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The Potential Source of Natural Antioxidant Agent of *Cassia alata* Microgreen

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Abstract

This study aimed to utilize *Cassia alata* (C. alata) as a microgreen and evaluate its potential as a source of natural antioxidant agents. The seeds of *Cassia alata* were cultivated in Rockwool at room temperature (27±1oC). Uppon the appearance of the first true leaves, approximately 21 days after planting, microgreens were harvested from triplicate trays using sterilized scissors. The antioxidant activity was assessed using the DPPH radical scavenging activity method and analyzed via UV-VIS spectrophotometry. The results showed that the IC50 values of *C. alata* microgreens was 1.789x103 µg/mL, categorizing it as a weak antioxidant. This study indicates that the extract of *C. alata* microgreens has the potential to be a natural source of antioxidant agents.

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Introduction

In current years, microgreen has popular and is often used as a culinary (Ghoora et al., 2020) for consumers concerned about their health (Supapvanich et al., 2020). Microgreens is a new type of vegetable, immature plants, and harvested at the first true-leaf stage on 10-14 days (Tan et al., 2020; Turner et al., 2020). It has been reported that microgreens are high in phytochemicals (Marton et al., 2010; Xiao et al., 2012) and antioxidants (Senevirathne et al., 2019). The antioxidant has a critical role in preventing cell damage (Yadav et al., 2016) and contributes to health benefits (Grosso et al., 2013). According to the study by Ghoora et al. (2020), some microgreen plant, like onion, mustard, carrot, and fennel, contain DPPH antioxidant activity IC_{50} *452.4±51.3; 168.4±14.8; 97.6±2.1; 94.3±0.7* µg/mL*, respectively.* However, there has been no further research related to *C. alata* microgreens.

C. alata (*Casia alata*) is a native plant from Argentina. In Indonesia, it is known as “Ketepeng Cina” (Fatmawati et al., 2020). This plant can grow in the tropics, mainly in South Kalimantan. *C. alata* is a type of herb plant (Chatterjee et al., 2013). In South Kalimantan, the extract of *C. alata* leaves is commonly used as the traditional herb for skin disease. According to Oladeji et al. (2020), the extract of *C. alata* leaves is commonly used as the traditional herb for typhoid, diabetes, malaria, asthma, ringworms, tinea infections, scabies, blotch, herpes, and eczema. The seeds and leaves of *C. alata* can be used as an antimicrobial (Abdulwaliyu et al., 2013), anti-inflammatory (Wongkaew and Sinsiri,

2014), antidiabetic (Abdulwaliyu et al., 2013), and antifungal (Wongkaew and Sinsiri, 2014).

Several studies have shown that *C. alata* is rich in antioxidants (Fatmawati et al., 2020), such as ascorbic acid, flavonoid, tocopherol, anthraquinone, and carotene (Chatterjee et al. (2013). Thus, this study was conducted to the utilization of *Cassia alata* as a microgreen. The aim of this study evaluates their potential source of natural antioxidant agents.

Materials and Methods

Plant Material

The seed of *Cassia alata* L. was obtained from PT. Sari Kaya Sega Utama, Banjarbaru, South Kalimantan. This study was adopted from Ghoora et al. (2020). The seed was cultivated in Rockwool for 21 days at room temperature (27±1°C). Before the seed was cultivated, it must be soaked in water for 5 hours. At the appearance of the first true leaves microgreens were harvested from a triplicate of trays with sterilized scissors. Microgreens were washed to remove extraneous dirt, washed with deionized water, and fan-dried for 5-10 min. Cleaned microgreens were frozen at -20±1°C before used.

Preparation of the extract

The extraction process was adapted from Sen et al. (2013). The frozen microgreens were air-dried prior to grinding. 250 gr powdered microgreens were extracted with 100% methanol (Sigma-Aldrich) using maceration methods. The extracts were stored at 4±1 °C.

DPPH radical scavenging activity assay

The DPPH radical scavenging activity assay using spectrophotometry (Hitachi, U2900) was adopted from *Senevirathne et al. (2019).* 50 µl of samples with various concentrations (1.00; 1.33; 1.66; 1.99 and 2.33 mg/mL), 1.0 ml of DPPH 0.4 mM, and 3.950 ml of ethanol were homogenized using the vortex for 30 minutes. The control consisted of 1.0 ml of DPPH (Sigma-Aldrich) and 4.0 ml of ethanol (Sigma-Aldrich). Vitamin E and Vitamin C (Sigma-Aldrich) are used as a comparison. The absorbance of samples was measured at 517 nm, and 50% inhibitory concentration (IC_{50}) was calculated.

Results and Discussion

Antioxidants are compounds that act to neutralize free radicals and prevent the damage of the normal cell. The performance of antioxidant activity is determined based on its ability to free radicals scavenging. Commonly, 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical that is used (Albaar,2015). Table 1 showed the measuring antioxidant activity using the DPPH free radicals scavenging method. The results showed that the absorption formed a calibration curve with a concentration range of 1.00; 1.33; 1.66; 1.99, and 2.33 mg/mL at 517 nm.

Table 1. Antioxidant activity of *C. alata* microgreen

Conc.(mg/ml)	Abs.			% Free radical scavenging (%)			Average
	1	2	3	1	2	3	
1.00	0.633	0.637	0.634	32.08	31.65	31.97	31.90
1.33	0.545	0.541	0.544	41.52	41.95	41.63	41.70
1.66	0.482	0.491	0.486	48.28	47.32	47.85	47.82
1.99	0.422	0.430	0.428	54.72	53.86	54.08	54.22
2.33	0.361	0.366	0.365	61.27	60.73	60.84	60.94

The results showed a linear relationship within the concentration and % free radical scavenging (%), described in the form of a linear regression equation y = 21.24x + 12.01 with R^2 0.991 (Figure 1). IC_{50} on microgreen *C. alata* was obtained by transforming the absorbance data (y) into %-free radical scavenging (%). The IC_{50} value of *C. alata* Microgreen was $1.789x10^3$ µg/mL.

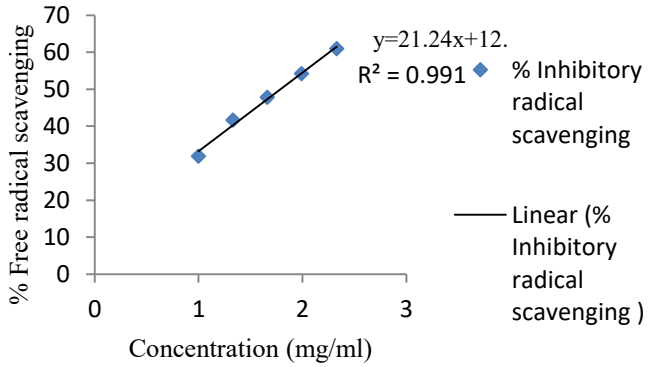

Figure 1. Correlation of concentration and % inhibitory radical scavenging

This result is higher than the study by Senevirathne et al. (2019), like finger millet and green peas, contain DPPH antioxidant activity IC_{50} 4339 ± 86, and 1830 ± 109 µg/mL, respectively. The IC_{50} value is the parameter of antioxidant activity. The higher antioxidant activity, the lower IC_{50} (Rivero-Cruz et al., 2020). According to Qusti et al. (2010), the category of antioxidants is classified very strong (IC_{50}<0.01 mg/mL), strong (0.01 mg/ml< IC_{50}<1 mg/mL), moderate (1 mg/ml< IC_{50}<7 mg/mL), and weak (IC_{50}>7 mg/mL). Meanwhile, according to Molyneux (2004), the antioxidants in a compound are weak because of IC_{50} values 200-1000 µg/mL. However, these compounds are considered a source of antioxidants. Based on the IC_{50} value, *C. alata* microgreen (figure 2) in this study had a potential source of natural antioxidant agents and was classified as a weak antioxidant.

Figure 2. *Cassia alata (C. alata)* microgreens at 21 days

Cassia alata contains strong antioxidants (Fatmawati et al., 2020). Based on the study of Chatterjee et al. (2013), the antioxidant activity in *C. alata* leaf extract was stronger 54±2.20 g/mL (equivalent to $54x10^6$ µg/mL) than a synthetic antioxidant compound BHT 72±1.18 g/mL. Commonly, *C. alata* leaf is extracted for medicine. There has been no further research utilization of *C. alata* to microgreens. In addition to this study, antioxidant activity in *C. alata* microgreen extracts was stronger than in *C. alata* leaf extracts.

Conclusion

This paper utilized *C. alata* to microgreen and determined the potential of *C. alata* microgreen as the source of natural antioxidant agents. Based on some studies, *C. alata* high in antioxidant and antioxidant potential. *C. alata* microgreen had the IC_{50} $1.789x10^3$ µg/mL. *C. alata* microgreen had potential as a source of natural antioxidant agents and was classified as a weak antioxidant. Thus, it would be recommended to complement mature leafy by microgreen to derive maximum antioxidant activity.

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