medium supplied extract from *Ipomoea batatas* (L.) Poir Control
A, B, C, D, E: 2, 4, 6, 8, 10 (ml/L)

Determination of plumbagin from adventitious root extract of *Plumbago zeylanica* L *in vitro*

The adventitious root extract of *Plumbago zeylanica* L from rooting experiments had plumbagin as well as pure plumbagin at Rf 6.5 (Figure 4).

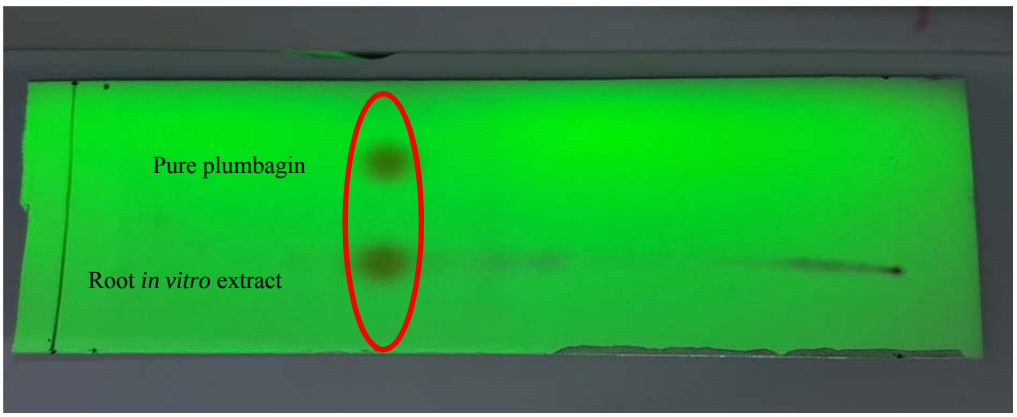

Figure 4. Products extracted in the chromatography system is solvent petroleum ether: ethyl acetate (6: 3) with pure plumbagin of Sigma and root *in vitro* extract

4. Discussion

In this research, the combination of auxin (IAA or NAA 0.1mg/L) and cytokinin (BA 1.0mg/L) affect shoot formation in *Plumbago zeylanica* L. stem *in vitro*. Phytohormones are signal molecules that individually or cooperatively direct the development of individual cells or carry information between cells and thus coordinate growth and development. Auxin are characterized principally by their capacity to stimulate cell elongation in excised stem and coleoptile sections, but they also influence a host of other developmental responses, including root initiation, vascular differentiation, tropic responses, and the development of axillary

buds, flowers and fruit. Cytokinins are characterized primarily by their ability, in combination with auxin, to stimulate cell division, or cytokinesis, in tissue culture. In addition to stimulating cell division, cytokinins also influence shoot and root differentiation in tissue culture, the growth of lateral buds and leaf expansion, chloroplast development and leaf senescence (Hopkins and Huner, 2004).

The extract from *Portulaca grandiflora* Hook and *Ipomoea batatas* (L.) Poir can use as auxin source influence root formation in stem culture. Which are beneficial for the purpose of producing adventitious roots, contributing to safe source of raw materials, increasing efficiency in pharmaceuticals, cost effective and environmentally friendly.

5. Conclusion

Micropropagation *Plumbago zeylanica* L. shoot achieved when stem culture on MS medium plus BA 1.0mg/L and IAA 0.10 or BA 0.1mg/L and NAA 0.10mg/L. *Portulaca grandiflora* Hook and *Ipomoea batatas* (L.) Poir extract at 6ml/L can be used to replace auxin in adventitious root formation of *Plumbago zeylanica* L. The extraction from adventitious root of *Plumbago zeylanica* L. content plumbagin■

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