RJC

RASĀYAN *J. Chem.*
Vol. 12 | No. 1 |146 - 151| January - March | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

BIOLOGICAL ACTIVITY OF METHANOL EXTRACT OF *Elaeocarpus mastersii* KING: ANTIOXIDANT, ANTIBACTERIAL, AND α-GLUCOSIDASE INHIBITOR

Tia Okselni, Adlis Santoni, Abdi Dharma and Mai Efdi*
[1]Department of Chemistry, Andalas University, Padang 25163, West Sumatra, Indonesia
*E-mail: maiefdi@sci.unand.ac.id

ABSTRACT

Plants are one of the natural resources that have great biological activity and have long been used in traditional medicine. In this research, methanol extracts of root, stem bark, and leaf of *Elaeocarpus mastersii* King were tested to determine phenolic and flavonoid content and evaluate their biological activities as the antioxidant, antibacterial, and antidiabetic using the method of DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, disc diffusion against *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus aureus* (*S. aureus*), *Salmonella typhosa* (*S. typhosa*), and *Escheria coli* (*E. coli*), and inhibition of α-glucosidase enzyme, respectively. All parts showed high phenolic content and antioxidant activity wherein the leaf extract was found to be the highest value (340.36 ± 2.09 mg GAE/g DW and IC_{50} 1.86 ± 0.00 µg/mL) followed by the stem bark (331.53 ± 6.96 mg GAE/g DW and IC_{50} 2.43 ± 0.01 µg/mL) and the root (216.27 ± 3.19 mg GAE/g DW and IC_{50} 3.37 ± 0.20 µg/mL), respectively. In contrast, their flavonoid values were very low. The stem bark exhibited the highest antibacterial activity against all of the tested bacteria. The activity of α-glucosidase inhibitor revealed that the stem bark had the highest activity among all parts with the IC_{50} of 14.56 ± 1.20 µg/mL. This study demonstrates that *Elaeocarpus mastersii* King has a great biological activity as the antioxidant, antibacterial, and α-glucosidase inhibitor.

Keywords: antioxidant, antibacterial, *Elaeocarpus mastersii* King, α-glucosidase inhibitory activity

INTRODUCTION

Elaeocarpaceae is a vast plant family consisting of 12 genera which their distribution areas are tropics and subtropics. The largest genus of *Elaeocarpaceae* family is *Elaeocarpus* comprising 350 species and one of the largest distribution areas is Indonesia[1]. Plants of the *Elaeocarpus* genus have a great impact to treat various diseases such as epilepsy, hypertension, asthma, and arthritis[2]. Several studies showed the great biological activity of *Elaeocarpus* plants from crude extracts and pure compounds as the antioxidant[3-6], antimicrobial[7-9], antidiabetic[10], antiinflammatory[2,11], cytotoxic[4,5,12,13], medicine of bronchial asthma[14], antiarthritic[15], antidepressant[16], and antiparkinsonian[17].

Elaeocarpus mastersii King belongs to the *Elaeocarpus* genus used as the natural medicine of diabetic and hypertensive disease. As long as our literature study, *E. mastersii* King was only reported by Ito et al.[18] and studied its cytotoxic activity. As the part of our ongoing program for studies about *E. mastersii* King plant[19], we evaluated the biological activity as the antioxidant, antibacterial, and α-glucosidase inhibitory activity from the root, stem bark, and leaf of *E. mastersii* King.

EXPERIMENTAL

Collection and Extraction of Plant

Plant materials (root, stem bark, and leaf) of *E. mastersii* King were obtained from the Riau Province, Sumatra-Indonesia. The plant materials were ground and macerated by methanol solvent. The solvent was evaporated by rotary evaporator yielding crude of methanol extracts.

http://dx.doi.org/10.31788/RJC.2019.1215019

Determination of Total Phenolic Content (TPC)
Modified Folin-Ciocalteu method[19] was used to determine the phenolic content of the extracts. The test tube composed of 2.5 ml Folin-Ciocalteu reagent (10%, *V/V*) and 2.0 mL sodium carbonate (2%, *W/V)* was added with 0.5 mL extract of 100 µg/mL in methanol solution. The mixed solution was stirred and incubated for 15 min at 45^0C. The absorbance of the mixture was determined by spectrophotometer (PD-303S Apel, Japan) at 765 nm. The calibration curve (gallic acid, 0-80 µg/mL) was used to obtain the phenolic value and the value was presented as gallic acid equivalent (GAE) of the dry weight of the extract.

Determination of Total Flavonoid Content (TFC)
Flavonoid content of the extract was evaluated by the colorimetric method[19] with slight modification. The extract of 2.0 mL (100 µg/mL, in methanol solution) was added with aluminum chloride [0.1 mL, 10% (*W/V)*] and sodium acetate (0.1 mL, 0.1 mM). The mixture was stirred and allowed to stand for 30 min at room temperature and its absorbance was assessed by spectrophotometer (PD-303S Apel, Japan) at 415 nm. Rutin (0-80 µg/mL) was used to get the calibration curve and the flavonoid value was presented as rutin equivalent (RE) of the dry weight of the extract.

Biological Activity
Antioxidant Activity by DPPH Assay
The DPPH method of Sen et al.[20] was used to evaluate the antioxidant activity of the extract.

Antibacterial Activity by Disc Diffusion Assay
Antibacterial activity was performed by disc diffusion method[21] with slight modification. *S. epidermidis* and *S. aureus* of gram-positive bacteria and *S. typhosa* and *E. coli* of gram-negative bacteria were used to determine the antibacterial activity of *E. mastersii* King extract. A Petri dish containing nutrient agar (NA) medium was added 100 µL of bacterial suspension. A cotton swab was used to inoculate the tested bacteria on the surface of an agar plate. Sterilized blank paper disc of 6 mm diameter was filled with 10 µL extract solution (10 mg/mL in DMSO) and placed on the top of the agar plate surface. The plate was incubated for 24 h at room temperature. The clear inhibition zone (including the diameter of the disc) was applied to determine the antibacterial activity. Gentamicin (10 µg/disc) and DMSO solution were used as the positive and negative control, respectively.

α- Glucosidase Inhibitory Activity
Antidiabetic activity was investigated by the α-glucosidase inhibitory method described by Sancheti *et al*[22]

Statistical Investigation
The analysis was conducted in triplication and performed as average ± standard deviation (SD). Statistical data were obtained by ANOVA and Duncan's multiple range test (SAS of 9.4 version) and *p*-values < 0.05 were considered as the significant difference.

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Content
Total phenolic and flavonoid value were determined by the curve of gallic acid ($y = 0.0083x - 0.0015$, R^2 = 0,9999) and rutin ($y = 0.0203 + 0.0345$, $R^2 = 0.9976$), respectively. All of the extracts exhibited the high phenolic and the low flavonoid value (Table-1).
Phenolic and flavonoid contents were measured by the colorimetric method based on the formation of complex compound as the reaction result of reagent and analyzed content which the blue complex is the result of reaction between Folin-Ciocalteu and phenolic compound[23] and the yellow complex shows the reaction result from the aluminum (III) ion with the carbonyl and hydroxyl groups of flavonoid[24].
This study showed that parts of the *E. mastersii* King plant had different phenolic value wherein the leaf extract was found to be the highest value (340.36 ± 2.09 mg GAE/g DW) followed by the stem bark and

root extract (331.53 ± 6.96 and 216.27 ± 3.19 mg GAE/g DW, respectively). The same pattern was also performed by flavonoid value wherein the highest flavonoid value was found in the leaf (21.59 ± 1.03 mg RE/g DW) followed by the stem bark extract (5.83 ± 0.75 mg RE/g DW), whereas the flavonoid value in the root was too small to be detected. These results indicated that the flavonoid was not the main phenolic compound of *E. mastersii* King.

Table-1: Phenolic and Flavonoid content of methanol extract from all parts of *E. mastersii* King

Part	TPC (mg GAE/g DW) ± SD	TFC (mg RE/g DW) ± SD
Root	216.27 ± 3.19 [c]	ND
Stem bark	331.53 ± 6.96 [b]	5.83 ± 0.75 [b]
Leaf	340.36 ± 2.09 [a]	21.59 ± 1.03 [a]

ND: not detected. The values were expressed as average ± SD (n=3). The different letters indicate the significant difference between the values ($P < 0.05$)

The Previous study reported the phenolic content from the fruit extract of *Elaeocarpus ganitrus* (232.24 mg GAE/g DW)[25]. This study revealed that *E. mastersii* King had more amount of phenolic content than the other species. Parts of plants have the different amount of phenolic due to the phenolic physiological functions associated with plant protection mechanisms against abiotic and biotic incursion[26,27].

Biological Activity

Antioxidant Activity

All parts of *E. mastersii* King revealed the strong antioxidant activity using DPPH method (Table-2).

Table-2: Antioxidant activity of methanol extract from all parts of *E. mastersii* King

Part	IC_{50} (µg/mL) ± SD
Root	3.37 ± 0.02 [c]
Stem bark	2.43 ± 0.01 [b]
Leaf	1.86 ± 0.00 [a]
Gallic acid	0.48 ± 0.00

The values were expressed as average ± SD (n=3). The different letters indicate the significant difference between the values ($P < 0.05$)

The DPPH method is based on the colorimetric principle in which the stabilization of free radical of 2,2-diphenyl-1-picrylhydrazyl (purple color) use an electron donor of the phenolic compound to form the 2,2-diphenyl-1-picrylhydrazine (yellow color)[28]. All parts of *E. mastersii* King showed the strong antioxidant activity with the similar regulation of phenolic value wherein the highest activity was obtained in the leaf (IC_{50} 1.86 ± 0.00 µg/mL) followed by the stem bark and root extracts (IC_{50} 2.43 ± 0.01 and 3.37 ± 0.20 µg/mL, respectively). It indicated that the phenolic compound had a significant effect on the antioxidant activity of *E. mastersii* King. The antioxidant activity of all parts of *E. mastersii* King was much better than a few species of *Elaeocarpus* genus, such as *Elaeocarpus floribundus* leaf and stem bark of methanol fractions (IC_{50}, 58.23 ± 0.04 and 7.36 ± 0.01 µg/mL, respectively)[4] and *Elaeocarpus sylvestris* leaf of methanol extract (IC_{50}, 11.3 ± 1.4 µg/mL)[6].

Antibacterial Activity

The antibacterial activities from all parts of methanol extract of *E. mastersii* King against human pathogenic bacteria are shown in Table-3.

Disc diffusion method had been used to investigate the antibacterial activity of all parts of the *E. mastersii* King methanolic extract against human pathogenic bacteria, such as *S. epidermidis* and *S. aureus* of gram-positive bacteria and *S. thyposa* and *E. coli* of gram-negative bacteria. The antibacterial activities were determined by the diameter of inhibition zones as the result of interaction between a disc containing an

antibacterial agent and the bacteria on the surface of an agar plate. The results showed that the extract of *E.mastersii* King contributed the inhibition zone diameters ranging from 6.90 to 8.90 mm for gram-positive bacteria and from 6.93 to 10.37 mm for gram-negative bacteria. These results exhibited that the extract of this plant was more sensitive to gram-negative bacteria. This result was supported by the previous study on the other species of *Elaeocarpus* genus which leaf extract of *Elaeocarpus ganitrus* also performed a good activity against gram-negative bacteria (eg. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*)[9]. Gram-negative bacteria consist of a thinner layer of peptidoglycan than gram-positive bacteria. Peptidoglycan has an important role in the survival of bacteria in the hypotonic environment. This layer is the attack center of antibacterial agents that can cause cell wall damage resulting in cell death[29]. Thereby, the cell wall of gram-negative bacteria can be damaged easily.

Table-3: Antibacterial activity of methanol extract from all parts of *E. mastersii* King

Part	Zone of Inhibition (mm)			
	Gram-positive		Gram-negative	
	S. epidermidis	*S. aureus*	*S. thyposa*	*E. coli*
Root*	7.00 ± 0.26^{b}	6.90 ± 0.10^{b}	7.27 ± 0.21^{b}	8.30 ± 0.30^{b}
Stem bark*	8.60 ± 0.62^{a}	8.90 ± 0.50^{a}	9.10 ± 0.46^{a}	10.37 ± 0.51^{a}
Leaf*	7.63 ± 0.47^{b}	7.40 ± 0.53^{b}	6.93 ± 0.12^{b}	7.13 ± 0.40^{c}
Gentamicin**	17.83 ± 0.35	16.50 ± 0.17	16.90 ± 0.10	12.47 ± 0.55

*Concentration of the extract (100 µg/disc), **concentration of gentamicin (10 µg/disc)
The values were expressed as average ± SD (n=3). The different letters indicate the significant difference between the values ($P < 0.05$)

Antibacterial activities of parts of *E. mastersii* King were in the following order stem bark > leaf > root for gram-positive bacteria, whereas the gram-negative bacteria were stem bark > root > leaf. The stem bark had the highest antibacterial activity against all bacteria. It could be due to the presence of ellagic acid, steroid, alkaloid and phenolic compounds which were found in the stem bark of *E. mastersii* King[18,19] and were known to provide the antibacterial activity[30-32].

α-Glucosidase Inhibitory Activity

The antidiabetic activity of all parts of *E. mastersii* King (Table-4) had been determined by the inhibition of the α-glucosidase enzyme.

Table-4: The α-glucosidase inhibitory activity of methanol extract from all parts of *E. mastersii* King

Part	IC_{50} (µg/mL) ± SD
Root	60.57 ± 2.84 ^{b}
Stem bark	14.56 ± 1.20 ^{a}
Leaf	96.36 ± 4.67 ^{c}
Acarbose	0.13 ± 0.00

The values were expressed as average ± SD (n=3). The different letters indicate the significant difference between the values ($P < 0.05$)

Antidiabetic properties of parts of *E. mastersii* King were obtained from the α-glucosidase inhibitory activity. Inhibition of α-glucosidase enzyme can prevent the carbohydrate digestion and delay the glucose absorption resulting in decreasing of blood glucose levels[33]. This study was investigated by the amount of p-nitrophenol released in the reaction results between 4-nitrophenyl α-D-glucopyranoside and α-glucosidase enzyme[22].

The results showed the good α-glucosidase inhibitory activity of *E. mastersii* King and as shown in Table 4, the highest IC_{50} value was found in the stem bark (IC_{50} 14.56 ± 1.20 µg/mL). This ability could be due to the presence of ellagic acid, steroid, alkaloid, and phenolic compounds in the stem bark[18,19] which were known to provide a significant antidiabetic activity[34,35]. The α-glucosidase inhibitory activity of *E.*

mastersii King was known to be better than the other species, as *Elaeocarpus sylvestris* with the IC_{50} value of 74.4 ± 0.9 µg/mL[10].

CONCLUSION

This study exhibited that all parts of *E. mastersii* King have high phenolic content and antioxidant activity. The stem bark had the highest antibacterial and α-glucosidase inhibitory activity among all parts. Therefore, *E. mastersii* King has a great biological activity as the antioxidant, antibacterial, and α-glucosidase inhibitor. This ability could be due to the presence of secondary metabolites such as phenolic, alkaloid, steroid, and ellagic acid compounds.

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Research and Technology of Indonesia for financial support (Grant number: 050/SP2H/LT/DRPM/2018).

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[RJC-5019/2018]