Eco. Env. & Cons. 27 (4) : 2021; pp. (1880-1884)
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ISSN 0971–765X

Comparison of antioxidant potential of *Gynura procumbens* adventitious root in vitro culture and ex vitro

Sugiharto*, Dannis Yuda Kusuma, Dwi Winarni, Idqa Nurtri Bhakti, Abdul Hakim Muhsyi and Yosephine Sri Wulan Manuhara*

Biology Department, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

(Received 20 October, 2020; Accepted 16 April, 2021)

ABSTRACT

The aim of this research was to compare antioxidant potential from methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro. Adventitious root *in vitro* biomass was obtained from root harvesting after 28 days of culture in liquid MS medium supplemented with 5 mg/l indole butric acid (IBA) in ballon type bubble bioreactor. Extraction was carried out by the maceration method with methanol as a solvent. The antioxidant potency assay were carried out by determined total phenolic content (TPC), total flavonoid content (TFC), analysis of flavonoids by High Performance Liquid Chromatography (HPLC), and analysis of antioxidant activity by 2,2-Diphenyl-picrylhydrazyl (DPPH) assay.Based on the data analyzed of TPC, TFC, and HPLC assay, they showed that methanol extract of adventitious root in vitro culture and ex vitro of *G. procumbens*, have potential as an antioxidant activity. Both in vitro and ex vitro the root of *G. procumbens* contain phenolic and flavonoids compound such as myricetin, kaempherol, quercetin, and catechin. Analysis by DPPH assay, *G. procumbens* adventitious root *in vitro* culture indicated moderate antioxidant activity and ex vitro was strong antioxidant activity.

Key words: *Adventitious root, Antioxidant activity, Gynura procumbens, In vitro and ex vitro*

Introduction

Indonesia is a mega biodiversity country and has many plants that have potential as medicinal plant, such as *Gynura procumbens*. People cultivate *G. procumbens* in their yards, because they are ussually consumed as fresh vegetable and as an appetizer to increase their appetite. *G. procumbens* is commonly found in Malaysia, Thailand, and tropical Asian countries (Keng *et al.,* 2009; Puangpronpitag *et al.,* 2010; Tan *et al.,* 2016).Traditionally, the leaves and roots of *G. procumbens* are parts that are often used as medicine plant by people in many tropical Asia countries, including Indonesia. The leaves of *G. procumbens* are widely used of health ailments such as fever, benign tumors, relieving itching due to caterpillars, diabetes mellitus, hypertension, and dysentery. While the roots of *G. procumbens* are widely used for the treatment as a painkiller and increases blood flow. These beneficial effects of *G. procumbens* are related to the presence of bioactive compound, such as phenolic, flavonoids, tannins, saponins, terpenes, coumarins, anthocyanins, and alkaloids (Dai *et al.,* 2007; Hu *et al.,* 2019a; Puangpronpitag *et al.,* 2010; Tan *et al.,* 2016).

G. procumbens has the potential as a natural anti-

oxidant plant. In several studies, extract of various *G. procumbens* callus culture organs indicated as antioxidant activity, because it contained phenolic and flavonoid compounds. In the DPPH test, the highest antioxidant activity was found in the root organ, worth of 84% (Krishnan *et al.,* 2015). In the ferric reducing test and DPPH assay, crude ethanolic extract of *G. procumbens* leaves was also shown to have antioxidant activity (Kaewseejan *et al.,* 2015).

Adventitious root culture especially from the balloon type bubble bioreactor technique has been used for the production of various secondary metabolites, because of its fast growth rate, production can be carried out at any time regardless of the season, and was capable of producing stable secondary metabolite content (Murthy *et al.,* 2014; Wu *et al.,* 2006). Several studies reported that the adventitious root in vitro culture of *G. procumbens* could produce more biomass and secondary metabolites (Saiman *et al.,* 2012), increased flavonoid content (Pramita *et al.,* 2018), increased biomass production and levels of flavonoids, quercetin and kaempferol (Manuhara *et al.,* 2019).

The aim of this research was to compare antioxidant potential from methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro. The antioxidant potency test were carried out by determined total phenolic content (TPC), total flavonoid content (TFC), analysis of flavonoids content by High Performance Liquid Chromatography (HPLC), and analysis of antioxidant activity by 2,2-Diphenyl-picrylhydrazyl (DPPH) assay.

Materials and Methods

Plant material and preparation adventitious roots *G. procumbens* in vitro culture

G. procumbens was obtained from Purwodadi Botanical Garden, Pasuruan, East Java, Indonesia. It was then nurtured in a polybag with a mix of soil and organic fertilizer (50:50) and incubated at room temperature. Adventitious root was obtained from leaf explants of *G. procumbens*.

The stock of adventitious roots *G. procumbens* in vitro, grown using 5 mg/l indole butyric acid (IBA) growth regulator in medium Murashige and Skoog (MS). The root stock is used as broodstock adventitious root, which will be used for the next root culture process. An adventitious root starter was used as the initial inoculum for root culture in a balloon type bubble bioreactor and placed in an incubation chamber. Adventitious root biomass was obtained from root harvesting after 28 days of culture in a laboratory scale bioreactor (Faizah *et al.,* 2018).

Methanol extract of *G. procumbens*

G. procumbens adventitious root in vitro culture and ex vitro were dried in oven at 50-60 °C for 5 days and crushed by mortar. Extraction was carried out by the maceration method with methanol as a solvent. A total of 25 g of dry root powder were immersed in 250 ml of methanol, the maceration process was carried out for 24 hours in a shaker. The supernatant was filtered through filter paper. This procedure was performed in triplicate, and then the filtrates were concentrated by evaporator.

Determination of total phenolic content (TPC) of *G. procumbens*

The TPC methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro were determined by Folin–Ciocalteau methods. Briefly, 200 mL of extract solution was mixed with 1 ml of diluted reagent solution (1:10 with distilled water) and incubated for 5 minutes. After incubated, 0.8 ml of 7.5% sodium carbonate solution was added and the mixture allowed to stand for 30 minutes at room temperature. Samples and blanks were measured by microplate reader Multiskan Go–Thermo scientific at 765 nm. Determination of TPC by calculating the absorbance of the sample through the regression equation obtained from the standard curve of gallic acid compound (Kaewseejan *et al.,* 2015).

Determination of total flavonoid content (TFC) of *G. procumbens*

The TFC methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro were determined by a modified colorimetric method. Briefly, 250 µl of extract solution mixed with 125 ml of distilled water and 75 µl of 0.5% sodium nitrate solution, then incubated for 6 minutes. 150 µl of 1% aluminum chloride solution was added and let stand for 5 minutes, and then 150 µl of sodium hydroxide 1M was added and made up to a total volume 2.5 ml with distilled water. Samples and blanks were measured by microplate reader Multiskan Go–Thermo scientific at 510 nm. Determination of TFC by calculating the absorbance of the sample through the regression equation obtained from the standard curve from compounds of quercetin and

kaempferol (Kaewseejan *et al.,* 2015).

Analysis of flavonoids *G. procumbens* by HPLC

HPLC analysis methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro were performed by Agilent 1100 series HPLC (auto sampler and DAD detector) with a Licrospher 100 RP-18 column (5 µm) 4.6x250 mm. Briefly, 200 µl of the ectract solution was filtered with a 0.45 µm nylon membrane and mixed with 200 µl of standard kaempferol, quercetin, catechin, and myricetin. The eluent was used acetonitrile:0.1% formic acid (60:40) for kaempferol and quercetin, while for catechin and myricetin using acetonitrile eluent:acetic acid 0.25% (20:80), flow rate 1 ml/minute (25 °C), with injection volume 20 µl. The absorbance were measured at 299 nm (for kaempferol and quercetin), 280 nm (for catechin), and 340 nm (for myricetin). Determination of flavonoids *G. procumbens* adventif roots by calculating the absorbance of the sample through the regression equation obtained from the standard curve of kaempferol, quercetin, catechins, and myricetin.

Analysis of antioxidant activity *G. procumbens* by DPPH

The antioxidant activity methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro were determined by DPPH assay with some modifications. Briefly, 100 µl of extract solution at various concentrations placed into 96 well plates with methanol solution as a control. Fifty microliter 100 µM DPPH was added followed by 30 minutes incubation in the dark. The percentage of scavenging activity measured at λ = 517 nm in a microplate reader. The results were expressed as an IC_{50} value (Sugiharto *et al*., 2019).

Data analysis

Data triplicate *G. procumbens* adventitious root in vitro culture and ex vitro for determination of TPC and TFC, analysis of flavonoids by HPLC, and analysis of antioxidant activity by DPPH assay were fitted to a regresion equation obtained from the standard curve.

Results and Discussion

In recent years, *in vitro* culture technique have an effective method for producing large amounts of biomass and secondary metabolites (Cui *et al.,* 2010). Some of the advantages of *in vitro* culture techniques for producing secondary metabolites are: (1) it can be carried out in a laboratory or industrial scale without affected by environmental conditions, (2) secondary metabolite products can be increased by various methods such as replenishment nutrition, precursors, biotic and abiotic environments, and (3) purification of secondary metabolites more easier. Adventitious root *in vitro* culture has been used for the production of various secondary metabolites. Adventitious root in vitro cultures show high genetic stability and biosynthetic ability and can be used for several consecutive generations. Therefore, it can be cultivated and can be applied for the commercial production of biomass and bioactive compounds (Murthy *et al.,* 2014; Lulu *et al.,* 2015; Wu *et al.,* 2006).

The results of antioxidant potential from methanol extract adventitious root *in vitro* culture and *ex vitro* of *G. procumbens* are shown in Table 1.

Based on the data analyzed of TPC, TFC, and HPLC assay, they showed that methanol extract of adventitious root *in vitro* culture and ex vitro of *G.*

Table 1. Antioxidant potential methanol extract of *G. procumbens* root

Data	Standard equivalents	Methanol extract of *G. procumbens* root	
		In vitro	*Ex vitro*
TPC	Gallic acid (mg/g DW)	120.24 ± 2.81	226.39 ± 5.65
TFC	Quercetin (mg/g DW)	289.44 ± 6.94	390.56 ± 7.69
	Kaempherol (mg/g DW)	1148.15 ± 23.13	1485.19 ± 25.66
HPLC	Myricetin (mg/l)	309.99 ± 0.19	324.76 ± 1.06
	Kaempherol (mg/l)	306.95 ± 1.61	429.49 ± 0.80
	Quercetin (mg/l)	179.38 ± 0.75	149.13 ± 0.76
	Catechins (mg/l)	151.50 ± 0.61	159.68 ± 0.99
DPPH	IC_{50} (µg/ml)	148.0	53.0

Results are expressed as mean ± SD (n = 3). DW=dry weight, TPC=total phenolic content, TFC= total flavonoid content, HPLC=High Performance Liquid Chromatography, DPPH=2,2-Diphenyl-picrylhydrazyl

procumbens, have potential as an antioxidant activity. Both *in vitro* and *ex vitro*, the root of *G. procumbens* contain phenolic and flavonoids compound such as myricetin, kaempherol, quercetin, and catechin. The antioxidant activity *G. procumbens* was strongly correlated with myricetin and kaempherol contents, because they showed the highest content. Flavonoids are the most potent scavenger of free radicals and potentially useful in the prevention of many disease due to oxidative damage. This data supported by several studies which reported that ethanol extract of the roots of *G. procumbens* showed antioxidant activity because it contains phenolic and flavonoids compounds, such as quercetin, myricetin, and kaempferol (Faizah *et al.,* 2018; Krishnan *et al.,* 2015). Hu *et al.,* (2019b) showed that isolation of *G. procumbens* roots were found three components of steroid compounds, namely β-sitosterol, daucosterol, and stigmasterol through Nuclear Magnetic Resonance (NMR) analysis.

The antioxidant activity of *G. procumbens* extract was assessed via DPPH assay to measure its free radical scavenging ability. Based on analyzed by DPPH assay, adventitious root in vitro culture of *G. procumbens* was determined IC_{50} at 148.0 µg/ml, indicated moderate antioxidant activity and ex vitro extract at 53.0 µg/ml was strong antioxidant activity. In comparative study, shoot of *G. procumbens* callus indicated the least inhibition concentration (66.89%) and root of *G. procumbens* showed the highest activity (84.00%) (Krishnan *et al.,* 2015). Meanwhile, *G. procumbens* leaves showed IC_{50} was 473.70 µg/ml for crude ethanolic extract and 220.58 µg/ml for ethyl acetate fraction (Kaewseejan *et al.,* 2015).

Conclusion

Methanol extract of *G. procumbens* adventitious root in vitro culture and ex vitro, have potential as an antioxidant activity. It's correlated with phenolic and flavonoid content. Analysis of antioxidant activity by DPPH assay, *G. procumbens* adventitious root in vitro culture indicated moderate antioxidant activity and *ex vitro* was strong antioxidant activity.

Acknowledgement

We gratefully acknowledge to Universitas Airlangga for supporting via a Research Group grant (No.434/UN3.14/PT/2020) and scholarship from Ministry of Education and Culture (PDD grant No. 853/UN3.14/PT/2020).

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