Effect of Growth Regulators on Cell Growth and Flavonoid Production in Cell Culture of *Elaecarpus grandiflorus*

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Abstract. *Elaecarpus grandiflorus* has the potential to be developed as a source of bioactive compounds. This study aims to obtain the most optimal medium for cell culture induction and flavonoid production in *Elaeocarpus grandiflorus* culture. Picloram (3.5, 5 and 7.5 ppm) and 2,4-D (1.5, 2.5 and 3.5 ppm) were used for induction of cell suspension culture. Cell suspension culture induction was observed through growth parameters (fresh and dry weight of cells) and cell suspension formation. In addition, it was found the production of flavonoids. Induction of cell culture is done by growing callus on Woody Plant Medium (WPM) with a variety of growth regulators. The culture was maintained in a shaker at a speed of 120 rpm for 30 days. At harvest, cells were filtered, weighed and dried. The spectrophotometer was used to determine the total flavonoid content. Quercetin was used as a standard compound. The best cell culture induction was obtained in cells maintained in WPM medium with the addition of 2,4-D 2,5 ppm. All cells in various treatment media can produce flavonoids with varying concentrations. WPM medium with the addition of 2,4 D and Picloram can be used for the production of flavonoids from *Elaecarpus grandiflorus* cells.

Keywords: *Elaecarpus grandiflorus,* cell culture, 2,4D, picloram, flavonoid production

1. Introduction

Elaecarpus grandiflorus contains bioactive compounds with anti-viral activity [1], antibacterial [2] and antidiabetic [3]. A study reported that the *E. grandiflorus* extract contained tannin, geraniin and 3, 4, 5- trimethoxy geraniin. Bioactive compounds found in this *E. grandiflorus* extract have anti-infective activity [4]. Other Elaeocarpus species are reported to contain alkaloids, flavonoids, glycosides, tannins, triterpenes, fatty acids, and cytotoxic compounds [5].

Plant cells have totipotential ability, so they can develop to form new intact plants and also to establish primary and secondary metabolite compounds as the parent plant if appropriately maintained [6]. The reason of in vitro secondary metabolites production is the existence of several compounds with complex structures making it hard to be synthetically produced, the high price of these secondary metabolite compounds as well as the scarcity of plants that synthesize these compounds or low levels of these compounds in plants [7].

In vitro flavonoid production reported done among others is in *Heliotropium indicum* culture [8], *Morus alba* culture [9], the callus and *Stelechocarpus burahol* cell culture [10,11,12], *Hypericum perforatum* cell suspension culture [13] , *Digitalis lanata* cell suspension culture [14] and *Ficus deltoidea* cell suspension culture [15]. The type and concentration of growth regulators is one of the factors that impact the production of bioactive compounds in vitro [10,11,12,16,17,18,19,20]. Hormone signals can regulate physiological processes in plant culture [21].

Flavonoids contained in *E. grandiflorus* has potential as a drug. Therefore, it is necessary to develop a method of producing flavonoids from *E. grandiflorus*. Cell culture is the right method for the production of flavonoids from *E. grandiflorus*. Induction of callus culture in E. grandiflorus has been reported in 2 different media, MS and WPM. 2,4-D and picloram treatments have succeeded in inducing callus formation with varying growth [22,23]. This research would provide a method of flavonoid production in the cell suspension culture of *Elaeocarpus grandiflorus*. The production of bioactive compounds in in-vitro culture is strongly influenced by the type and concentration of growth regulators. Therefore, it is necessary to optimize the type and concentration of growth regulators in the culture of *Elaeocarpus grandiflorus* so that it can produce high amounts of the targeted flavonoids.

2. Methods

Plants: Leaf stalk explants were taken from the 2-year-old *Elaeocarpus grandiflorus* plant which is maintained at Semarang State University. *Material for callus and cell suspension culture*: fungicide, bactericide, bleach solution (containing 5.25% NaClO) as explant sterilization material. WPM medium as a growth medium, 2,4-D (sigma), and picloram (Phytotech) as growth regulators. Ingredients for the determination of compound types: methanol (pa, Merck), NaNO2 (pa, Merck), AlCl3 (pa, Merck), Quercetin.

Independent variable: type and concentration of growth regulators. Two types of auxin used were 2,4-D with concentrations of 1.5, 2.5, and 3.5 ppm and Picloram with concentrations of 3.5, 5 and 7.5 ppm. Dependent variables: fresh and dry weight of the cells and production of flavonoid.

Control variables: medium pH and temperature of the planting room and the incubation room: 23-25°C.

Research procedure. WPM medium stock was taken according to the composition and dissolved in distilled water and then mixed with growth regulators and added distilled water until it reaches the desired volume. The mixture was stirred with a magnetic stirrer. The mixture was added with 3% sucrose, then shook again. The pH was adjusted to 5.8. The high-temperature media solution was poured into a 100 ml Erlenmeyer of 20 ml each, then closed tightly. Erlenmeyer with the media were sterilized in an autoclave with a temperature of 121°C and pressures between 1.1 - 1.5 kg / cm^2 for 20 minutes. The 30 days old callus with 5 times subculture used for cell cultur induction. Cell suspension culture formation is carried out by transferring 1 g of callus into a 100 ml Erlenmeyer containing 20 ml of WPM medium. The culture was shaken at a speed of 120 rpm. The culture was maintained for 30 days in dark condition. At harvest, cells were filtered and weighed. The cell was then dried in the oven for 48 hours at 60°C. The weight of dry cells was observed. Growth was determined by the fresh and dry weight of the cell.

The compound content test is done as follows: dry cells were crushed with mortar and pestle to be powder. The powder was macerated with methanol. The extract was then dried and resuspended in methanol. The sample was then analyzed by a spectrophotometer to determine the total flavonoid content. Quercetin was used as a standard flavonoid compound.

3. Results and Discussion

The growth of cell suspension culture shown by the fresh and dry cell weight is shown in Table 1. Based on the data in Table 1, it can be seen that cell culture grows most optimally on medium with 2.5 ppm of 2,4D while the lowest growth was observed in the medium with 1.5 ppm of 2,4D growth regulator. In addition to providing good growth, the addition of 2,4D encourages the formation of suspension cell culture with callus breakdown. In a medium with picloram, the callus tends to be compact and not fragmented to form cell suspension culture.

In *Papaver bracteatum* cell suspension culture, MS medium with the addition of 1 mg/L NAA, 1 mg/L BAP, and 15 mg/L ascorbic acid produces maximal growth compared to other treatments. Among auxins, NAA is a growth regulator that is more effective than 2,4-D for growth of *P. bracteatum* cell suspension cultures [24]. Medium with the 0.1 mg/L BA + 0.1 mg/L TDZ gives the results of fast and crumb *Elaeagnus angustifolia* cell growth [25]. In *Centella asiatica* culture, the formation of cell suspension culture gives the best results observed in the medium with the addition of

2 mg/L 2,4-D and 1 mg/L kinetin [26]. Picloram (7.5 ppm) has been reported to induce the growth of *stelechocarpus burahol* cell suspension cultures [11].

Based on the results of research that have been done and information from various literature, it can be concluded that the growth of cell suspension culture is strongly influenced by the type and concentration of growth regulators.

Table 1 *Elaecarpus grandiflorus* cell culture growth on WPM medium with variations in growth regulators

PGR (ppm)		Fresh weight (g)	Dry weight (g)
2,4 D	1,5	0,60	0,27
	2,5	1,52	0,48
	3,5	0,93	0,31
Picloram	3,5	0,84	0,30
	5,0	1,51	0,46
	7,5	1,00	0,36

The total flavonoid content in cell suspension culture was measured using a spectrophotometer with the standard quercetin. The results of determining the flavonoid content in cell suspension culture are presented in Table 2. Table 2 also provides the total-amount of content by calculating the dry weight of the harvested cells.

Table 2 The total flavonoid and phenolic content of *Elaecarpus grandiflorus* cell culture on WPM medium with variations in growth regulators

PGR (ppm)		Flavonoid (mg QE/g DW)	Flavonoid Total-Content (µg)
2,4 D	1.5	1.310	366.80
	2.5	1.575	456.75
	3.5	1.300	260.00
Picloram	3.5	1.299	337.74
	5.0	1.362	394.98
	7.5	1.360	421.60

Based on Table 2, the highest flavonoid production was obtained in cell cultures maintained on WPM medium with the addition of growth regulators 2,4D 2.5 ppm (1.575 mg QE/g DW). The lowest flavonoid production was obtained in cell cultures maintained on WPM medium with the addition of growth regulators 2,4D 3.5 ppm. The flavonoid production from this culture is lower than the flavonoid production in *Stelechocarpus burahol* culture [11].

Habibah et al. [10, 11, 12] reported that the production of flavonoids in *Stelechocarpus burahol* culture with various explants was influenced by ZPT. Ikenaga et al. [27] reported that the production of saponins in the *Solanum aculeatissimum* callus culture was influenced by ZPT. Plant hormones also significantly affect the accumulation of isoflavonoids in soybean callus tissue. The addition of NAA of 5 mg/L optimizes the production of isoflavonoids to 1100 nmol/g fresh weight [28]. Gurel et al. [20] reported that hormone composition in culture medium influences the production of lanatoside C and digoxin in *Digitalis davisiana* culture. Raising metabolites secondary to auxin are also reported in the culture of *Morus alba*. In *Morus alba* culture, the addition of IAA to the medium, instead of optimizing the development of adventitious callus and roots, it also increases routine content. The addition of 5 mg/L IAA raised routine production 87.5% during the first four weeks after the callus induced [9].

4. Conclusion

The growth of cell suspension culture of *Elaecarpus grandiflorus* is strongly influenced by the type and concentration of growth regulators. WPM medium with the addition of 2,4 D and Picloram can be used for the production of flavonoids from *Elaecarpus grandiflorus* cells.

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