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Production of biomass and flavonoid of *Gynura procumbens* (Lour.) Merr shoots culture in temporary immersion systemAyu Dewi Pramita^a, Alfinda Novi Kristanti^b, Sugiharto^a, Edy Setiti Wida Utami^a, Yosephine Sri Wulan Manuhara^{a,*}^aLaboratory of Plant Tissue Culture, Biology Department, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia^bLaboratory of Organic Chemistry, Chemistry Department, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

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ABSTRACT

Gynura procumbens (Lour.) Merris one of medicinal plant which was carried out used as antioxidant, anticancer, anti-inflammatory, hepatoprotective, and antimicrobial. Many strategies were used to increase the production of biomass and valuable compounds. This study was to investigate the variation effect of growth regulators and immersion frequency on production of biomass and flavonoid contained of *G. procumbens* shoots culture in temporary immersion bioreactor. Stem nodes were used as an explants and induction of shoots were done in solid MS medium supplemented with many kinds of growth regulator. The best treatments were used to produce biomass and flavonoid compounds in temporary immersion bioreactor; there are combination of IAA 2 mg/L and BA 4, 6, 8 mg/L and immersion frequency (5 min each 3 h; 15 min each 12 h). Results showed that the growths of *G. procumbens* shoots in solid MS medium were influenced by supplementation of growth regulators. MS medium supplemented with single cytokinin (6 mg/L kinetin) and combination of auxin (IAA) and cytokinin (BA) caused increasing of shoots growth. Production of biomass of *G. procumbens* in temporary immersion bioreactor was achieved in long immersion interval (12 h) and highest flavonoid production was obtained in combination treatment of immersion frequency 15 min each 12 h and MS medium supplemented with IAA 2 mg/L, BA 8 mg/L.

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1. Introduction

Gynura procumbens is one of medicinal plant that has been known to treat many diseases such as, anti-hyperglycemic [1], anti-hypertension [2], antimicrobial, antioxidant, anti-inflammatory, anticancer, cardio protective, and improving fertility [3]. Many kind of secondary metabolites that has been explored from *G. procumbens* are kaempferol, quercetine [4], rutin, myricetin, quercetin, apigenin [5] and stigmaterol [6], they are flavonoid compounds. Many flavonoids in *G. procumbens* were used as phytoalexin that was produced to response of elicitors, so the plant had disease resistant. Many flavonoids have an antioxidant bioactivity.

Secondary metabolites in plant were obtained from roots, stems, leaves, flowers and fruits. Over exploitation of plant to obtain secondary metabolites cause plant in eradication. Besides that, production of secondary metabolite in natural habitat was influenced by plant growth stage, environmental stress, nutrition

and plant genetic [7]. Plant tissue culture is an alternative technique to solve these problems because in this system, we controlled nutrition and environmental stress.

In recent years, biomass production of organ cultures has been developed in liquid culture, even to produce secondary metabolite. Micropropagation in liquid culture has been developed in many types of bioreactor such as balloon type bioreactor and temporary immersion bioreactor. Balloon type bubble bioreactor has been successfully done in micropropagation of *Morindacitrifolia* (L.) [8], *Eurycomalongifolia* [9], *Panax ginseng* C.A. Meyer [10,11], *Cyclopia-genistoides* (L.) Vent [12], *Hypericum perforatum* [13], *Aloe barbadensis* [14], and *Dendrobium candidum* Wall ex Lindl. [15]. Plant biomass production in balloon type bubble bioreactor has many profits, such as faster production, good quality, produce higher secondary metabolite and low cost, but in this bioreactor, the organ was submerged, so it will contain more water; this condition called hyperhydricity (a physiological disorder occurring in plant tissue culture characterized by high water retention capacity due to adverse culture condition). Besides that, the culture also became lack of oxygen. Temporary immersion system could solve this problem by way of the immersion frequency. Tissue or organ

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