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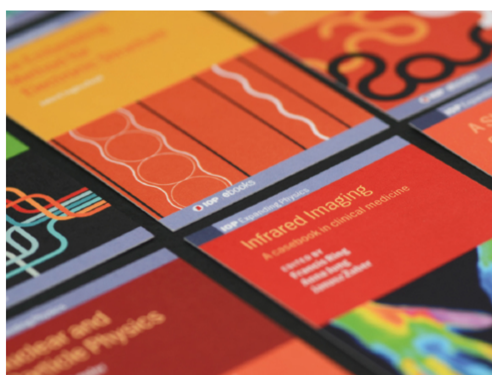

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Bioactive compounds from callus culture of *Elaeocarpus grandiflorus*

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Abstract *E. grandiflorus* has potential as a source of bioactive compounds. This study aims to analyze the content of flavonoid and phenolic bioactive compounds in the callus culture of *E. grandiflorus* in various concentrations of PGR. Callus culture induction was carried out by maintaining *E. grandiflorus* leaf stalk explants on solid Murashige & Skoog (MS) with the addition of growth regulators namely 2,4-dichlorophenoxyacetic acid (2,4-D) and picloram in different concentrations. The results of the study showed that callus maintained on MS medium with the addition of 2,4-D and picloram could produce flavonoids and phenolics. Flavonoid and phenolic concentrations produced in each treatment varied. Overall, it can be concluded that the MS medium with additional growth regulators, 2,4-dichlorophenoxyacetic acid (2,4-D) or picloram can be used for bioactive compound production of callus *Elaeocarpus grandiflorus*.

1. Introduction

Secondary metabolites production in callus is strongly influenced by hormones. The physiological processes in plant culture are regulated by existing hormone signals [1] and secondary metabolites production can be altered by exogenous growth regulators by regulating expression at the transcription stage of secondary metabolites genes [2], [3]. Transcription factors that play an important role in various development processes include defense responses namely NAM / ATAF / CUC (NAC), these are regulated by hormone concentration. The influence of hormones on the production of secondary metabolites in cultures has been reported by Rosa et al., [3] who explained that the increasing concentrations of auxin in culture medium induce PmNAC1 at the transcription level. The accumulation of mRNA CHALCONE SYNTHASE (CHS) and FLAVONOL SYNTHASE (FLS), which are enzymes involved in flavonoid synthesis, also occur due to the addition of IAA [4].

Phenolic in plants consists of simple phenols, lignins, coumarins, lignans, tannins, flavonoid and phenolic acids [5]. Phenolic compounds are reported to have various biological effects including anticancer, antiviral, antioxidant and anti-inflammatory activities. Phenolic acid has also been reported to inhibit colon cancer cell proliferation and induce cancer cell apoptosis [6]. The bioactive ability of flavonoids varies greatly as an anti-uric acid [7], anti-hypertension [8] and anti-bacterial [9]. Flavonoids are divided into several main groups namely chalcone, flavanones, flavones, flavanols, proanthocyanidin, flavandiol, aurone, and catechins [10]. Flavonoids in plants have a critical role in

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the integrity of plant structure, UV photoprotection, internal regulation of physiology, reproduction and plant cell signaling [10]. Besides, flavonoids also act as signaling molecules in plant responses to environmental signal, especially during stress of biotic and abiotic stress [11], as well as signaling of plant interactions with microbes [12]. Flavonoids, which have effective antioxidant activities, also control key stages in cell growth and differentiation so that they can regulate the overall development of plants and organs individually [13]. Although flavonoid biosynthesis in plants follows the same pathway, the type and concentration of flavonoids produced are different in each species. This occurs because the pattern of gene expression associated with key enzymes and transcription factors involved in flavonoid biosynthesis varies depending on the stage of organ development, hormonal treatment response and response to wounding stimulation [14]. In *Withania somnifera* culture, the type and concentration of auxin greatly affect biomass accumulation and withanolides production [15].

Callus induction on *Elaeocarpus grandiflorus* was successfully carried out on Murashige & Skooge (MS) medium and also Woody Plant Medium (WPM) with varying growths. The growth of callus *Elaeocarpus grandiflorus* in both media was very influenced by the growth of regulators used, namely 2,4D and picloram [16-18].

2. Methods

MS medium was prepared according to the composition and then added with growth regulators. The 3% sucrose was added and the pH was adjusted to 5.8 by adding a few drops of NaOH solution if it is too low or a few drops of HCl solution if it is too high. The 7 grams of agar powder were added to one liter of the medium mixture.

The petiole was sterilized following several procedures: the petiole was washed with running water, soaked in a fungicide and bactericidal solution for 10 minutes and rinsed with autoclaved water. Sterilization in LAF was done by immersion in a bleach solution (containing 5.25% NaClO) with a concentration of 20% for 10 minutes and washed using sterile aquadest for 3 times. The petiole was then cut into 2 cm size and planted on solid MS medium supllemented by growth regulators (2,4-D 1.5; 2.5 and 3.5 ppm) or picloram 3.5; 5.0; and 7.5 ppm) [19]. A bottle containing explants was placed on a culture rack in an incubation chamber with a temperature of 24-25ºC. The 5-month-old culture was then harvested and the callus was dried in the oven (60° C for 48 hours) to be analyzed for its flavonoid and phenolic content.

The flavonoid extraction method used followed by Hao et al.'s [19]. Dry callus (0.2 g) was crushed with mortar and pestle to make powder. Callus was extracted using methanol and incubated for one hour at 90°C. The dried extract was resuspended in methanol. The content of flavonoid was quantitatively analyzed using a spectrophotometer. The Zou et. Al. Method [20] was used to determine the total flavonoid content. Sample or standard solution (0.5 mL) was mixed with 2 mL aquadest and 0.15 mL of 5% $NaNO_2$ solution was added. After 6 minutes, 10% $AlCl_3$ solution (0.15 mL) was added and left for 6 minutes, then 4% NaOH solution (2 mL) was added to the solution . The water was added up to reaching the total volume of 5 ml. The mixture was vortexed to make it homogeneous and left for 15 minutes. The absorbance of the mixture was determined at λ 510 nm. Quercetin was used as a standard.

The determination of Total Phenol Content adopted Folin-Ciocalteu's Method [21]. Extract (100 mg) was dissolved to 10 mL with distilled water to obtain a concentration of 10 mg/mL. The concentration was pipetted in 1 mL and diluted with distilled water up to 10 mL. 1 mg/ mL concentration extract was obtained and pipetted in 0.2 mL extract. 15.8 mL of aquades and 1 mL Reagan Folin-Ciocalteu were added then shook. Let stand for 8 minutes then add 3 mL of 10% Na_2CO_3 to the mixture. Leave the solution for 2 hours. The absorption was measured with a UV-Vis spectrophotometer at a maximum absorption wavelength of 765 nm. Three repetitions were carried out so that the phenol content obtained was in mg equivalent to gallic acid/g of extract.

3. Results and Discussion

E. grandiflorus petiole grown on MS medium supllemented by 2.4 D growth regulator or picloram with various concentrations produced callus with varying growths. This is consistent with research that has been reported by Wijawati et al. [16]. The best growth rate was obtained in the medium supllemented by 5 mg/L picloram. The analysis results of secondary metabolites showed that the *E. grandiflorus* callus that grew on medium supllemented by 2,4D and picloram in all treatments produced flavonoids and phenolics with varying concentrations. The highest total flavonoid production per gram extraxt was produced in callus grown on medium with the addition of picloram 3.5 mg / L (Table 1). This shows that the production of flavonoids is influenced by growth regulators given.

This is in line with various studies that have been reported. Gurel et al. [22] revealed that concentration of hormone in *Digitalis davisiana* culture medium influenced the lanatoside C and digoxin production. In callus cultureof *Morus alba*, addition of IAA increased routine production by 87.5% [23].

Table 1. The flavonoid content of *Elaeocarpus grandiflorus* callus culture on MS medium with variations in growth regulators

PGR (ppm)		Flavonoid (mg/mL)	Total Flavonoid (mg quercetin equivalents/g extract)
2,4 D	1.5	1.5814	1.3534
	2.5	0.9869	1.8786
	3.5	1.1495	3.1333
Picloram	3.5	1.0394	3.3516
	5.0	0.2911	0.2389
	7.5	1.7172	1.7196

Phenolic production in a variety of plant callus cultures has been widely reported among others in the culture of Thevetia peruviana [27]; *Celastrus paniculatus* culture which is one of the medicinal plants in India [25]; and *Gossypium hirsutum* culture [26]. Callus grown on MS medium with an additional 2.4 D and picloram with various concentrations could produce phenolic compounds. Phenolic production in the *E. grandiflorus* callus varies according to the kind and concentration of growth regulators added (Table 2).

Table 2 The phenolic content of *Elaeocarpus grandiflorus* callus culture on MS medium supplemented by 2,4 D and Picloram

PGR (ppm)		Phenolic mg/mL	Total Phenolic (mg Gallic acid equivalents/g extract)
2,4 D	1.5	3.059	2.008
	2.5	2.293	3.522
	3.5	6.256	7.901
Picloram	3.5	5.128	14.681
	5.0	6.436	4.867
	7.5	3.463	3.279

4. Conclusion

Callus planted in medium with picloram and 2,4-D produced flavonoids and phenolics with varying concentrations. The kind of growth regulators and its concentrations influence the production of flavonoids and phenolic in E. grandiflorus callus culture.

5. Acknowledgment

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