# Secondary metabolites in elaeocarpus grandiflorus cell culture in WPM medium with various concentrations of PGR

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**Abstract.** This study aims to analyze the influence of plant growth regulator (PGR) for secondary metabolites production in Elaeocarpus grandiflorus cell culture. Picloram (3.5, 5 and 7.5 ppm) and 2,4-D (1.5, 2.5 and 3.5 ppm) were used as PGR of cell suspension culture. Cell cultures were obtained from callus grown in liquid WPM medium with various concentrations of PGR. Culture is maintained in shakers at a speed of 120 rpm in dark conditions. Harvesting is done after the age of culture reaches 30 days. Extraction was done by the maceration method with methanol as a solvent. A qualitative test using a thin layer chromatography test (TLC) was conducted to analyze the presence of various secondary metabolites. Phenolic quantitative tests were performed using a spectrophotometer using gallic acid standard compounds. The results showed that the cell culture contained alkaloids, phenolics, terpenoids, flavonoids. All treatments showed the presence of phenolic with varying total phenolic concentrations. PGR affects the type and concentration of secondary metabolites produced by E. grandiflorus cell suspension culture.

## 1. Introduction

Bioactive compounds synthesis in cell culture varies greatly between species. Some cell cultures could produce bioactive compounds but some cannot. Some cultures can produce more secondary metabolites than intact plants but some are not. The secondary metabolite production in vitro culture is affected by several factors including the concentration of exogenous growth-regulating substances [1-3]. Secondary metabolites production in callus is greatly determined by hormones. Hormone signals can regulate physiological performance in plant culture [4]. Exogenous growth regulators could adjust the number of secondary metabolites by controlling the expression of genes tangled in the secondary metabolites synthesis.

The control of gene expression is done at the transcription stage by regulation of gene transcription factors that play a crucial role in plant development the process including the synthesis of secondary metabolites [5] [6]. Specific transcription factors for NAM/ATAF/CUC (NAC) plants play an essential role in various developmental processes including guard responses. Factors of NAC domain transcription are thought to be controlled at the level of transcription by environmental factors, such as hormone concentration. Induction at the level of PmNAC1 transcription is related to an increase in auxin concentrations in the culture medium6. Auxin has also been known to stimulate enzyme coding genes in the flavonoid biosynthesis pathway. The addition of 1 μm IAA induces accumulation of

mRNA CHALCONE SYNTHASE (CHS) and FLAVONOL SYNTHASE (FLS) three times greater than controls [7]. Saponins production in callus culture of Solanum aculeatissimum was affected by ZPT [8]. Callus produces 0.8% saponin (per dry weight), or 0.32% aculeatiside A and 0.48% aculeatiside B at the addition of 0.05 ppm or 0.1 ppm NAA and 10 ppm BA. Plant hormones also highly influence the number of isoflavonoids in soybean callus tissue. The addition of 5 mg/L of NAA raises the isoflavonoids production to 1100 nmol/g wet weight [9]. The composition of hormones in culture medium determines the production of lanatoside C and digoxin in the culture of Digitalis davisiana 3. Increased metabolites secondary to auxin are also claimed in Morus alba culture. In the culture of M. alba, the increment of IAA to the medium to increase the development of roots and adventitious callus also surges custom content. The administration of 5 mg/L IAA raised custom production 87.5% along the first 4 weeks later the callus was induced [10].

Elaeocarpus grandiflorus has long been used as an anti-diabetes drug. Scientific evidence in this regard has been widely reported. E. grandiflorus extract had antidiabetic activity [11]. In addition, it was also reported that E. grandiflorus accommodates bioactive compounds that have anti-viral activity [12], antibacterial [13] and anti-infectious [14]. Secondary metabolites detected in various species of Elaeocarpus include flavonoids, alkaloids, tannins, triterpenes, glycosides, fatty acids, and cytotoxic compounds [15].

## 2. Materials and method

WPM medium is made by means of stock WPM medium taken according to the composition and added with growth regulators according to treatment (2,4-D with concentrations of 1.5, 2.5 and 3.5 ppm and Picloram of 3.5 5 and 7, 5 ppm). The mixture is then added with distilled water until reaching the expected volume. The mixture was also administered with 3% sucrose, and the pH was adjusted to 5.8. A total of 20 ml of media solution was put into a 100 ml Erlenmeyer and tightly closed, then sterilized by an autoclave with 121oC, the pressure between 1.1-1.5 kg/cm2 for 20 minutes.

The cell suspension culture is done by transferring callus into a 100 ml Erlenmeyer containing 20 ml of WPM medium with various ZPT concentrations. The culture is shaken to the shaker at 100 rpm. The culture was kept for 30 days in the dark. After 30 days the cells are harvested and dried by oven at 60°C for 48 hours. The test of compound content is performed by pounding dry cells using pestle and mortar so that it becomes powder. The powder then was macerated by methanol for 2 days. The extract is later dried and resuspended in methanol. Subsequently, the sample was filtered using a 0.45 µ filter. The sample then was analyzed qualitatively by the TLC method to detect classes of bioactive compounds. Quantitative phenolic compounds were tested using a spectrophotometer, using gallic acid as a standard compound. Data analysis was performed descriptively.

## 3. Result and discussion

### *3.1. Result*

By using TLC method can be detected the presence of alkaloids, flavonoids and phenolics but not for steroids, saponins and tannins in cell suspension culture (CLC) after the 2,4-D administration (Table 1). Nevertheless, TLC is not capable to show the concentration of alkaloids, flavonoids, and phenolics. The terpenoid presence just detected in addition of 2.5 ppm 2,4-D, but not in 1.5 and 3.5 ppm. These results are corresponding to some previous researchers who used 2,4-D as PGR in cell suspension culture [15-20]. Increased of secondary metabolites to IAA addition are reported in the culture of Morus alba, especially routine content [10]. The data suggested that PGR addition have an effect to secondary metabolites production particulary for alkaloids, flavonoids and phenolics.

In case of Picloram, the addition has an effect on the production of alkaloids, flavonoids, and phenolics, but does not have an effect on the production of steroids, saponins and tannins on CLC (Table 1). This data indicated that 2,4-D and picloram give the similar effect on the secondary metabolite production. The optimal phenolic production and total phenolic content were 2.5 ppm 2,4-D and 7.5 ppm picloram, respectively. Nevertheless the effect of increasing the concentration of

picloram on the production of alkaloids, flavonoids, and phenolics cannot be demonstrated because TLC only provides qualitative information. Therefore, it is necessary to further investigate quantitatively to determinate the content of secondary metabolites found in the E. grandiflorus in CSC. Secondary metabolites contained in CSC E. grandiflorus almost similar with their relatives. Phytochemical screening of leave extracts (aqueous, ethanol and methnol) of E. serratus L., showed positive result with the presence of many significant phytochemicals like saponins, tannins, cardiac glycosides, flavonoids, steroids [21]. E. floribundus leaves and fruits phytochemical analysis (mesocarp-epicarp parts) revealed the presence of secondary metabolites i.e. alkaloids, phytosterol, tannin, saponins, terpenoids, phenol, and fixed oil as on various extracts [22]. These data indicate the potential of E. grandiflorus as a valuable source of secondary metabolites for medicine.

Secondary metabolites possess some significant role in living cells. In addition to antioxidant properties, flavonoids can directly interact with proteins, making them ideal small molecules for the enzymes' modulation, cell surface receptors, and transcription factors [23]. Flavonoids could modulate the tumor-associated macrophage function. Flavonoids have several pharmacological activities as antiparkinson, anti-ulcer, anti-depressant spasmolytic, anti-bacterial, anti-inflammatory, anti-hypertensive, anti-diabetic, and anti-cancer [24]. Plant-derived alkaloids have anti-colitis activity and potential for the treatment of inflammatory bowel disease (IBD) [25].

The phenolic content of E. grandiflorus in CSC was 1.357-2.094 mg/mL and Total Phenolic Content 0.0524-0.0739 mg Gallic acid equivalents/g extract in range (Table 2). Phenolic content and total phenolic content were fluctuate based on the concentration of PGR and there are similarities in the pattern of production between 2,4-D and picloram. The biochemical analysis performed in line with all phenolic contents in Crataegus azarolus was accessed in callus cultured on MS medium with 2.0 mg/L 2,4-D [18]. Based on some reports on the medicinal plant, phenolic compounds demonstrate free peroxide decomposition, radical inhibition, oxygen scavenging or metal inactivation in biological systems and prevent oxidative disease burden [26].

Some researchers used 2,4-D in the culture of the cell suspension to produce secondary metabolites. In the establishment of cell suspension culture, 2 mg/L 2,4-D and 1 mg/L kinetin were the best PGRs in encouraging cell growth and flavonoid production [16]. 2,4-D addition success to enhance of alkaloid accumulation in Catharanthus roseus [17]. There was an improvement of secondary metabolites in Spilanthes acmella cell culture, especially the scopoletin [27]. The addition of 2 mg/L 2,4-D in Gymnema Sylvestre cell suspension culture results in high production of phenolic compounds [19]. Cell suspension culture was established using green friable calli from explants of leaf cultured on MS medium with 2.0/mg/l 2,4-D [28]. Administration 2 mg/L picloram in Ficus deltoidea cell suspension culture result in flavonoids with the high content [29].

Biosynthesis and accumulation of secondary metabolites undergo in any cells of a plant; the secondary metabolites of interest can be cultured [30]. In case the storage and alkaloid biosynthesis are in a particular tissue or organ; then performing in vitro cultures of a specific tissue or organ is likely to obtain the expected substance [31].

Plant cell cultures with the high-value secondary metabolites production are promising potential alternative sources for the pharmaceutical agents' production of the industrial concern. Medicinal plant cell suspension cultures (MPCSC), which are characterized by fermentation with plant cell totipotency, could be a bright alternative ''chemical factory'' [31]. Based on the discussion above, the E. grandifloris plant has the prospect of being developed as a bioreactor of bioactive compounds, particularly alkaloids, flavonoids and phenolics by cell suspension culture.

**Table 1**. Detection of secondary metabolites in Elaeocarpus grandiflorus cell culture at WPM with the addition of 2,4-D and Picloram

| PGR (ppm) | | alkaloid | terpenoid | steroid | flavonoid | saponin | phenolic | tannin |
|---|---|---|---|---|---|---|---|---|
| 2,4 D | 1,5 | + | - | - | + | - | + | - |
| | 2,5 | + | + | - | + | - | + | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3,5 | + | - | - | + | - | + | - |
| Picloram | 3,5 | + | + | - | + | - | + | - |
| | 5,0 | + | + | - | + | - | + | - |
| | 7,5 | + | + | - | + | - | + | - |

**Table 2.** The total content of phenolic in Elaeocarpus grandiflorus cell culture on WPM with growth regulators variations

| PGR (ppm) | | Phenolic mg/mL | Total Phenolic (mg Gallic acid equivalents/g extract) |
|---|---|---|---|
| 2,4-D | 1,5 | 1.566 | 0.0524 |
| | 2,5 | 2.094 | 0.0650 |
| | 3,5 | 1.651 | 0.0739 |
| Picloram | 3,5 | 1.562 | 0.0691 |
| | 5,0 | 1.357 | 0.0596 |
| | 7,5 | 1.763 | 0.0566 |

## 4. Conclusion

These findings suggested that there were four secondary metabolites found in E. grandifloris cell suspension culture that are alkaloids, flavonoids, phenolics, and terpenoids. Both of PGR, 2,4-D, and picloram can be applied to induce secondary metabolites in E. grandifloris. The addition of 3.5 mg/L of 2,4-D was the optimum concentration to produce total phenolic content in cell suspension culture.

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## References

[1] Amoo S O and Staden J V 2012 *Plant Cell Tiss. Organ Cult*.**112** 249-256
[2] Amoo S O, Aremu AO & Staden J V 2012 *Plant Cell Tiss Organ Cult*. **111** 345–358
[3] Gurel E, Yucesan B, Aglic E, Gurel S, Verma S K., Sokmen M, and Sokmen A 2011 *Plant Cell Tiss. Organ Cult.* **104** 217–225
[4] Molchan O, Romashko, S and Yurin V *Plant Cell Tiss. Organ Cult.* **108** 535–539
[5] Zhao S, Sun H, Gao Y, Sui N and Wang B 2011 *In Vitro Cell. Dev. Biol. Plant* **47** 391–398
[6] Rosa Y B C J, Aizza L B C, Bello C C M, and Dornellas M C 2013 *Plant Cell Tiss. Org Cult*.
[7] Lewis D R, Ramirez M V, Miller N D, Vallabhaneni P, Ray W K, Helm R F, Winkel B S J and Muday G K 2011 *Plant Physiol.***156**(**1**) 144–164
[8] Ikenaga T, Handayani R and Oyama T 2000 *Plant Cell Rep*. **19** 1240–1244
[9] Downey P J, Levine L H, Musgrave M E, Keon-Bennett M, and Moane S 2013 *Microgravity Sci. Technol.* **25** 9–15
[10] Lee Y, Lee D E, Lee H S, Kim S K, Lee WS, Kim S H and Kim M W 2011 *Plant Cell Tiss Org.* **105** 9–19
[11] Bualee C, Ounaroon A and Jeenapongsa 2007 *Naresuan Univ. J.* **15** (**1**) 17-28
[12] Xu H, M Wan, B Loh, O Kon, P Chow dan K Sim 1996 *Phytotherapy Res.* **10** 207-210
[13] Goun E, Cunningham G, Chu D, Nguyen C, and Miles D 2003 *Fitoterapia* **74** 592-596
[14] Rahman A, Wahyuono S, and Bates R 1998 *Indo. J. Pharmacy* **9**(**3**)139-145
[15] Hardainiyan S, Nandy B C, and Saxena R 2015 *Int. J. Pharmacy Pharmaceut. Sci.* **7**(**6**) 415-418

[16] Tan S H, Musa R, Ariff A, and Maziah M 2010.*Am. J. Biochem. Biotechnol*. **6** (**4**) 284-299
[17] Verma A K, Singh R R, and Singh S 2012 *Botanica Serbica* **36** (**2**) 123-130
[18] Chaabani G, Tabart J, Khan M I and Karray-Bouraoui N 2015 Acta Physiol Plant **37** (**16**) 1-9
[19] Chung I M, Rajakumar G, Subramanian U, Venkidasamy B and Thiruvengadam M 2019.Appl. Sci. **9** 2165
[20] Pourianezhad F, Rahnama H, Mousavi A, Khosrowshahli M, and Mafakheri S 2019 Bioresour. Bioprocess **6** 23
[21] Das P, Kar P, Hasnu S, Nath S and Tanti B 2017 J Pharmacog. Phytochem. **6**(**4**) 866-869.
[22] Deivasigamani R and Devi M S 2018 J. Pharmacy Pharmaceut. Sci. **7**(**10**) 1452- 1457.
[23] Sudhakaran M, Sardesai S and Doseff AI 2019 Antioxidants **8** 103
[24] Rana A C and Gulliya B 2019 Chemistry and pharmacology of flavonoids- a review. Ind. J.Pharmaceut. Edu. Res. **53**(**1**) 8-20.
[25] Peng J, Zheng T T, Li X , Liang Y, Wang L J, Huang, Y C, and Xiao H T 2019 *Front. Pharmacol.* **10** 351
[26] Oberoi H S and Sandhu S K 2015 *Crit. Rev. Food Sci. Nutr*. **55** 319–337.
[27] Abyari M, Nasr N, Soorni J, and Sadhu D 2016 *Braz. Arch. Biol. Technol*. **59** e16150533, Jan/Dec
[28] Sathish S, Vasudevan V, Karthik S, Pavan G and Manickavasagm M 2019 *Plant Biotechnol. Rep*. **2** (**5**) 1-9
[29] Haida Z, Syahida A, Arif S M, Maziah, M and Hakima M 2019 *Sci. Rep.* **9** 9533
[30] Wink M and Roberts M F 1998 *Comparison of alkaloid synthesis, transport, and storage*. In: Roberts M.F., Wink M., editors. Alkaloids: Biochemistry, Ecology, and Medicinal Applications.New York, NY: Plenum Press; 1998. pp. 239-262
[31] Ratnadewi D 2017 *Alkaloids in plant cell cultures*. In: Alkaloids–Alternatives in Synthesis, Modification, and Application. Chapter 1 1-25. InTechOpen.www.intechopen.com