

## Evaluation of Flavonoid Content from Shoot Culture of *Gynura procumbens* in Balloon-type Bubble Bioreactors

Ira Nailas Saadah<sup>1\*</sup>, Yosephine Sri Wulan Manuhara<sup>2</sup>, Alfinda Novi Kristanti<sup>3</sup>

<sup>1</sup> Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Walisongo, Semarang, Indonesia

<sup>2</sup> Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

<sup>3</sup> Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

### ABSTRACT

*Gynura procumbens* has been widely used to treat several health disorders, such as hyperglycemia, hypertension, diabetes, and allergies. This study aimed to determine the flavonoid content of *G. procumbens* cultured in a balloon-type bubble bioreactor using various sucrose concentrations (10, 30, and 50 g/L) and explant densities (5, 10, and 15 explants). The plant parts used were stem nodes of *G. procumbens*, grown in a balloon-type bubble bioreactor using Murashige and Skoog (MS) liquid media with IAA 2 mg/L and BAP 4 mg/L. The total flavonoid content was measured using a UV-Vis spectrophotometer ( $\lambda = 510$  nm), and the flavonoid profile was tested using thin-layer chromatography (TLC). The administration of 10 g/L sucrose produced the highest total flavonoid production. In the treatment of explant density, the most increasing total flavonoid production was obtained in the treatment of 5 explants. The TLC results showed that each treatment forms spots like standard. This study proved that sucrose and explant densities culture on balloon-type bubble bioreactor method had optimized the production of flavonoids of plant shoots of *G. procumbens* culture.

**Keywords :** *Gynura procumbens*; flavonoid; sucrose concentrations; explant densities

### INTRODUCTION

Plants produce several chemical compounds beneficial for health purposes called secondary metabolites (Chattopadhyay et al., 2002). Flavonoid is a secondary metabolite produced through the shikimate and malonate acetate pathways (Markham, 1982). A medicinal plant of *Gynura procumbens* contains flavonoids that have proved as many biological activities, such as antihyperglycemic (Algariri et al., 2014), antihypertensive (Kim et al., 2006), antimicrobial, antioxidant, anti-inflammatory, anticancer, cardioprotective, and increasing fertility (Tan et al., 2016). Ethanolic extract of *G. procumbens* was also observed as an anti-aging candidate by detaining matrix metalloproteinases (MMPs) dan reactive oxygen species (ROS) production in fibroblast cells exposed to ultraviolet (U.V.) radiation (Kim et al., 2006). The flavonoid group, quercetin, and kaempferol are the factors that play a role in inhibiting both MMPs and ROS (Lim & Kim, 2007).

In nature, to obtain secondary metabolites, large-scale plants must be destroyed (Chattopadhyay et al., 2002) because they are only produced by plants during certain stages of growth and development, during certain seasons, and under specific environmental or nutritional stress

(Baque et al., 2013). That is why an alternative propagation method needs to optimize the production of secondary metabolites without extensive plant destruction, such as balloon-type bubble bioreactors (BTBB) cultures. The use of BTBB has been widely developed for mass micropropagation of *Morinda citrifolia* (L.) (Jang et al., 2013), *Eurycoma longifolia* (Lulu et al., 2015), *Cyclopia genistoides* (L.) Vent. (Kokotkiewicz and Bucinski, 2015), *Hypericum perforatum* (Kwiecien et al., 2015), *Aloe barbadensis* (Mariateresa et al., 2014;), and *Dendrobium candidum* Wall ex Lindl. (Cui et al., 2014).

As an energy source, sucrose maintains to build cell organelles that make up plant organs that can affect the plant's metabolites. To optimize the flavonoid production of *G. procumbens*, shoot culture in Balloon Type Bubble Bioreactor (BTBB) needs to be treated. The differences in sucrose concentration and varieties of inoculum density were chosen to know the proper protocol for producing flavonoids.

### METHODOLOGY

#### Materials and Instruments

The mother plants were cultivated for at least six months before using soil media. The third to fifth nodal stem from the apex of *Gynura procumbens* was generated to be a shoot and conducted as an explant (Figure 1). Chemicals that

\*Corresponding author : Ira Nailas Saadah  
Email : ira\_saadah@walisongo.ac.id

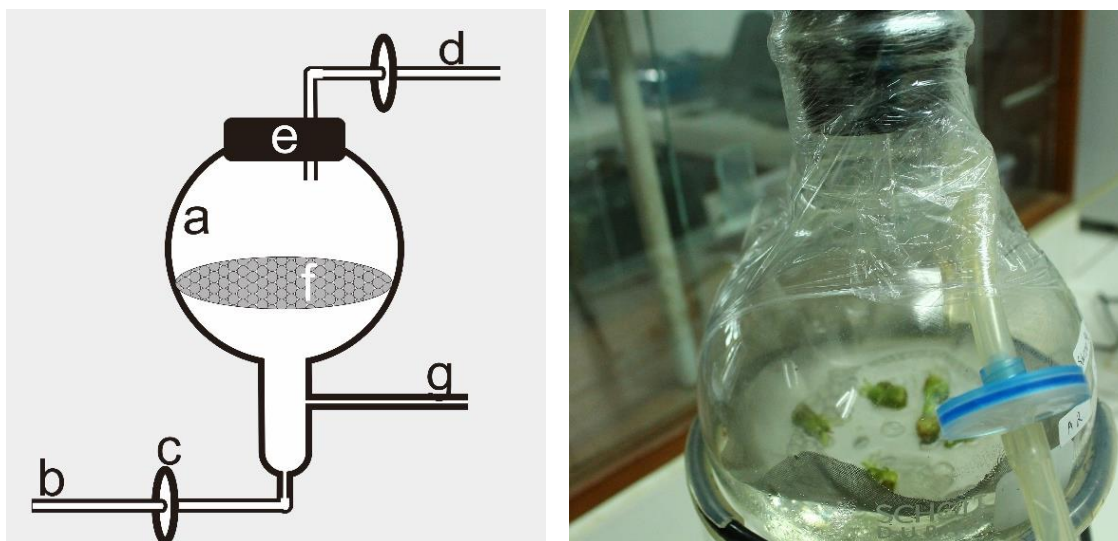


Figure 1. Left: Schematic diagram of a BTBB: (a) a vessel; (b) intake air aeration duct; (c) filter; (d) air outlet; (e) inlet explants; (f) mass; (g) sampling drain. Right: explants in BTBB.

are composing Murashige and Skoog media (Murashige and Skoog, 1962), indole-3-acetic acid (IAA), 6-benzyl amino purine (BAP) (Merck with pro analyze standard), sucrose, agar, and distilled water are used for culturing in BTBB (Figure 1), as mentioned in Saadah et al. (2019). Ethanol p.a (Merck), ethyl acetate p.a (Bratachem), glacial acetic acid p.a (Bratachem), formic acid p.a (Bratachem), and TLC silica gel 60 F<sub>254</sub> (Merck) are used to analyze total flavonoids content and profile.

### Methods

Shoots of *G. procumbens* is cultured using BTBB for four weeks under controlled condition (light intensity 1900 lux and temperature 28<sup>o</sup>±3<sup>o</sup>C) in different sucrose concentration (10, 30, and 50 g/L) and varieties of inoculum density (5, 10, and 15 shoots) (Saadah et al., 2019). Then, the shoots were dried using an oven at 60<sup>o</sup>C for 48 hours for flavonoid analysis. Extraction of flavonoids was performed using a 0.1 g drying sample and 10 mL ethanol (100 %) as a solvent in a water bath (temperature of 60<sup>o</sup>C) for 5 minutes and filtered with filter paper. The ethanol extract was concentrated to a volume of 2 mL. The total flavonoid content of the sample was analyzed by taking each extract from 0.25 mL of ethanol extract, then added by 1.25 mL of distilled water and 75 µl of 5% sodium nitrite, mixed, and allowed to stand for six minutes. Afterward, 150 µl of 10% aluminum chloride was added and mixed slowly. After five minutes, 0.5 mL of 1 M NaOH was added.

Next, distilled water was added to get a total volume of 2.5 mL (Kaewseejan *et al.*, 2015).

Ethanol was used as a blank solution. A UV-Vis spectrophotometer analyzed the absorbance of the solution at a wavelength of 510 nm (Kaewseejan *et al.*, 2015). The absorbance data obtained is the value of Y, which is used to calculate the value of X (total flavonoid content) and subject to the formula obtained from the standard curve of kaempferol that has been made (data not shown). A further test was presented to determine the flavonoid profile by thin-layer chromatography (TLC) with kaempferol as a standard (Faizah *et al.*, 2018). A TLC silica gel plate eluted the sample with ethyl acetate: n-hexane (4:6). The plate was sprayed with 1% ethanolic aluminum chloride solution and visualized under U.V. light at 365 nm (Gwatidzo *et al.*, 2018).

### RESULT AND DISCUSSION

The highest flavonoid content was obtained at ten g/L sucrose in the sucrose treatment. In the explant density treatment, the highest total flavonoid content was obtained at a density of 5 explants (Figure 2). However, the overall flavonoid yield of ex-vitro plants was still higher than the culture treatment in a bioreactor for four weeks. The TLC test results are shown in Figure 3.

The higher the sucrose content added in the culture medium, the higher the carbon source available in the medium. The number of carbon sources available in the medium could increase the shoot biomass of *G. procumbens*, as reported by

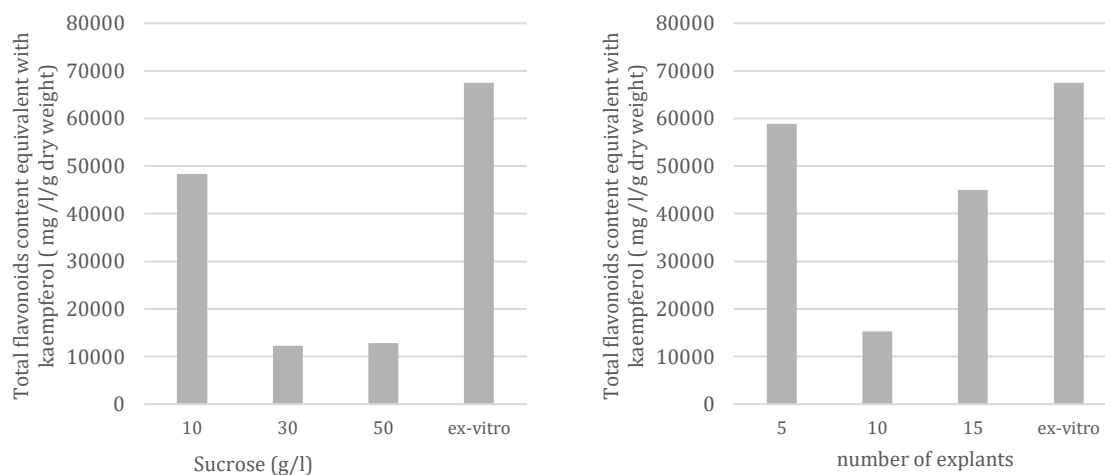


Figure 2. Total flavonoid content based on sucrose concentration and explant density.

Saadah et al. (2019). Sucrose will be hydrolyzed to glucose and free fructose by the enzyme invertase before being used (Salisbury and Ross, 1995). Some sucrose in the tissue culture medium is degraded into free fructose and glucose (Ball, 1953). Sucrose is easily accessible by plants for cell metabolism processes and building cell organelles. According to Markunas et al. (2011), sucrose also plays a vital role in forming secondary metabolites used as raw medicinal ingredients. Sucrose can increase the expression of flavonoid biosynthesis genes encoding phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), and isoflavone synthase (IFS) (Markunas et al., 2011).

The highest total flavonoid content was obtained in the sucrose treatment of 10 g/L (Figure 2). Sucrose acts as a carbon source, controlling the osmotic of plant cells and as a signal coding gene for the formation of secondary metabolites, requiring an in-depth study of the biosynthetic process and its regulation in each plant species (Ferreyra et al., 2012). In this case, the plant is experiencing nutritional stress due to the low sucrose levels available in the media. According to Baque et al. (2015), plants that experience anxiety due to a lack of nutrients can trigger the formation of secondary metabolites (flavonoids).

The culture period influences the formation of secondary metabolites of cultured plants (Baque et al., 2013). In the density treatment of five explants, the plants had sufficient space to grow well and reached three growth phases: lag, exponential, and stationary. It can cause the total

flavonoid content of the plant shoot culture of *G. procumbens* to achieve optimal results in treating five explants. On the contrary, the density treatment of 15 explants had limited space where plant explants competed for growing room and nutrients. The lack of condition can trigger plant stress leading to flavonoid production (Baque et al., 2013).

Overall, the yield of total flavonoid content from ex-vitro plants was higher than thus of plants cultured in a bioreactor. However, a bioreactor produced flavonoids during a culture period of 4 weeks, shorter than conventionally cultivated ex-vitro plants. Ex-Vitro plants require a long time to use as raw materials to obtain secondary metabolites (flavonoids). This conventional method cannot meet the demand for plants as raw materials in pharmaceuticals, aromatherapeutic products (Keng et al., 2009), and raw materials for the food industry (Mariateresa et al., 2014).

In Order to prove the presence of the flavonoids, each treatment was tested by the TLC with the kaempferol standard. The results of the chromatogram under 366 nm UV (Figure 3) showed a color of fluorescence that indicates the presence of flavonoids (kaempferol) (Gwatidzo et al., 2018). Figure 3 shows that each treatment forms a similar spot to the standard (kaempferol) spot, but the fluorescence strength is slightly different from the others. The ex-vitro plant seems different from others and may contain more complex metabolites. Faizah et al. (2018) reported the presence of flavonoids in the root culture of *G. procumbens* by the kaempferol as the standard. The effect of carbon source.

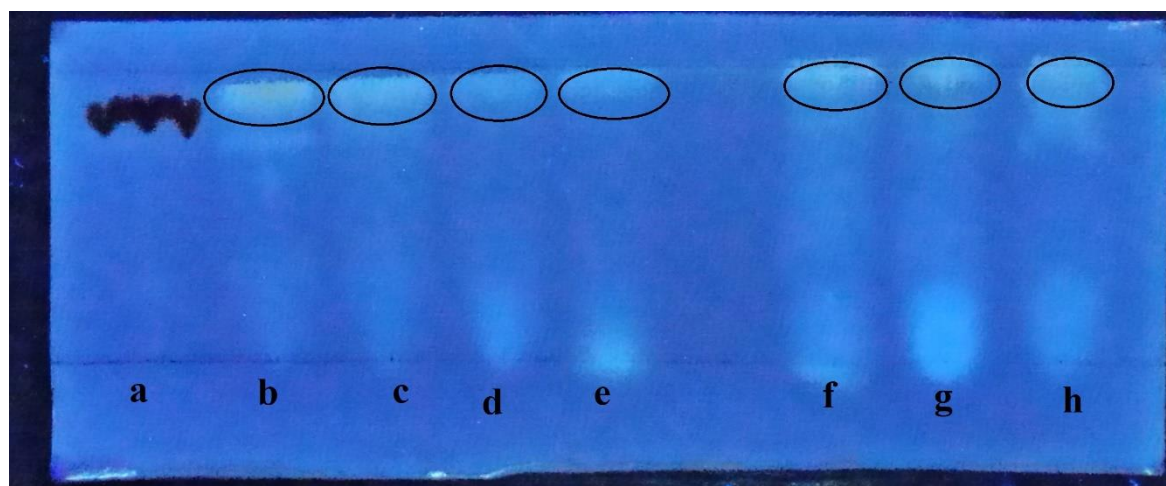


Figure 3. Chromatogram of Flavonoid of *G. procumbens* in BTBB. a. ex vitro plant; b. standard (kaempferol); c. sucrose 10 g/L; d. sucrose 30 g/L; e. sucrose 50 g/L; f. 5 explants; g. 10 explants; and h. 15 explants.

## CONCLUSION

From this research, we conclude that based on the sucrose content, the best total flavonoid production was obtained in the treatment with ten grams per liter of sucrose and the density of explants with five. The chromatogram results showed that each treatment forms a spot similar to standard.

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