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Research Article

ANTIOXIDANT ACTIVITY FROM TEN SPECIES OF MYRTACEAE

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ABSTRACT

Objective: Antioxidants are compounds that can inhibit free radical reactivity. They become very interesting to be observed because they can prevent some diseases such as goat arthritis, cancer, cardiovascular disease, Alzheimer's disease, and macular degeneration. Since Indonesia is rich for its biodiversity, there are a lot of plants that have potential to be developed as new alternative antioxidants. The aim of this research was to evaluate antioxidant activity from 10 species of Myrtaceae (*Syzygium cumini, Syzygium samarangense, Syzygium aqueum, Syzygium aromaticum, Syzygium polyanthum, Syzygium jambos, Syzygium malaccense, Psidium guajava, Eucalyptus deglupta,* and *Melaleuca leucadendra*).

Methods: Continuous extraction with Soxhlet apparatus was selected as extraction method. Three solvents (n-hexane, ethyl acetate, and methanol) with different polarity were used in this process. 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was used to evaluate antioxidant activity with ascorbic acid as a standard drug.

Results: Based on the experiments, methanol extracts showed higher activity than other extracts with their inhibitory concentration 50% (IC_{50}) was below than 25 µg/ml. The lowest IC_{50} was exhibited by methanol extract of *S. jambos*, which was 7.8 µg/ml.

Conclusion: It can be concluded that *S. jambos* is potential to be developed as a new alternative antioxidant.

Keywords: Antioxidants, *Eucalyptus*, *Syzygium*, *Melaleuca*, *Psidium*, 2,2-diphenyl-1-picrylhydrazyl.

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INTRODUCTION

Antioxidants are substances that may protect cells from the damage caused by unstable molecules (free radicals) [1]. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of cancer, cardiovascular diseases, cataracts, immune system decline, and brain dysfunction [2]. In general, free radicals have been implicated in the pathogenesis of at least 50 diseases [3]. Several studies have shown that plant-derived antioxidant nutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects [4-6].

Eucalyptus species from Myrtaceae family has been widely explored on their various pharmacological activities such as analgesic, antifungal, anti-inflammatory, antibacterial, antidiabetic, antioxidative, antiviral, antitumor, antihistaminic, anticancer, cytochrome p450 inhibitor, and hepatoprotective properties [7].

The previous studies on fruits from plants of Myrtaceae family showed that phenolic compounds, such as ellagic acid, rutin, and quercetin, contributed to antioxidant activity. They could be found in *Eugenia brasiliensis*, *Melaleuca cauliflora*, *Melaleuca vexator*, *Syzygium curranii*, *Syzygium cumini*, *Syzygium malaccense*, *Syzygium samarangense Syzygium jambos,* and *Psidium guajava* [8,9].

Previous studies confirmed that seeds of *Syzygium aromaticum* have a number of polyphenols including ellagic acid (9.08 mg/kg), ellagic acid derivative (3.46 mg/kg), gallic acid (28.55 mg/kg), and quercetin glucoside (3.53 mg/kg) [10]. Eugenol of *S. aromaticum* has suppressive effects on arthritic rats [11].

Part of *Syzygium aqueum* (fruit) has been confirmed to have 4.1 mg/100 g of ascorbic acid content, 35.0 mg/100 g of total phenol content, and 31.0 mg/100 g of ascorbic acid equivalent antioxidant activity, meaning have mild antioxidant activity [12].

Until now, the information regarding antioxidant activity from the leaves of Myrtaceae was still limited. The aim of this study was to observe antioxidant activity from the leaves of *Eucalyptus deglupta*, *S. mallaccenses*, *S. samarangense*, *P. guajava*, *S. cumini*, *S. aquaeum*, *S. aromaticum*, *Melaleuca leucadendra,* and *S. polyanthum*.

METHODS

Plants material

The leaves of *S. jambos*, *E. deglupta*, *S. mallaccenses*, *S. samarangense*, and *S. cumini* were collected from Indonesian Institute of Sciences, Center for Plant Conservation - Bogor Botanical Gardens (Bogor, West Java, Indonesia) and the leaves of *P. guajava*, *S. aqueum*, *S. aromaticum*, *M. leucadendra,* and *S. polyanthum* were collected from various places in Bandung, West Java, Indonesia.

Extraction

The dried plant material was powdered using a grinder. It was weighed (300 g) and extracted with various solvents, which had gradual polarity such as hexane, ethyl acetate, and methanol using Soxhlet apparatus. The solvents were evaporated under reduced pressure using a rotary evaporator, and a greenish-black colored sticky residue would be obtained.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the plant's extracts and preparation of DPPH solution were adopted from Blois [13] by making some modifications. The diluted working solutions of the test extracts and DPPH were prepared in methanol. Each extract (various concentrations) was mixed

with DPPH solution at a concentration rate of 50 µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at a wavelength 515-517 nm using spectrophotometer ultraviolet-visible. Methanol p.a. (1.5 ml) and DPPH solution (50 µg/ml, 1.5 ml) (1:1) were used as a blank. Based on the reduction of DPPH absorbance, the antioxidant activity of each extract was determined by calculating percentage of radical scavenging activity, as follow:

$$\text{Radical scavenging activity}(\%) = \frac{\text{Ao-As}}{\text{Ao}} \times 100$$

Where Ao is absorbance of DPPH in methanol and As is absorbance of DPPH in methanol plus sample solution (1:1). Inhibitory concentration 50% (IC_{50}) was obtained by plotting the correlation of extract concentration and radical scavenging activity (%). Ascorbic acid was used as the reference. Analysis was performed in triplicate for each extract and standard.

RESULTS

The DPPH radical scavenging activity is one of the most widely-used methods to screen the antioxidant activity on plant extracts. Table 1 shows the antioxidant activity of hexane, ethyl acetate, and methanolic extracts of 10 Myrtaceae plant leaves.

DISCUSSION

Methanol extracts produced moderate to high DPPH scavenging activity with values of IC_{50} were 7.90-26.03 µg/ml. The highest DPPH scavenging activity was observed in *S. jambos* (7.90). In ethyl acetate extracts, the highest scavenging activity was observed in *S. jambos* (11.52). While in hexane extract, the highest scavenging was observed in *S. cumini* (12.58).

Table 1: Results of antioxidant activity of plant extracts

Plants (Leaves)	Solvents	IC_{50} (µg/ml)
E. deglupta	Methanol	9.71±0.09
	Ethyl acetate	36.13±1.34
	Hexane	21356.24±10778.96
M. leucadendra	Methanol	22.46±0.69
	Ethyl acetate	69.04±1.64
	Hexane	289.53±3.70
P. guajava	Methanol	16.59±0.51
	Ethyl acetate	53.54±2.23
	Hexane	651.40±20.31
S. aqueum	Methanol	20.24±0.65
	Ethyl acetate	65.12±0.60
	Hexane	515.48±16.70
S. aromaticum	Methanol	21.51±0.42
	Ethyl acetate	22.27±0.69
	Hexane	13.70±0.05
S. cumini	Methanol	16.91±0.37
	Ethyl acetate	48.06±4.89
S. jambos	Hexane	12.58±22.99
	Methanol	7.90±0.21
	Ethyl acetate	11.52±0.47
S. malaccences	Hexane	514.79±7.66
	Methanol	10.77±0.17
	Ethyl acetate	31.12±0.81
S. polyanthum	Hexane	3121.37±227.73
	Methanol	26.03±0.28
	Ethyl acetate	73.15±2.23
S. samarangense	Hexane	96.42±1.98
	Methanol	13.85±0.42
Ascorbic acid	Ethyl acetate	100.47±1.34
	Hexane	693.30±21.06
	Methanol	3.94±0.09

IC_{50}: Inhibitory concentration 50%, *E. deglupta: Eucalyptus deglupta, M. leucadendra: Melaleuca leucadendra, P. guajava: Psidium guajava, S. aqueum: Syzygium aqueum, S. aromaticum: Syzygium aromaticum, S. cumini: Syzygium cumini, S. jambos: Syzygium jambos, S. malaccenses: Syzygium malaccense, S. polyanthum: Syzygium polyanthum, S. samarangense: Syzygium samarangense*

The result showed that methanol extracts have higher antioxidants, compared to other solvents in DPPH assay. The antioxidant activity of observed methanol extract in *S. jambos* and *E. deglupta* was nearly the same, compared to ascorbic acid (3.94 µg/mL). The antioxidant activity with an IC_{50} value of <10 µg/ml could be considered as a good antioxidant and an IC_{50} value of 10-50 µg/ml could be considered as a powerful antioxidant [14].

A previous study by Reynertson *et al*. [8] exposed that methanol-formic acid (9:1) fruit extract of *S. cumini*, *S. jambos*, *S. malaccense,* and *S. samarangense* has IC_{50} values of 389, 92, 269, and 78 µg/ml, respectively. Despite the fact that there are slight differences in the solvent and extraction method, it can be seen that leaves have higher antioxidant potential, compared to fruits.

The result on this study showed that extract of *S. aqueum* has IC_{50} values of 20.24 µg/ml for leaves and from a previous study has IC_{50} values of 12.00 µg/ml for fruits [12], confirming that fruits have higher antioxidant potential, compared to leaves.

A previous study by El-Ahmad *et al*., confirmed that guava leaf oil reduced DPPH radicals with an IC_{50} value of 3.59 µg/ml [15]. Supporting the result from that previous study, this study confirmed that leaves of *P. guajava* have potential antioxidant activity. In line with the previous studies [16-20], this study also indicated that leaves of *M. leucadendra* and *S. polyanthum* have mild potential antioxidant activity.

The essential oil of *S. aromaticum* (buds) was significantly found in antioxidants, such as superoxide dismutase, glutathione reductase, and glutathione S-transferase [21]. This confirmed that *S. aromaticum* has an antioxidant activity, both in leaves and buds.

However, the chemical constituents that were responsible for this activity would require further investigation, since the observed antioxidant activity might be due to the presence of several constituents.

CONCLUSIONS

It can be concluded that methanol extracts have better antioxidant activities, compared to other solvents. The IC_{50} values of methanol extracts were varied between 7.90 and 26.03 µg/ml, while IC_{50} values of ascorbic acid were 3.94 µg/ml. The methanol extract of *S. jambos* showed the highest antioxidant activity among all extracts with an IC_{50} value of 7.90 µg/ml.

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