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DETERMINATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC CONTENT, AND TOTAL FLAVONOID CONTENT OF ROOTS, STEM BARK, AND LEAVES OF *Elaeocarpus mastersii* KING

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ABSTRACT

In this research, antioxidant activity, total phenolic, and flavonoid content of n-hexane, ethyl acetate, and methanol fractions of roots, stem bark, and leaves of *Elaeocarpus mastersii* King were determined by the colorimetric method using DPPH assay (1,1-diphenyl-2-picrylhydrazyl), Folin-Ciocalteu reagent, and aluminum chloride reagent, respectively. Phytochemical screening was evaluated by specific reaction of constituents to the specific reagent. Ethyl acetate fraction had the best value of phenolic content and antioxidant activity among all fractions which the best value was obtained from the leaves (380.99 ± 2.14 mg GAE/g DW and IC_{50} 1.95 ± 0.01 µg/mL) followed by the roots (362.88 ± 1.89 mg GAE/g DW and IC_{50} 2.05 ± 0.01 µg/mL) and the stem bark (341.89 ± 3.97 mg GAE/g DW and IC_{50} 2.36 ± 0.02 µg/mL). All fractions of this plant showed the low value of flavonoid content. The phytochemical screening exhibited that this plant was dominated by phenolic and alkaloid compounds. These results demonstrate the great potential of *Elaeocarpus mastersii* King as a natural antioxidant and active compounds.

Keywords: antioxidant activity, *Elaeocarpus mastersii* King, total phenolic content, total flavonoid content

INTRODUCTION

Free radicals promote oxidative damage on cells, tissues, lipids, proteins, and DNA[1,2]. Oxidative damage is related to the human disease such as cancer, cardiovascular, rheumatoid arthritis, ischemia/reperfusion, atherosclerosis, diabetes mellitus, and neurodegenerative (Alzheimer's disease and Parkinson's disease). Free radicals can be prevented by the presence of antioxidant agents obtained from synthetic compounds (e.g. butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, octyl gallate, dodecyl gallate) and natural product[2-4]. Synthetic antioxidants have been suspected as an agent of carcinogenesis, liver damage, and DNA damage when used at high levels[5-7]. Therefore, a natural antioxidant is more desirable to replace the synthetic antioxidant. The natural antioxidant is found in the plant which has secondary metabolites, such as flavonoids, stilbenes, terpenoids, and phenolic compounds[8-10]. Phenolic compounds are categorized as the primary antioxidant agent because the phenolic compounds can donate a hydrogen atom to the radical compounds and produce the stable radical[4].

Elaeocarpaceae family have a large number of phenolic compounds, especially the genus of *Elaeocarpus*[11-13]. Previous studies showed that *Elaeocarpus* genus plants have a great biological activity as an antioxidant[12-17]. *Elaeocarpus mastersii* King is classified into *Elaeocarpus* genus that has been used as folk medicine for treatment of diabetic and hypertensive disease. Previous research reported that the *E. mastersii* has phenolic compounds (a derivate of ellagic acid) with their pharmacological properties[18].

The aim of this research is to determine total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of n-hexane, ethyl acetate, and methanol fractions from the roots, stem bark, and leaves of *E. mastersii*. In addition, it is the first report to determine TPC, TFC, and antioxidant activity from the various fractions and parts of *E. mastersii*.

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EXPERIMENTAL

Plant Material

Roots, stem bark, and leaves of *E. mastersii* were collected in Riau, Indonesia. The plant was identified at Herbarium of Andalas University with the collection number of ANDA: 040.

Extraction

The plant materials were extracted by maceration method at room temperature using methanol solvent. The crude extracts of each part were concentrated by a rotary evaporator and partitioned using n-hexane and ethyl acetate solvent.

Determination of Total Phenolic Content

TPC of the *E. mastersii* extracts was measured by Folin-Ciocalteu method[19] with slight modifications. The extract of 100 µg/mL in ethanol solution was added into a test tube containing Folin-Ciocalteu reagent (10%, V/V) and sodium carbonate (2%, W/V) with the volume ratio of 0.5:2.5:2. The mixture was shaken and incubated at 45^0C for 15 min. Absorbance was measured at 765 nm using spectrophotometer (Apel PD-303S, Japan). Phenolic contents were obtained from the calibration curve of gallic acid (0-80 µg/mL) and expressed as gallic acid equivalent (mg GAE/g) of dry weight.

Determination of Total Flavonoid Content

TFC was measured by a colorimetric method using aluminum chloride reagent[20] with slight modifications. The extract was dissolved by ethanol solution (100 µg/mL). The extract solution (2.0 mL) was mixed with 0.1 mL of aluminum chloride (10%, W/V) and 0.1 mL of sodium acetate (0.1 $mmol.L^{-1}$). The mixture was shaken and incubated at room temperature for 30 min. Absorbance was measured at 415 nm using spectrophotometer (Apel PD-303S, Japan). Flavonoid contents were obtained from the calibration curve of rutin (0-80 µg/mL) and expressed as rutin equivalent (mg RE/g) of dry weight.

Antioxidant Activity by DPPH Assay

The antioxidant activity of the extract was investigated by DPPH assay[19] with slight modifications. The solution of DPPH in ethanol (1.0 mL, 0.1 $mmol.L^{-1}$) was added into the tube containing 3.0 mL of extract in various concentrations. The mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using spectrophotometer (Apel PD-303S, Japan). The antioxidant activity was estimated based on the inhibition percentage of radical using the equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) \times 100]/A_{control}$$

Where A control is the absorbance of control containing all of the reagents except the sample, while A sample is the absorbance of the sample.

Statistical Analysis

All analyses were done in triplicate and the values were presented as average with their standard deviations (SD). The correlation coefficients (R) between TPC, TFC, and antioxidant activity of the *E. mastersii* extracts were calculated to determine their relationship. Statistical analysis was investigated by One-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SAS (version 9.4) software. *P*-value < 0.05 was recognized as a significant difference.

Phytochemical Screening

The secondary metabolites, as phenols, terpenoid, steroid, and alkaloid compounds of the *E. mastersii* extracts were investigated based on the standard method.[21]

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Content

The phenolic values were obtained from the calibration curve of gallic acid, $y = 0.0081x + 0.0024$ with $R^2 = 0.9997$. The flavonoid values were obtained from the calibration curve of rutin, $y = 0.0196x + 0.0377$ with $R^2 = 0.9963$. The results are shown in Table-1.

Table-1: Total Phenolic Content and Total Flavonoid Content of the *E. mastersii* extract

Fraction	Part	TPC (mg GAE/g DW) ± SD	TFC (mg RE/g DW) ± SD
n-hexane	Roots	21.77 ± 0.26 [h]	5.77 ± 0.51 [f]
	Stem bark	15.47 ± 0.38 [i]	2.53 ± 0.29 [g]
	Leaves	79.26 ± 0.33 [g]	6.62 ± 2.06 [f]
Ethyl acetate	Roots	362.88 ± 1.89 [b]	30.09 ± 1.28 [b]
	Stem bark	341.89 ± 3.97 [c]	18.35 ± 0.78 [d]
	Leaves	380.99 ± 2.14 [a]	26.34 ± 0.29 [c]
Methanol	Roots	152.18 ± 3.56 [f]	16.14 ± 1.06 [e]
	Stem bark	287.16 ± 2.14 [d]	30.94 ± 0.78 [b]
	Leaves	273.58 ± 2.14 [e]	44.03 ± 0.51 [a]

The value was presented as mean ± SD (n=3) and values with different superscript letters imply the significant differences ($P < 0.05$)

TPC was measured by Folin-Ciocalteu reagent based on the principle of formation of the blue phosphotungstic-phosphomolybdic complex as the result of a reaction between Folin-Ciocalteu and phenolic compounds[22]. The results showed that *E. mastersii* extracts had the high amount of phenolic content. The highest phenolic content was obtained from the ethyl acetate fraction for all parts of this plant. In the ethyl acetate and n-hexane fraction, the highest number of phenolic contents was found in the leaves with the value of 380.99 ± 2.14 and 79.26 ± 0.33 mg GAE/g DW, whereas the methanol fraction was found in the stem bark (287.16 ± 2.14 mg GAE/g DW). In general, n-hexane fraction of all parts had the low value of phenolic content. It is related to the polarity properties of phenolic compounds which are more dissolved in the polar solvents. Several previous studies showed that the solvent has a significant effect on the amount of phenolic contents[20,23-25]. Each part of the plant has a variation of phenolic content. It is due to the physiological function of phenolic compounds related to the defense mechanism of plants against biotic and abiotic attack[26,27].

TFC was investigated by colorimetric method using aluminum chloride reagent based on the formation of a yellow complex between the ion of aluminum (Al^{3+}) and the carbonyl and hydroxyl groups of flavonoid[28]. TFC test results showed the low flavonoid value of all parts of *E. mastersii* which the highest value (44.03 ± 0.51 mg RE/g DW) was found in leaves of the methanol fraction. It indicated that the flavonoid was the minor compounds of the *E. mastersii* plant.

Antioxidant Activity by DPPH Assay

Antioxidant activity of the *E. mastersii* extract had been examined by DPPH assay. The results (Table-2) showed that most of the extracts had strong antioxidant activity.

Table-2: Antioxidant activity by DPPH assay of the *E. mastersii* extract

Fraction	Part	IC_{50} (µg/mL) ± SD
n-hexane	Roots	85.70 ± 0.52 [e]
	Stem bark	187.11 ± 1.22 [f]
	Leaves	14.21 ± 0.05 [d]
Ethyl acetate	Roots	2.05 ± 0.01 [a]
	Stem bark	2.36 ± 0.02 [a]
	Leaves	1.95 ± 0.01 [a]
Methanol	Roots	6.38 ± 0.02 [c]
	Stem bark	2.75 ± 0.02 [a b]
	Leaves	3.21 ± 0.00 [b]
Ascorbic acid	-	2.44 ± 0.03

The value was presented as mean ± SD (n=3) and values with different superscript letters imply the significant differences ($P < 0.05$)

The principle of the DPPH method is to stabilize free radical of DPPH (1,1-diphenyl-2-picrylhydrazyl) with the presence of an electron donor from a hydrogen atom of the phenolic compound. An increase in

the number of phenolic compounds leads to an increase in the number of OH group on the aromatic ring, thereby enhancing the ability to inhibit free radicals[29]. The results showed that the ethyl acetate fraction of all parts provided the strongest antioxidant activity among all fractions. In the ethyl acetate and n-hexane fraction, the strongest antioxidant activity was found in the leaves with the IC_{50} value of 1.95 ± 0.01 and 14.21 ± 0.05 µg/mL, whereas the methanol fraction was found in the stem bark (IC_{50} 2.75 ± 0.02 µg/mL). Utami et al.[13] reported that the strongest antioxidant activity of *Elaeocarpus floribundus* was found in the methanol fraction of stem bark (IC_{50} 7.36 ± 0.01 µg/mL) and Prihantini et al.[17] reported the best IC_{50} value of *Elaeocarpus sylvestris* (7.7± 0.8 µg/mL) found in the methanol fraction of leaves. It showed that *E. mastersii* have a stronger antioxidant activity than a few species of *Elaeocarpus*.

Correlation of Phenols, Flavonoid and Antioxidant Activity

The correlation between TPC, TFC, and antioxidant activity of n-hexane, ethyl acetate, and methanol fractions of *E. mastersii* plant had been investigated by the correlation coefficient (*R*) using statistical analysis. The results are shown in Table-3.

Table-3: Correlation between TPC, TFC, and Antioxidant Activity of n-Hexane, Ethyl Acetate, and Methanol Fractions of *E. mastersii* Plant

Fraction	$R_{TPC\text{-}TFC}$	$R_{TPC\text{-}DPPH}$	$R_{TFC\text{-}DPPH}$
n-Hexane	0.63093 (*P* = 0.01429)	0.86096 (*P* = 0.00011)	0.84534 (*P* = 0.00037)
Ethyl acetate	0.69262 (*P* = 0.13012)	0.96238 (*P* = 1.59E-06)	0.84118 (*P* = 9.82E-07)
Methanol	0.83266 (*P* = 0.50240)	0.99890 (*P* = 5.46E-12)	0.82119 (*P* = 0.00011)

$R_{TPC\text{-}TFC}$ is correlation coefficient value between phenolic content and flavonoid content
$R_{TPC\text{-}DPPH}$ is correlation coefficient value between phenolic content and antioxidant activity
$R_{TFC\text{-}DPPH}$ is correlation coefficient value between flavonoid content and antioxidant activity

A correlation analysis between phenolic and flavonoid contents showed that the correlation of n-hexane fraction was found to be 0.63093 ($P < 0.05$), while the ethyl acetate and methanol fractions did not show the correlation ($P > 0.05$). This result indicated that flavonoid was not the main and major of phenolic compounds from *E. mastersii* plant.

As shown in Table 3, the correlations of TPC, TFC to the antioxidant activity of *E. mastersii* plant revealed that TPC had a higher correlation to antioxidant activity than TFC which the highest correlation coefficient (*R)* value was found to be 0.99890 obtained from the methanol fraction. It indicated that phenolic compounds have an important role in the antioxidant activity of *E. mastersii* plant.

Phytochemical Screening

The phytochemical screening had been conducted to determine the secondary metabolite compounds that could be involved in the antioxidant activity of *E. mastersii* (Table-4). The results are shown in Table-4.

Table-4: Phytochemical Screening of the *E. mastersii* Extract

Fraction	Part	Chemical Constituent			
		Phenols	Terpenoid	Steroid	Alkaloid
n-hexane	Roots	−	−	+	+
	Stem bark	−	−	+	+
	Leaves	−	−	+	+
Ethyl acetate	Roots	+	−	−	+
	Stem bark	+	−	−	+
	Leaves	+	−	−	+
Methanol	Roots	+	−	−	−
	Stem bark	+	−	−	−
	Leaves	+	−	−	−

(+) indicates the presence of constituents, (-) indicates the absence of constituents

The phytochemical screening is based on the specific reaction of each compound to the specific reagent[21]. The results showed that *E. mastersii* plant was dominated by phenolic and alkaloid compounds (Table 4). Alkaloids are the most common compounds that had been isolated of *Elaeocarpus* genus such as (±)-elaeocarpine, (±)-isoelaeocarpine, (±)-3-oxoisoelaeocarpine, (±)-elaeocarpine N-oxide, and (-)-isoelaeocarpiline of *Elaeocarpus sphaericus*[30], elaeocarpenine of *Elaeocarpus fuscoides*[31], and habbemines A and B of *Elaeocarpus habbemensis*[32]. Alkaloids are also known to provide a positive correlation in the antioxidant activity.[33,34]

CONCLUSION

This study revealed that the ethyl acetate and methanol fractions of all parts of *E. mastersii* have strong antioxidant activity and a great potential as a source of natural antioxidant. This ability could be due to the presence of phenolic compounds. The ethyl acetate fraction has the best value of phenolic content and antioxidant activity of *E. mastersii*. Therefore, the ethyl acetate fraction may have more active compounds among all fractions.

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REFERENCES

1. A.P. Wickens, *Respiration Physiology,* **128**, 379 (2001), **DOI:** 10.1016/S0034-5687(01)00313-9
2. M. Valko, D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, and J. Telser, *Int. J. Biochem. Cell Biol.*, **39**, 44 (2007), **DOI:** 10.1016/j.biocel.2006.07.001
3. A. Augustyniak, G. Bartosz, A. Čipak, G. Duburs, Ľ. Horáková, W. Łuczaj, M. Majekova, A.D. Odysseos, L. Rackova, E. Skrzydlewska, M. Stefek, M. Štrosova, G. Tirzitis, P.R. Venskutonis, J. Viskupicova, P.S. Vraka, and N. Žarkovic, *Free Radic. Res.,* **44(10)**, 1216 (2010), **DOI:** 10.3109/10715762.2010.508495
4. F. Shahidi and P. Ambigaipalan, *J. Funct. Foods,* **18**, 820 (2015), **DOI:** 10.1016/j.jff.2015.06.018
5. J.E.N. Dolatabadi and S. Kashanian, *Food Res. Int.*, **43**, 1223 (2010), **DOI:** 10.1016/j.foodres.2010.03.026
6. H.C. Grice, *Food Chem. Toxicol.,* **26(8)**, 717 (1988), **DOI:** 10.1016/0278-6915(88)90072-5
7. H.P. Witschi, *Food Chem. Toxicol.,* **24(10)**, 1127 (1986), **DOI**: 10.1016/0278-6915(86)90298-X
8. A. Matkowski, *Biotechnol. Adv.,* **26**, 548 (2008), **DOI:** 10.1016/j.biotechadv.2008.07.001
9. D. Shahwar and M.A. Raza, *Asian Pac. J. Trop. Biomed.,* **2(7)**, 547 (2012), **DOI:** 10.1016/S2221-1691(12)60094-X
10. J.M. Lorenzo and P.E.S. Munekata, *Asian Pac. J. Trop. Biomed.,* **6(8)**, 709 (2016), **DOI:** 10.1016/j.apjtb.2016.06.010
11. S. Hardainiyan, B.C. Nandy, and R. Saxena, *Int. J. Pharm. Pharm. Sci.,* **7(6)**, 415 (2015).
12. S. Saikia, N.K. Mahnot, and C.L. Mahanta, *Food Biosci.,* **13**, 15(2016), **DOI:** 10.1016/j.fbio.2015.11.003
13. R. Utami, N. Khalid, M.A. Sukari, M. Rahmani, A.B. Abdul, and Dachriyanus, *Pak J. Pharm. Sci.*, **26**, 245 (2013).
14. M.N. Parvin, S. Sarwar, S.A. Chowdhury, H.M. Zakaria, and N.H. Huda, *Stamford J. Pharm. Sci.*, **2(2)**, 86 (2009), **DOI:** 10.3329/sjps.v2i2.5829
15. R. Shamna, J.M. Sasikumar, E.S. Adithya, P.H. Christabel, and M.S. Lakshmi, *Food*, **5(1)**, 96 (2011).
16. L. Jayasinghe, N.R. Amarasinghe, B.G.S. Arundathie, G.K. Rupasinghe, N.H.A.N. Jayatilake, and Y. Fujimoto, *Nat. Prod. Res.*, **26**, 717 (2012), **DOI:** 10.1080/14786419.2010.551514
17. A.I. Prihantini, S. Tachibana, and K. Itoh, *Procedia. Environ. Sci.*, **28**, 758 (2015), **DOI:** 10.1016/j.proenv.2015.07.089
18. A. Ito, H. Chai, D. Lee, L.B.S. Kardono, S. Riswan, N.R. Farnsworth, G.A. Cordell, J.M. Pezzuto, and A.D. Kinghorn, *Phytochemistry*, **61**, 171 (2002), **DOI:** 10.1016/S0031-9422(02)00232-7
19. S. Sen, B. De, N. Devanna, and R. Chakraborty, *Chin. J. Nat. Med.*, **11(2)**, 149 (2013), **DOI:**

10.1016/S1875-5364(13)60042-4
20. Q.D. Do, A.E. Angkawijaya, P.L. Tran-Nguyen, L.H. Huynh, F.E. Soetaredjo, S. Ismadji, and Y. Ju, *J. Food Drug Anal.*, **22(3)**, 296(2013), **DOI:** 10.1016/j.jfda.2013.11.001
21. J.B. Harborne, Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, Chapman and Hall, New York (1973), **DOI:** 10.1007/978-94-009-5921-7
22. P. Schofield, D.M. Mbugua, and A.N. Pell, *Animal Feed Science and Technology,* **91**, 21 (2001), **DOI:** 10.1016/S0377-8401(01)00228-0
23. F. Barros, L. Dykes, J.M. Awika, and L.W. Rooney, *J. Cereal. Sci.*, **58(2)**, 305 (2013), **DOI:** 10.1016/j.jcs.2013.05.011
24. S.A.O. Santos, C.S.R. Freire, M.R.M. Domingues, A.J.D. Silvestre, and C.P. Neto, *J. Agric. Food Chem.*, **59**, 9386 (2011), **DOI:** 10.1021/jf201801q
25. A. Fernández-Agulló, E. Pereira, M.S. Freire, P. Valentão, P.B. Andrade, J. González-álvarez, and J.A. Pereira, *Ind. Crops. Prod.*, **42**, 126 (2013), **DOI:** 10.1016/j.indcrop.2012.05.021
26. L.J. Jing, M. Mohamed, A. Rahmat, and M.F.A. Bakar, *J. Med. Plants. Res.*, **4(1)**, 27 (2010), **DOI:** 10.5897/JMPR09.308
27. A. Bhattacharya, P. Sood, and V. Citovsky, *Mol. Plant Pathol.*, **11(5)**, 705 (2010), **DOI:** 10.1111/J.1364-3703.2010.00625.X
28. M. Popova, V. Bankova, D. Butovska, V. Petkov, B.N. Damyanova, A.G. Sabatini, G.L. Marcazzan, and S. Bogdanov, *Phytochemical Analysis,* **15**, 235 (2004), **DOI:** 10.1002.pca.777
29. M.G. Miguel, *Flavour Fragr. J.*, **25**, 291 (2010), **DOI:** 10.1002/ffj.1961
30. C. Zhou, X. Wang, J. Mo, J. Zhang, and L. Gan, *Helv. Chim. Acta.*, **94**, 347 (2011), **DOI:** 10.1002/hlca.201000200
31. P.L. Katavic, D.A. Venables, T. Rali, and A.R. Carroll, *J. Nat. Prod.*, **70**, 872 (2007), **DOI:** 10.1021/np060607e
32. P.L. Katavic, D.A. Venables, T. Rali, and A.R. Carroll, *J. Nat. Prod.*, **70**, 866 (2007), **DOI:** 10.1021/np060577f
33. S. Khamtache-abderrahim, M. Lequart-Pillon, E. Gontier, I. Gaillard, S. Pilard, D. Mathiron, H. Djoudad-Kadji, and F. Maiza-Benabdesselam, *Ind. Crop. Prod.*, **94**, 1001 (2016), **DOI:** 10.1016/j.indcrop.2016.09.016
34. A. Zahari, A. Ablat, Y. Sivasothy, J. Mohamad, M.I. Choudhary, and K. Awang, *Asian Pac. J. Trop. Med.*, **9(4)**, 328 (2016), **DOI:** 10.1016/j.apjtm.2016.03.008

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