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Biomass and Flavonoid production of *Gynura procumbens* adventitious roots induced by sucrose, Phenylalanine and Tyrosine

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Adventitious roots culture of *Gynura procumbens* was successfully cultured in liquid medium by agitation in order to increased biomass and flavonoid content. Adventitious roots were induced from leaf explants on Murashige and skoog (MS) medium with 3% sucrose and various indol butyric acid (IBA) concentrations. Duration of initation, number, length and fresh weight of adventitious roots were evaluated. The best IBA concentration was achieved at 5 mg/L. In liquid culture, 2 g adventitious roots were used as inoculum. Various treatments of sucrose, phenylalanine, and tyrosine in different concentration were applied separately in culture using MS liquid medium was supplemented with 3% sucrose and 5 mg/L IBA. Culture were maintained in shaker incubator with 100 rpm at 25±3^{o}C for 28 days without light. Results show maximum biomass was achieved on culture with 50 g/L sucrose, 50 mg/L phenylalanine, and 50 mg/L tyrosin. The highest content of quersetine and kaempferol was obtained in culture with 50 g/L sucrose, 200 mg/L phenylalanine and 200 mg/L tyrosine. Base on the data, production of biomass and flavonoid of *G. procumbens* adventitious roots needs two-phase culture system for optimum yield. First phase cultures to achieve maximum biomass and second phase cultures to increase the content of G*. procumbens* adventitious roots.

Keywords: *Gynura procumbens*, adventitious roots, sucrose, phenylalanine, tyrosin, flavonoid.

INTRODUCTION

Technology of organ culture has been interested in recent years in order to produced specific secondary metabolite, because organ culture has many advantages such as genetic stability, easy culture, and stable in metabolite yield. Some of secondary metabolites in plant was found in roots, but harvesting of roots is destructive for the plants. *Gynura procumbens* is vegetable and medicinal plant which has been used in Indonesia, Malaysia, Thailand, and other region of South East Asia. As a medicinal plant, *G. procumbens* has potential as antioxidant (Kaeswejaan et al. 2015), and vasodilatation (Hoe et al. 2011; Ng et al. 2013). Kaempferol, quercetin, rutin, and myricetin are flavonoid compounds which have been isolated from *G. procumbens* leaves (Saiman et al. 2012). Flavonoid compounds have potency as an antioxidant, especially myricetin and kaempferol

(Kaewseejan et al. 2015).

In vitro propagation of organ culture has developed in agar-based system and liquid-based system. Liquid culture systems have significant effects on multiplication rates. Many researcher used adventitious roots to produce biomass and secondary metabolite in liquid culture, such as in *Glycyrhiza uralensis*, *Eurycoma longifolia*, *Hypericum perforatum*, *Prunella vulgaris* L., and *Eleutherococcus koreanum* (Yin et al. 2014; Lulu et al. 2015; Cui et al. 2010; Fazal et al. 2014; Lee et al. 2014). Adventitious roots culture of *G. procumben*s has been developed in liquid culture used by temporary immersion system (TIS) (Kusuma et al. 2016). In TIS culture, biomass of *G. procumbens* adventitious roots had been increased significantly, but production of secondary metabolite was not detected yet quantitatively.

Flavonoid biosynthesis can be undertaken through two pathways, the shikimate and malonic acid pathway. Both of this biosynthesis plays a role in determining the carbon flavonoid framework (Taiz and Zeiger, 2002). Phenylalanine and tyrosine are the intermediate compound of flavonoid biosynthesis in shikimate pathway. Sucrose is the main source of carbon to induce the cell growth and it also help the molecule signals to stimulate the genetic expression in coding the enzymes involved in isoflavonoid biosynthesis (Morkunas et al. 2014). In this research the effect of sucrose, phenylalanine, and tyrosine on biomass and secondary metabolite of *G. procumbens* adventitious roots was evaluated in shake flask system. The goal is improvement of biomass and flavonoid content of *G. procumbens* adventitious roots.

MATERIALS AND METHODS

Materials

G. procumbens was obtained from florist in Surabaya, East Java, Indonesia. The plant was identified and confirmed by Botanical Garden Purwodadi, Indonesian Institute of Science, Pasuruan East Java, Indonesia.

Induction of adventitious roots on solid medium

Leaves of *G. procumbens* were washed by mild detergent and rinsed with tap water, then were sterilized with clorox 10% (v/v) containing 5.25% sodium hypochloride for 7 min and rinsed with sterile distilled water three times. Sterile leaves were cut 4 cm^2 and planted in MS (Murashige and Skoog) medium supplemented with various IBA (indol butiric acid) 1, 3, 5, 7 mg/, sucrose 30 g/L, and agar 8%. Cultures were incubation at 25±3^oC in the dark condition for 28 days. Duration of initation of adventitious root was observed during cultivation. Number, length and fresh weight of adventitious root were counted, measured and weighed respectively in the end cultivation. Fresh weighing method according to Hao and Guan (2012). Water on surface of adventitious root was absorbed using filter paper, and then weighed using analytical balance (Ohaus PA214).

Cultivation of adventitious roots in shake flask

Adventitious roots which were produced from the best concentration of IBA in the early step were used as an explant for cultivation in shake flask. Two grams of adventitious roots were planted in 100 mL MS liquid medium and were placed in 250 mL Erlenmeyer flask. There were nine flasks for three treatments. The first treatments were cultured in MS liquid medium supplemented with various sucrose (10, 30, 50 g/L), the second treatments were supplemented phenylalanine (50, 100, 200 mg/L) and sucrose 30 g/L, and the third treatment were supplemented tyrosin (50, 100, 200 mg/L) and sucrose 30 g/L. Each culture medium had IBA at the best concentration for induction. Cultured were maintained in shaker incubator with 100 rpm at 25±3^oC for 28 days without light. Total sugar content, pH and conductivity of liquid medium were measured before sterilized, early and the end of culture using hand refragtometer (Atago Master 10T), pH meter (Hanna HI 220) and hand conductometer (Ezodo Cond521).

Flavonoid detection of adventitious roots

Adventitious roots from each treatment were dried at 50^oC in incubator oven (Memmet INB200) for 5 days and then were grinded. One hundred mg (dry weight) of adventitious roots were transferred into a 50 mL flask which containing 10 mL ethanol (Merck) and were heated at 60^oC for 5 min and then were filtered using filter paper whatman No 1). The extracts were concentrated until 2 mL and then were analyzed using modified colorimetry method (Kaewseejan et al. 2015). Extracts were taken 0.25 mL, added into 1.25 mL of demineralized water and 75 µL of 5% sodium nitrate solution, and dissolved for 6 min. Then, 0.5 mL of NaOH 1 M and demineralized water were added into the solution until it reached a volume of 2.5 mL. The absorbance value was obtained

using UV spectrophotometer at 510 nm (BOECO S-22, Germany). The blanks were used absolute ethanol. The absorbance values obtained were counted using linear regression equations based on the standard quercetine and kaempferol.

RESULTS

Indol butiric acid (IBA) in various concentration influenced induction and growth of *G. procumbens* adventitious roots. The fastest induction was achieved in the medium supplemented IBA 5 mg/L and the best growth was also known in its medium. It was showed by amount, length, and fresh weight of adventitious roots (Table 1). In low concentration of IBA induction of adventitious roots was slow and the data of amount, length, and fresh weight also low, but the slowest induction was happened on IBA concentration 3 mg/L, the lowest amount of roots was on IBA 1 mg/L, the shortest of roots was on IBA 3 mg/L and 7 mg/L, and the lowest of fresh weight was on IBA 1 mg/L and 3 mg/L (Figure 1).

Various concentration of sucrose, phenylalanine, and tyrosine influenced biomass production of *G. procumbens* adventitious roots. Increased sucrose concentration was followed by biomass weight. Sucrose treatment until 50 g/L could improve fresh and dry weight of Adventitious roots. Contrary, this result was disappear on culture with phenylalanine and tyrosin treatment. The cultures were supplemented with phenylalanine and tyrosin at 100 and 150 mg/L had biomass reduction. The best biomass production at sucrose treatments was achieved in MS medium supplemented with 30 g/L sucrose, whereas at phenylalanine and tyrosine treatments were achieved in MS medium supplemented with 50 mg/L and tyrosine 50 mg/L respectively (Table 2). The highest of biomass from all treatments were obtained in MS medium supplemented with phenylalanine 50 mg/L. Biomass production was 11 fold from initial inoculum. *G. procumbens* adventitious roots morphology in different treatment could be seen in Figure 2.

Production of flavonoid compound was identified by kaempferol and quercetine content. In various concentration of sucrose, kaempferol and quercetine increased along with increasing sucrose concentration. The highest flavonoid compound was achieved at sucrose concentration 50 g/L and at phenylalanine concentration 200 mg/L. Supplemented various concentration of tyrosine could also increase kaempferol and quercetine content, but its improvement was small or not significant. In all treatments showed that kaempferol and quercetine content of adventitious root in vitro was higher than adventitious roots ex vitro (mother plant), except in supplemented of sucrose 10 g/L treatment (Table 2).

Medium properties such as pH, total sugar and conductivity at each treatment were measured in early and the end of cultivation. Data were showed at Table 3. At the end of cultivation, pH of medium in all treatment decreased, but the decrease of ph on treatment sucrose 50 g/L , phenylalanine 50 mg/L, and tyrosin 50 mg/L was not below 5.0. It's showed that medium conditions in all three treatments were still able to support the adventitious root growth which was indicated by the high acquisition of biomass compared to other treatments (Table 2). In other treatments, pH medium were lower than 5 therefore growth of adventitious roots were not good which were showed by the lower biomass. Data of conductivity medium in all treatment showed decreased. Its mean adventitious roots could absorb inorganic compound from medium effectively. Beside that total sugar in medium also decreased in all treatments.

Table 1. Effect of growth regulator (IBA) on the induction of adventitious roots

IBA (mg/L)	Duration of roots formed (days)	Number of roots	Roots length (cm)	Fresh weight (g)
1	16.20±6.09^{ab}	1.38±1.40^{c}	3.99±4.86^{b}	0.01±0.02^{b}
3	19.00±5.04^{a}	3.38±2.88^{b}	2.96±2.24^{b}	0.01±0.02^{b}
5	11.13±1.46^{b}	8.00±3.46^{a}	8.72±1.53^{a}	0.44±0.14^{a}
7	18.13±5.99^{a}	7.50±4.41^{ab}	2.99±2.22^{b}	0.07±0.11^{b}

Means with the same superscripts letter are similar at P≤0.05 by Tukey's multiple comparison test

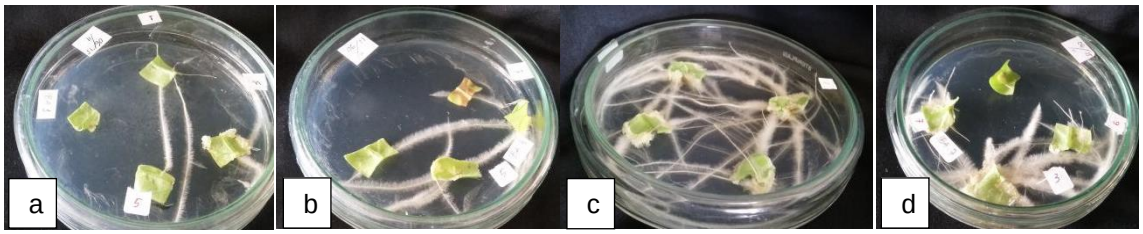

Figure 1. Adventitious roots of *G. procumbens* cultured in MS solid medium supplemented with various concentration of IBA after 28 days cultivation; (a) 1 mg/L, (b) 3 mg/L, (c) 5 mg/L, d. 7 mg/L.

Table 2. Effect of sucrose, phenylalanine, and tyrosine on biomass and flavonoid content of adventitious roots in liquid culture

Treatments		Fresh weight (g)	Dry weight (g)	Kaempferol (mgL^{-1}/g dry weight)	Quersetine (mgL^{-1}/g dry weight)
Sukrosa (g/L)	10	5,00±1,20	0,13±0,03	155,56	8,33
	30	6,50±1,34	0,14±0,08	600,00	125,00
	50	7,80±1,98	0,28±0,01	733,33	165,00
Phenylalanine (mg/L)	50	22,08±5,21	0,63±0,14	730,56	191,67
	100	13,61±4,15	0,47±0,11	838,39	224,17
	200	6,18±4,60	0,22±0,11	911,11	245,83
Tyrosine (mg/L)	50	14,37±3,48	0,40±0,10	863,89	231,67
	100	12,27±4,24	0,33±0,12	783,33	207,50
	200	10,64±5,68	0,26±0,17	869,44	233,33
Ex-vitro adventitious roots (mother plant)		-	-	377,78	58,33

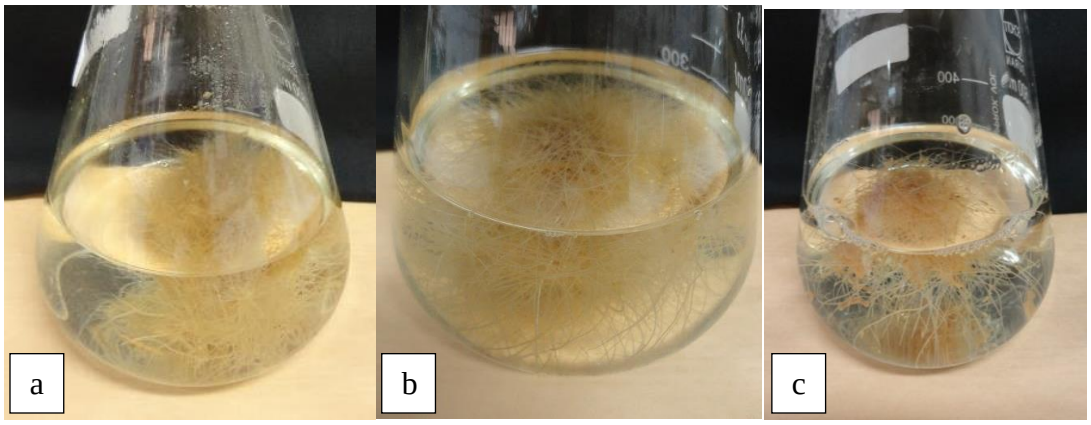

Figure 2. *G. procumbens* adventitious roots morphology in different treatments after 28 days cultivation in shake flask; (a) sucrose 50 g/L, (b) phenylalanine 50 mg/L; (c) tyrosine 50 mg/L

DISCUSSION

Induction of adventitious roots in medium supplemented with exactly concentration IBA will increase, such as in MS medium supplemented IBA 5 mg/L which could increase *G. procumbens* adventiitous roots optimally. *G. procumbens* adventitious roots growth directly without formed callus, good developed, have structure thin and long, and roots branch grow through the medium. De Klerk *et al.* (1999) reported, IBA had high effectiveness to induce roots than IAA on *in vitro* culture. External supplementation of IBA could increase endogenous IAA (indole acetic acid) in plant tissue, but addition of excess IBA did not increase endogenous hormone (Nordstrom *et al.,* 2004; Ludwig-Muller, 2005). In this research growth of adventitious roots decreased when medium was supplemented with 7 mg/L IBA. This result was indicated IBA 7 mg/L could inhibit cell proliferation and elongation. High concentrations of auxin could inhibit cell lengthening, because hormonal imbalance between endogenous auksin and exogenous auksin (Ahmad et al. 2015). High dose of auxin has activity like herbicides that inhibit the initiation of adventitious roots (Evans et al. 2003).

The effect of sucrose concentration on *G. procumbens* adventitious root-growth had been demonstrated in this study. Increased concentration of sucrose until 70 g/L could increase biomass (fresh and dry weight) and flavonoid content (kaempferol and quersetine) of *G. procumbens* adventitious roots. In plant cells, sucrose acts as a substrate of glycolysis in cytoplasm and will be changed to piruvic acid, then transfer to mitochondria for producing ATP (adenosine tri phosphate). Besides that, Glucose may affect the absorption of nitrate by inducing nitrate reductase in nitrate assimilation for amino acid synthesis. Low concentration of sucrose could decrease nitrogen absorption and cell growth, so stationer phase will be achieved earlier (Yeoman and Yeoman, 1996). Sucrose also used as source of energy by explant for proliferation and production of secondary metabolite (Murthy et al. 2016).

In this research, the effect of addition of phenylalanine and tyrosine on biomass and secondary metabolite content of *G. procumbens* adventitious roots was also evaluated. Treatment of phenylalanine and tyrosin separately at 50 mg/l achieved the best biomass accumulation of *G. procumbens* adventitious root. Biomass gain was achieved by phenylalanine was higher than tyrosin(Table 2). Jibouri et al. (2016) reported the same result on callus cultures of *Verbascum thapsus* L. was supplemented with phenylalanine 50 mg/L. Concentration of phenylalanine and tyrosin at 50 mg/L may induce *G. procumbens* adventitious root to synthesize growth hormone with an optimal concentration for its growth. Phenylalanine and tyrosin are aromatic amino acids which have function as bioblock of protein and play important role for hormone synthesis, such as auxin and salicylate (Tzin and Galili, 2010). Addition of both amino acids could also accelerate primer metabolism process through the conversion phosphoenolpyruvic acid to pyruvic acid. In biosynthesis pathway, phenylalanine and tyrosine involved in primary metabolism to change phosphoenolpyruvic acid to pyruvic acid. Pyruvic acid has been known as a key compound in some biochemical pathways. The induction of growth hormone synthesis at optimum concentration and increased concentration of pyruvic acid may be the mechanism of penilalanine and thyroxine for accumulation biomass of *G. procumbens* adventitious roots.

Production of quercetine and kaempferol of *G. procumbens* adventitious roots on sucrose, phenylalanine, and tyrosine treatments were different. Kaempferol and quercetine content showed increasing trend with increasing sucrose concentration in culture medium (Table 2). The addition of initial sucrose at 50 g/L in medium produced the highest quercetine and kaempferol. The same result was reported by Zhang et al. (1996), medium culture with initial sucrose concentration above normal concentration (30 g/L) could increase saponin and polysaccharida content in cell cultures of *Panax ginseng.* In this research, increasing quercetine and kaempferol was in line with increasing biomass. This result was indicated no osmosis dan oxidative stress on *G. procumbens* adventitious roots until 50 g/L sucrose which could damage membranes and macromolecules (Mittler, 2002), Finally It will decrease biomass and increase secondary metabolites. Otherwise, Baque et al. (2011) showed growth of *Morinda citrifolia* adventitious root was enhanced at 50 g/L sucrose while flavonoid contend was enhanced at 1% sucrose. It was also indicated sucrose has double role, being a carbon source and a regulator signal of metabolism (Koch, 2004).

Phenylalanine and tyrosine were intermediate compounds in flavonoid metabolism. In this research, both compounds could increase quercetine and kaempferol. Flavonoid was produced from two pathway such as shikimat and

malonic acetic pathway. Phenylalanine and tyrosine were intermediate compounds from shikimat pathway. Differences of phenylalanine and tyrosine were lies on enzyme that was used to come in shikimate pathway. Phenylalanine used phenylalanine ammonia lyase, whereas tyrosine used tyrosine ammonia lyase (Docimo et al. 2013). Production of secondary metabolite that was induced by phenylalanine showed in hairy roots cultures of *Psoralea corylifolia* L using elicitation and precursor feeding (Shinde et al. 2009). High concentration of phenylalanine (250-500 mg/L) could increase quercetine in cell culture of *Citrullus colocynthis* (Linn.) Schrad was reported by Meena et al. (2014).

Beside nutrient composition, the medium properties such as pH, conductivity and total sugar content also affect the growth and production and of secondary metabolites of adventitious roots. The three properties of medium used to detect growth and development of adventitious roots. In this research, initial pH medium was set at 5.8 and at the end of cultivation had range pH 4.0 - 5.0. This pH level was available for growing explants in liquid medium (Cui et al. 2010). Decreasing of pH in MS liquid medium caused by absorption of ammonium (Thorpe et al. 2008; Danesh et al. 2006). Macronutrient and micro nutrient were absorbed by explants and the value of conductivity medium used to detect how much nutrient have already absorbed. Decreasing value of conductivity and total sugar content in medium was supplemented of phenylalanine and tyrosine was showed in Table 3. It indicated adventitious roots had active metabolism and growing cells and also being indicator of adventitious roots biomass.

CONCLUSION

Cultivation of *G. procumbens* adventitious root in shake flask system with sucrose, phenylalanine and tyrosine treatment had been successfully done. The result showed production of biomass and flavonoid of *G. procumbens* adventitious roots has potency to more improve in bioreactor system for scale up production.

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

AUTHOR CONTRIBUTIONS

RN and RLKS designed and conducted the experiments and also wrote manuscript. ANT performed flavonoid analysis and reviewed the manuscript. AY and YSWM performed data analysis and reviewed the manuscript. All author read and approved the final version.

REFERENCES

Ahmad, N., Fatima, F., Ahmad, I. and Anis, M., 2015. Effect of PGRs in adventitious root culture in vitro: present scenario and future prospects. Rend. Fis. Acc. Lincei, 26 (3): 307–321.

Baque,M.A., Aigirban, E-J.L. and Paek, K-Y., 2011. Sucrose regulated enhanced induction of anthraquinone,phenolics, flavonoids biosynthesis and activities of antioxidantenzymes in adventitious root suspension culturesof *Morinda citrifolia* (L.). Acta Physiologiae Plantarum, 34(2): 405-415.

Cui, X., Chakrabarty, D., Lee, E., and Paek, K., 2010. Production of adventitious roots and secondary metabolites by *Hypericum perforatum* L. in a bioreactor. Bioresource Technology 101(4):4708–4716.

Danesh, Y. R, Galtapeh, E.M. and Alizadeh, A. 2006. Study on the growth patterns of transformed carrot hairy roots in on optimized system. J. Agric. Technol. 2(1): 89-97.

De Klerk, G-J., Van der Krieken, W. and De Jong, J.C., 1999. The Formation of Adventitious Root: new conceps, new posseblities. In vitro Cell. Dev.biol.-Plant. 35: 189-199.

Docimo, T., Consonni R., Coraggio I., and Mattana, M., 2013. Early Phenylpropanoid Biosynthetic Steps in *Cannabis sativa* : Link between Genes and Metabolites: Article, International Journal Sciences. 14: 13626-13644.

Evans, D.E., Coleman, J.O. and Kearns, A. 2003. Plant cell cultures. BIOS scientific publishers, London and New York.

Fazal, H., Abbasi, B.H., and Akhmad, N. 2014.

Optimization of adventitious root culture for production of biomass and secondary metabolites in Prunella vulgaris L. Applied Biochem. Biotechnol., 174: 2086-2095.

Hao, J-H and Guan, Q. 2012. Synthesis of saikosaponins in adventitious roots of *Bupleurum chinense* by semi-continuous culture. Plant Cell Tiss Organ Cult, 108:159–165.

Hoe, S.Z., Lee, C.N., Mok, S.L., Kamarudin, M.Y., and Lam, S.K., 2011. *Gynura procumbens* Merr. decreased blood pressure in rats by vasodilatation via inhibition of calcium channels. Clinics 66: 143-150.

Jibouri, A.M.J.A, Abed,A.S., Ali,A.J.A. and Majeed, D.M., 2016. Improvement of Phenols Production by Amino Acids in Callus Cultures of *Verbascum Thapsus* L. American Journal of Plant Sciences. 7 :84-91.

Kaewseejan, N., Sutthikhum,V.,and Siriamornpun, S., 2015. Potential of *Gynura procumbens* leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity. Journal of Funcional Food. 12(1): 120-128.

Koch, K. 2004. Sucrose metabolism: regulatory mechanisms andpivotal roles in sugar-sensing and plant development. Curr Opin Plant Biol, 7: 235–246.

Kusuma, D.Y., Kristanti, A.N., Manuhara, Y.S.W. 2016. Effect of sucrose and immersion frequency on production of adventitious roots and secondary metabolites of Gynura procumbens (Lour.) Merr. In temporary immersion bioreactors. Asian Journal of Plants Sciences 16:24-36.

Lee, U.J., Park, S.Y., and Paek, K.Y. 2014. Enhancement strategies of bioactive compound production in adventitious root culture of Eleotherococcus koreanum Nakai subjected to methyl jasmonate and salicylic acid elicitor through airlift bioreactors. Plant Cell Tissue Org. Cult. 120:1-10.

Ludwig-Muller, J., Vertocnik, A., and Town, C.D., 2005. Analysis of Indole-3-Butyric Acid-Induced Adventitious Root Formation on *Arabidopsis* Stem Segments, Journal of Experimental Botany 56(418): 2095-2105.

Lulu,T, Park, S.Y., Ibrahim, R., and Paek, K.Y. 2015. Production of biomass and bioactive compound from adventitious roots by optimization of culturing conditions of *Eurycoma longifolia* in balloon-type bubble bioreactor system. J. Biosci. Bioeng., 119:712-717.

Meena, Mahesh Chand, Rishi Keshi Meena and Vidya Patni. 2014. Effect of elicitor on quersetin production in cell cultures of *Citrullus colocynthis* (Linn.) Schrad. The Pharma Innovation. Vol 3 No 2.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Mol Biol, 29: 479–489.

Morkunas, I, Formela, M., Samardakiewicz, S.,Marczak, L., Nowak, W., Narozna, D., Bednarski, W. and Maluski, A. K., 2014. Effects of endogenous signals and *Fusarium oxysporum* on the mechanism regulating genistein synthesis and accumulation in yellow lupine and their impact on plant cell cytoskeleton. Molecules. 19(5): 13392-13421.

Murthy, H. N., Dandin, V.S. and Paek, K.Y. 2016. Tools for biotechnological production of useful phytochemicals from adventitious root cultures. Phytochem Rev. 15: 129-145.

Ng, H.K., Ph, T.F., Lam, S.K., and Hoe, S.Z. 2013. Potassium channel openers and prostacyclin play a crucial role in mediating the vasorelaxant activity of *Gynura procumbens*, BMC Complement. Altern. Med., vol. 13. 10.1186/1472-6882-13-188.

Nordstrom, A-C., Jacobs, F.A., and Cohen, J.D., 2004. Hormone Biosynthesis, Metabolism And Its Regulation, *in* Davies, P.J., ed, Plant Hormones: Biosynthesis, Signal Transduction, Action!*,* 3rd Edition, 36-62.

Saiman, M. Z., Mustafa, N.R., Schulte, A.E., Verpoorte, R., Choi, Y.H., 2012. Induction, characterization, and NMR-based metabolic profiling of adventitious root cultures from leaf explant of *Gynura procumbens.* Plant Cell Tiss Organ Cult. 109(3):465-475.

Shinde AN., Malpathak N, and Fulzele DP,. 2009. Enhanced production of phytoestrogenic isoflavones from hairy root cultures of *Psoralea corylifolia* L. using elicitation and precursor feeding. Biotechnol. Bioprocess Eng. 14: 288-294.

Taiz, L. and Zeiger, E. 2002. Plant physiology 3rd edition. Sinauer Associates. Sunderland.

Thorpe, T., Stasolla, C., Yeung, E.C., de Klerk G-J., Robert A and George, F. 2008. The components of plant tissue culture media II : organic additions, osmotic and pH effect, and support systems, *In* George E.F., Hall M.A and de Klerk G-J., ed. Plant propagation by tissue culture, 3rd edition, vol 1:115-173. Springer. Netherlands.

Tzin, V and Galili, G. 2010. The Biosynthetic

Pathways for Shikimate and Aromatic Amino Acids in *Arabidopsis thaliana*. The Arabidopsis Book, 8: e0132. Published online 2010 May 17.

Yeoman, M.M. and Yeoman C.L. 1996. Manipulating secondary metabolism in cultured plant cells. New Phytol. 134(4): 553-569.

Yin, S., Zhang, Y., Gao, W., Wang, J., Man, S, and Liu, H., 2014. Effects of nitrogen source and phosphat concentrationon biomass and metabolites accumulation in adventitious root culture of *Glycyrrhiza uralensis* Fisch. Acta Physiologiae Plantarum, 36:915-921.

Zhang, Y.H., Zhong, J.J and Yu, J.T. 1996. Enhancement of ginseng saponin production in suspension cultures of *Panax notoginseng*: Manipulation of medium sucrose. *J. Biotechnol,* 51: 49–56.