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RESEARCH ARTICLE

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Antioxidant Assay with Scavenging DPPH Radical of *Artocarpus anisophyllus* Miq Stem bark extracts and Chemical compositions and Toxicity Evaluation for the Most Active Fraction

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ABSTRACT:

The purpose of this study was to determine the antioxidant activity of crude extract and their fractions, the content of secondary metabolite types of crude extract, and the chemical content and toxicity of the most active fractions as antioxidants from the stem bark of *Artocarpus anisophyllus* Miq. (Mentawa). The methods used are phytochemical screening, toxicity tests against *Artemia salina* by the Brine Shrimp Lethality Test (BSLT), antioxidant activity assay with DPPH radical reduction method, and determination of chemical compound by GC-MS analysis. Phytochemical test results showed that crude extract containing alkaloids, triterpenoids, phenolics and flavonoids. The results of the antioxidant activity of the n-hexane, ethyl acetate and methanol fractions obtained IC₅₀ values of 127.69, 28.65 and 79.43ppm, respectively. Ethyl acetate (as the most active fraction) was then fractionated using a vacuum chromatography column and the fractions obtained were E1 (268.8mg), E2 (337.1), E3 (234.3mg) and E4 (431.2mg). The antioxidant activity test showed that E2 was the most active compared to other fractions with an IC₅₀ value of 37.24ppm. While the toxicity test results showed that E2 was very active with an LC₅₀ value of 6.23ppm indicating that E2 was also potentially developed as an anticancer drug. Based on GC-MS spectrum analysis, of the several main compounds, four of which are aromatic compounds that have the potential to be developed as antioxidants, namely 2- (Benzyloxy) phenol (phenolic compound) (3.96%) (51), 1, 2- Benzenedicarboxylic acid, dinonyl ester (2.44%) (52), Linderazulene (2.43%) (30), p-Nonylphenol (2.36%). Some other aromatic minor compounds can also be active as antioxidants.

KEYWORDS: *Artocarpus anisophyllus* Miq., Secondary Metabolites, Mentawa, Toxicity, Antioxidant, DPPH.

INTRODUCTION:

Plants are actually very important for human life, one of which is an important source of compounds as medicinal ingredients. Empirically, medicinal plants are still used to cure various types of diseases as an alternative treatment¹. Indonesia consists of seven bioregions and it has the second largest biodiversity after Brazil².

Artocarpus, one of the main genus of Moraceae that is found in tropical forests of Indonesia and spread on various islands, such as Kalimantan, Sulawesi, Sumatra, Java and others. Some species of *Artocarpus* have been used in traditional medicines which have effects such as Antibacterial, anticancer, antidiabetic, fever medication, worms, antiviral, anti-inflammatory, diuretic, and anti-hypertensive. This pharmacological effect may be due to the presence of aromatic compounds such as flavonoids, stilbenoids, xanones and arylbenzofurans^{3,4,5,6}

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Artocarpus anisophyllus Miq (Mentawa) is one of the forest plants that produce edible fruit that is commonly found on the island of Borneo. A mixture of Mentawa leaves ash with coconut oil can be used to treat burns by applying it to the skin of burns⁷. The plant leaves are also used by people to treat boils and itching⁸. Crude extracts of Mentawa stem have strong antioxidant activity and it contains secondary metabolite compounds such as flavonoids, phenolics and triterpenoids⁹, root extracts exhibited toxicity against *Artemia salina*¹⁰. While the crude extracts of Mentawa leaves have antibacterial and antioxidant activities¹¹ and from the leaves and heartwood this plant have been isolated prenylated flavonoid compounds which are antioxidant and tyrosinase inhibitory activities.¹²

As a continuation of our research on *A. anisophyllus* Miq, in this article we will report the antioxidant properties of total extracts and their fractions from Mentawa bark and the chemical composition of the most active fractions as antioxidants.

METHODOLOGY:

Sample Collection and Preparation:

Mentawa (*Artocarpus anisophyllus*) was collected from Tanah Merah village, Samarinda, East Kalimantan. The plant was identified in the Plant Anatomy and Systematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda. The sample (stem bark of *A. anisophyllus*) was cleaned, dried and mashed by a smoothing machine to obtain dry powder samples.

Extraction and fractionation:

1.082gram dried powder samples were extracted using maceration method ¹⁰ methanol for 2x 24 hours. The filtrate obtained was concentrated by a rotary evaporator to give crude brown extract (10grams). Crude extract was further partitioned with n-hexane and ethyl acetate respectively. The most active fraction as an antioxidant will be fractionated using vacuum column chromatography.

Phytochemical Test:

The phytochemical test was conducted as a preliminary test to find out the types of secondary metabolite compounds in crude extracts which covered the test of alkaloid, steroid and triterpenoid, flavonoid, phenolic and saponin compounds. The research procedure refers to previous studies^{13,14}.

Toxicity Test with the Brine Shrimp Lethality Test (BSLT) Method:

Fraction most active as antioxidant obtained from vacuum column chromatography will be further tested for toxicity against *Artemia salina* larvae. Sample was made in various concentrations, then *A. salina* larvae were added (500ppm; 250ppm; 125ppm; 62.5ppm; 31.2 ppm; 15.6ppm and 7.8ppm). Sample was tested three times (triplo). The number of dead shrimp larvae was counted after 24 hours of treatment. The larval mortality data obtained were analyzed using the SAS probit analysis program to determine the LC₅₀ value.^{15,16,17}

Antioxidant Activity Test:

Samples were made in concentrations of 20ppm, 40 ppm, 60ppm and 80ppm. Each sample was put 4mL into a cuvette and then added 1 ml of a 0.024mg/mL DPPH solution, homogenized and then incubated for 30 minutes. Then the absorbance samples were measured at a wavelength of 517nm using a UV-Vis spectrophotometer. The IC₅₀ calculation refers to the previous research procedure^{18,19,20,21,22,23}.

GC-MS analysis:

GC-MS used is Thermo Scientific Trace 1310 Gas Chromatograph-High Resolution Mass Spectrometry (Thermo Scientific ISQLT Single Quadrupole Mass Spectrometer). The specifications of this instrument is covering auto injector TriPlus type RSH, UHP helium used as carrier gas, and use the software Chromeleon own library for easy knowing that compound was detected when there are no standards in accordance with the tested compounds.

RESULTS AND DISCUSSION:

Extraction and partitioning results obtained crude extracts, fractions of n-hexane; ethyl acetate and methanol were 10, 0.98, 4.21, and 2.49 grams, respectively. The phytochemical test exhibited that crude extracts containing alkaloids, triterpenoids, phenolics and flavonoids. Based on the results of antioxidant tests using DPPH radical reduction showed that the ethyl acetate fraction had the lowest IC₅₀ value compared to the n-hexane and methanol fractions with IC₅₀ values of 28.57, 127.67 and 79.43ppm, respectively. Furthermore, fractionation is carried out of the ethyl acetate fraction (the most active as an antioxidant) using liquid column chromatography by increasing the eluent polarity gradually to give E1 (268.8mg), E2 (337.1), E3 (234.3 mg) and E4 (431.2mg). The IC₅₀ of the four fractions (E1-E4) are showed in table 1. E2 has the highest antioxidant properties compared to other fractions with an IC₅₀ value of 37.24ppm.

Table 1. The IC₅₀ value of the fractionation results from ethyl acetate fraction (Average of three replicates performed for each concentration)

Fractions	Concentration (ppm)	Absorbance	% inhibition	Linear regression equation	IC ₅₀ (µg/mL)
E1	20	0.198	29.985	Y = 0.7188x + 17.003	45.91
	40	0.150	47.368		
	60	0.110	61.403		
	80	0.076	73.333		
E2	20	0.204	28.401	Y = 1.1348x + 7.737	37.24
	40	0.130	54.364		
	60	0.058	79.425		
	80	0.012	96.692		
E3	20	0.193	32.435	Y = 0.9149x + 11.505	42.08
	40	0.160	43.618		
	60	0.088	68.579		
	80	0.042	84.732		
E4	20	0.331	20.241	Y = 0.414x + 10.396	95.52
	40	0.309	25.542		
	60	0.276	33.494		
	80	0.201	45.232		
Vitamin C	2	0.547	18.358	Y = 12.291x + 9.4776	3.07
	4	0.153	77.164		
	6	0.045	93.284		
	8	0.034	94.925		

Table 2. The LC₅₀ value of E2 (Average of three replicates performed for each concentration)

Sample	concentration	Log concentration	Total larvae	Dead larvae	% Mortality	Probit	LC ₅₀ (ppm)
E2	500	2.6989	9.67	9.67	100.00	8.09	6.23
	250	2.3979	9.33	8.67	92.92	6.48	
	125	2.0969	10.33	9.00	87.12	6.13	
	62.5	1.7959	10.67	8.67	81.26	5.88	
	31.25	1.4948	8.67	5.00	57.67	5.20	
	15.63	1.1938	10.33	7.00	67.76	5.47	
	7.81	0.8928	9.00	5.67	63.00	5.33	

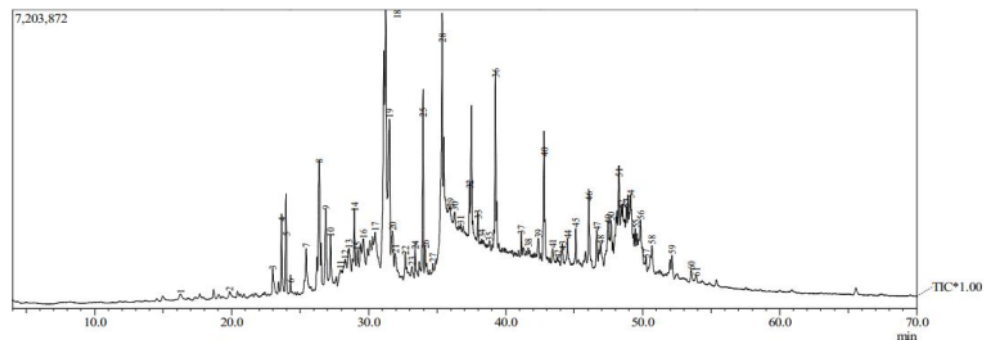


Fig. 1. GC-MS chromatogram of E2

Table 2. Chemical composition of E2

S.No	Peak	% Area	Molecular formula	Molecular weight	Compound
1	16.284	0.19	C ₄ H ₈ O ₃	104	Propanoic acid, 2-hydroxy-2-methyl-
2	19.874	0.18	C ₁₀ H ₂₂ O	158	1-Octanol, 2,7-dimethyl-
3	23.020	0.59	C ₁₂ H ₂₂	166	Hexane, 1-(isopropylidenecyclopropyl)-
4	23.656	1.31	C ₁₅ H ₂₆ O	222	Globulol
5	23.992	1.11	C ₁₃ H ₂₀ O	192	15 a-Ionone
6	24.325	0.23	C ₁₄ H ₂₂ O	206	Phenol, 3,5-bis(1,1-dimethylethyl)-
7	25.452	1.26	C ₈ H ₁₄ O	126	2-Hexanone, 3-methyl-4-methylene-
8	26.402	3.44	C ₁₁ H ₂₂	152	Bicyclo[2.2.1]heptane, 2-(1-methylpropyl)-
9	26.886	1.60	C ₈ H ₁₂ O	124	30 Octadien-2-one, (E,E)-
10	27.230	1.19	C ₁₀ H ₁₈	138	Cyclopentene, 1,4-dimethyl-5-(1-methylethyl)-
11	27.958	0.89	C ₁₈ H ₂₆ O ₂	250	Sclarrolide
12	28.299	1.18	C ₁₀ H ₁₂ O ₃	180	22 Epoxypropyl para-methoxyphenyl ether
13	28.571	1.40	C ₁₀ H ₁₆ O ₂	168	2-Cyclohexen-1-one, 2-hydroxy-6-methyl-3-(1-methylethyl)-

14	28.968	1.71	C ₁₆ H ₃₄	226	Hexadecane
15	29.199	0.89	C ₁₄ H ₂₂ O	206	Phenol, 4-(2,2,3,3-tetramethylbutyl)
16	29.625	2.36	C ₁₅ H ₂₄	220	p-Nonylphenol
17	30.486	5.34	C ₁₅ H ₂₆ O	222	Palustrol
18	31.253	9.52	C ₁₇ H ₃₀ O	168	3-Decen-2-one, 3-methyl
19	31.522	3.81	C ₁₃ H ₁₈ O ₃	222	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)
20	31.751	1.22	C ₁₇ H ₃₄ O ₂	270	Isopropyl myristate
21	31.958	0.82	C ₈ H ₁₄ O	126	2-Hepten-2-one, 4-methyl-
22	32.689	0.72	C ₁₆ H ₃₂ O ₄	278	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
23	33.158	0.22	C ₁₂ H ₂₆ O	186	1-Octanol, 2-butyl-
24	33.414	0.42	C ₂₀ H ₄₂	282	Eicosane
25	33.980	2.29	C ₁₇ H ₃₄ O ₂	270	29-Undecanoic acid, methyl ester
26	34.138	0.46	C ₁₈ H ₃₈	292	Methyl-3-(3,5-diterbutyl-4-hydroxyphenyl) propionate
27	34.692	0.15	C ₁₄ H ₂₂ O ₄	278	Dibutyl phthalate
28	35.380	9.58	C ₁₈ H ₃₆	252	1-Octadecene
29	35.958	2.69	C ₁₇ H ₃₄ O ₂	270	Isopropyl myristate
30	36.292	2.43	C ₁₅ H ₁₄ O	210	Linderazulene
31	36.725	3.70	C ₁₃ H ₁₅ NO ₃	233	4-Oxazolecarboxylic acid
32	37.381	4.49	C ₁₇ H ₃₀ O ₂	266	9,12-Hexadecadienoic acid, methyl ester
33	37.992	1.02	C ₁₅ H ₃₀ O ₂	298	Octadecanoic acid, methyl ester
34	38.260	1.18	C ₁₃ H ₂₈	184	Undecane, 4,5-dimethyl-
35	38.825	0.53	C ₁₉ H ₄₀	268	Octadecane, 2-methyl
36	39.262	2.97	C ₂₂ H ₄₄	308	1-Docosene
37	41.153	0.40	C ₂₀ H ₄₂	282	Eicosane
38	41.657	0.17	C ₁₆ H ₃₂ O ₂	256	Methyl 12-methyltetradecanoate
39	42.389	0.31	C ₁₀ H ₂₁ Cl	176	Decane, 1-chloro-
40	42.815	1.78	C ₂₂ H ₄₄	308	1-Docosene
41	43.438	0.29	C ₂₅ H ₄₂ O ₂	374	Octadecanoic acid, phenylmethyl ester
42	43.825	0.13	C ₁₄ H ₃₂	212	Tetradecane, 4-methyl-
43	44.158	0.32	C ₂₂ H ₃₆ O ₃ Si	386	Hexadecanoic acid, 3- (trimethylsilyloxy) propyl ester
44	44.535	0.47	C ₂₀ H ₄₂	282	Eicosane
45	45.129	0.55	C ₂₄ H ₃₈ O ₄	390	Di-n-octyl phthalate
46	46.085	1.53	C ₂₇ H ₅₆ O	396	1-Heptacosanol
47	46.660	0.60	C ₁₃ H ₁₀ F ₂ O ₂	236	18-Hol, 2-benzyloxy-3,6-difluoro
48	46.934	0.85	C ₂₃ H ₄₄ O ₄	384	1,3-Dioxolane, 4- (2-methoxy-4-hexadecenyl)oxy methyl-2,2-dimethyl-
49	47.488	1.49	C ₁₅ H ₁₄ O ₃	242	Benzaldehyde, 3-methoxy-4-(phenylmethoxy)-
50	47.692	0.98	C ₁₉ H ₄₀	268	Nonadecane
51	48.292	3.96	C ₁₃ H ₁₂ O ₂	200	2-(Benzyloxy)phenol
52	48.440	2.44	C ₂₆ H ₄₂ O ₄	418	1,2-Benzenedicarboxylic acid, dinonyl ester
53	48.827	1.73	C ₂₈ H ₄₆ O ₄	446	1,2-Benzenedicarboxylic acid
54	49.124	3.95	C ₂₉ H ₆₀ O	424	Nonacosanol
55	49.463	1.24	C ₂₈ H ₄₆ O ₄	446	1,2-Benzenedicarboxylic acid, diisodecyl ester
56	49.852	1.69	C ₁₄ H ₃₀ O ₂	300	28-Ianoic acid, 3,3-dimethyl-5-[(phenyl methyl)seleno]-
57	50.258	0.23	C ₂₈ H ₃₆ O ₄	446	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester
58	50.687	0.75	C ₁₀ H ₁₂ O ₂	164	15-Ethoxyphenylacetone
59	52.146	0.63	C ₁₆ H ₁₈ O ₂	242	Benzene, 1,1'-(1,2-ethanediyl) bis[4-methoxy-
60	53.552	0.27	C ₃₄ H ₆₈ O ₂	488	Cholesta-4,6-dien-3-ol, benzoate
61	53.925	0.14	C ₁₄ H ₂₂ O	206	Iron alpha

Toxicity test results showed that E2 was very toxic against *Artemia salina* larvae with LC₅₀ value of 6.23 ppm. Based on the results of previous studies indicate that the toxicity test against *A. salina* is not specific to anticancer but it can be used as a screening to determine the anticancer potential of the extract or fraction. In addition, this method is inexpensive, easy to do, and quickly known the results¹⁵

31 The results of the GC MS E2 analysis were showed in Table 2. The mayor chemical content of E2 (above 2%) consists of 1-Octadecene (28), 3-Decen-2-one, 3-methyl (18), Palustrol (17), 9,12-Hexadecadienoic acid, methyl

19r (32), 2-(Benzyloxy) phenol (51), Nonacosanol (54), 2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl) (19), 4-Oxazole carboxylic acid (31), Bicyclo [2.2.1] heptane, 2-(1-methylpropyl) - (8), 1-Docosene (36), Isopropyl myristate (29), 1,2-Benzenedicarboxylic acid, dinonyl ester (52), Linderazulene (30), p-Nonylphenol (16) and Hexadecanoic acid, methyl ester (25). Four of them are aromatic compounds that have antioxidant potential, namely 2- (Benzyloxy) phenol (51), 1,2-Benzenedicarboxylic acid, dinonyl ester (52), Linderazulene (30), p-Nonylphenol (16).

In addition, other minor aromatic compounds which have potential as antioxidants such as 1,2-Benzenedicarboxylic acid (53), Pentanoic acid, 3,3-dimethyl-5-[(phenyl methyl)seleno]- (56), Benzaldehyde, 3-methoxy-4-(phenylmethoxy)- (49), 1,2-Benzenedicarboxylic acid, diisodecyl ester (55), 2,3-Epoxypropyl para-methoxyphenyl ether (12), Phenol, 4-(2,2,3,3-tetramethylbutyl) (15), 2-Methoxyphenylacetone (58), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (22), Phenol, 2-benzyloxy-3,6-difluoro (45), Benzene, 1,1'-(1,2-ethanediyl)bis[4-methoxy- (59), Di-n-octyl phthalate (45), Octadecanoic acid, phenylmethyl ester (41) Phenol, 3,5-bis(1,1-dimethylethyl)- (6), 1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester (57) and Dibutyl phthalate (27).

CONCLUSION:

E2 obtained from fractionation by vacuum column chromatography of the ethyl acetate fraction of Mentawa stem bark was the most active fraction as an antioxidant against DPPH radicals with IC₅₀ values of 37.24ppm. This fraction is also very toxic against *Artemia salina* (LC₅₀ value of 6.23ppm) which indicates anticancer potential. Several aromatic compounds have been identified from GC-MS data analysis which has potential as antioxidants.

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