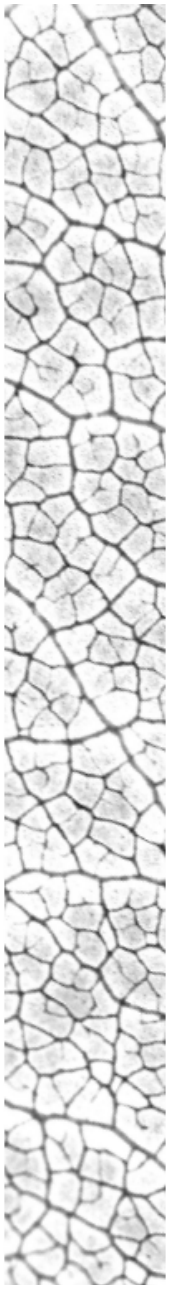

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Toxicity test, antioxidant activity test and GC-MS profile of the active fraction of *Coptosapelta tomentosa* (Blume) root (Merung)

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Abstract
Merung (*Coptosapelta tomentosa* (Blume)) is one of the plants of the genus *Coptosapelta*, commonly found in the forests of Borneo. People in Kalimantan, especially in East Kalimantan and South Kalimantan, use the extract of merung root as traditional medicine for aphrodisiacs, blood clots (menstruation), inflammatory or swollen pain, rheumatism, and diarrhea. This study aims to determine the toxicity with the brine shrimp lethality test (BSLT) method and antioxidant activity with the DPPH radical scavenging method of crude extracts and their fractions from merung roots and to determine the chemical content of the most active fractions using GC-MS. Based on the results of the toxicity test and antioxidant activity test showed that the ethyl acetate fraction was the most active extract compared to the others with LC_{50} and IC_{50} values of 123.83 µg/mL and 31.160 µg/mL, respectively. GC-MS spectrum analysis results of ethyl acetate fraction compared with the database obtained major compounds namely Ethanone, 1- (1,3,4,4a, 5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalen-8-ol) - (32.08%), Squalene (26%), Lupeol (24.94%), 7-Hexadecyn-1-ol (2.88%), 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z) - (1.24%), 9,10-Anthracenedione, 1-hydroxy-2-(hydroxymethyl) - (1.23%), and 4-isoquinoline, 3-ethoxy- (1.14%). 9,10-Anthracenedione, 1-hydroxy-2- (hydroxymethyl)- and 4-isoquinoline, 3-ethoxy- potentially as antioxidants. There are also several other minor aromatic (phenolic) compounds which can have antioxidant potential.

Keywords: Merung, *Coptosapelta tomentosa* (Blume), antioxidants activity, DPPH radical, toxicity, chemical composition

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INTRODUCTION

Indonesia is one of the countries rich in biodiversity. The people of Indonesia have long recognized the use of plants in traditional medicine. *Jamu* (herbal medicine) is one example of the heritage of traditional medicine consumed, and it is believed to cure certain diseases. Merung plant (Coptosapelta tomentosa Valeton K. Heyne) (Fitryana 2018) is one of the plants used in traditional medicine, especially in Kalimantan. Merung (another local name is Manuran/Maniren) by the people of South Kalimantan has long been used in traditional medicine as an aphrodisiac drug and to reduce blood (menstruation). Merung root is used to treat inflammation or swelling, rheumatism, and diarrhea by the people of East Kalimantan (Hermanda et al. 2016). The plant may contain substances beneficially to ameliorate symptoms caused by bacteria, hepatotoxin, inflammation, virus, diuretics, cough, and hypoglycemi (Kardinan and Kusuma 2004).

Previous studies indicate that Merung has a variety of bioactivity for further study. Root extract has antibacterial activity against test bacteria Escherichia coli, and Staphylococcus aureus (Hermanda et al. 2016). Both root and stem extracts are very active as antiplasmodia (Arnida and Supomo 2017, Arnida et al. 2017). While extracts of all parts of plants can be used as an anti-inflammatory, tonic, and can reduce blood glucose levels (Minh et al. 2014, Nugrahani 2012).

Dayak Kenya (East Kalimantan), since the first harness Merung root as a medicine leucorrhea. Leucorrhea is one of the early symptoms of cervical cancer (Supriningrum et al. 2016). Phytochemical

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Table 1. Antioxidant activity of extracts of *Coptosapelta tomentosa* (Blume) root

Extract	Concentration (ppm)	Absorbance	% inhibition	IC_{50} (µg/mL)	
Crude	20	0.213	19.623	93.166	High
	40	0,188	29.056		
	60	0,169	36,226		
	80	0.147	44.195		
n-hexane	20	0,161	39,245	49.100	Very high
	40	0,139	47.672		
	60	0.124	53.207		
	80	0,103	61.132		
Ethyl acetate	20	0,145	45,283	31.160	Very high
	40	0,124	53,207		
	60	0.097	63.395		
	80	0,077	70.943		
methanol	20	0,189	28.678	83.097	High
	40	0,171	36.138		
	60	0,162	38.867		
	80	0.130	50.817		
Vitamin C	2	0.220	16.851	5,399	Very high
	4	0,167	36,981		
	6	0,113	57358		
	8	0,070	73.635		

screening and toxicity test results show that all parts of Merung contain phenolic and flavonoids, and it shows that root is the most toxic against *Artemia salina* Leach compared to others with LC_{50} value of 173.09 ppm (Karolina et al. 2018). This study aims to measure antioxidant activity and profile chemical compounds of the ethyl acetate fraction.

MATERIALS AND METHODS

Extraction and Separation

The dried powder of merung root (6 kg) extracted by maceration using methanol for 24 hours, repeated two times. The obtained filtrate was then evaporated under low pressure with rotavapor and obtained a brown crude extract (164.67 grams). The crude extract is redissolved with methanol and then partitioned using n-hexane, and the partition is continued using ethyl acetate. Fractions of n-hexane, ethyl acetate, and methanol were obtained 5.33, 61.13, and 71.13 grams, respectively.

Toxicity Test

The sample toxicity test used the Brine Shrimp Lethality Test against *Artemia Salina* Leach shrimp larvae (Erwin et al. 2018, Karolina et al. 2018, Meyer et al. 1982).

Antioxidant Activity Test

The antioxidant activity test was carried out by DPPH radical scavenging method. The standard vitamin C solutions were prepared in concentrations of 2, 4, 6, and 8 µg/mL, respectively. The sample solution was prepared in concentrations of 20, 40, 60, and 80 µg/mL, respectively. 2 mL sample/vitamin C and 2 mL of 0.024 µg / mL DPPH solution was put into the test tube, respectively. After homogenization, the samples were incubated for 30 minutes, then measured using a UV-Vis Spectrophotometer at the optimum wavelength of 515 nm. The same treatment is carried out in making blanks without adding samples.

$$\% \text{ Inhibition} = \frac{\text{Absorbsorbance of blank-Absorbance of sample/vitamin C}}{\text{Absorbance blank}} \times 100\%.$$

IC_{50} values were calculated using the linear regression equation Y = a + bX; if Y is equal to 50, then the value of X is IC_{50} (Erwin 2015, Erwin et al. 2018, Supomo et al. 2019).

GC-MS Recording

The GC-MS (Shimadzu GCMS-QP2010 Plus) records the spectrum of active fractions. The equipment specification includes the mobile phase of Helium Gas, Stationary Phase/Column: RTX-5-MS 30M x 0.15 mm ID x 0.25 um. The peak obtained from the chromatogram was then compared with the internal database.

RESULTS AND DISCUSSION

Based on the extraction and fractionation of crude extracts, n-hexane fraction, ethyl acetate fraction, and methanol fraction obtained 164.67, 5.33, 61.13, and 71.13 grams, respectively. The toxicity test was performed using the brine shrimp lethality test (BSLT) method, and the LC_{50} values obtained for a fraction of n-hexane, ethyl acetate, and methanol were 162.28; 123.83 and 287.12 µg / mL, respectively. LC_{50} values obtained indicate that all extracts are toxic to shrimp *Artemia salina* L. ($31<LC_{50}<1000$ ppm). However, the ethyl acetate fraction has the lowest LC_{50} value, so that it has the highest toxicity compared to the others (Meyer et al. 1982).

Based on the results of antioxidant activity tests against DPPH radical scavenging, IC_{50} values for crude extracts, n-hexane, ethyl acetate, and methanol fractions were 93.166, 49.100, 31.160, and 83.097 µg / mL, respectively. Ethyl acetate extract has the smallest IC_{50} value. As a consequence, it has the highest antioxidant properties compared to other extracts (Molyneux 2004).

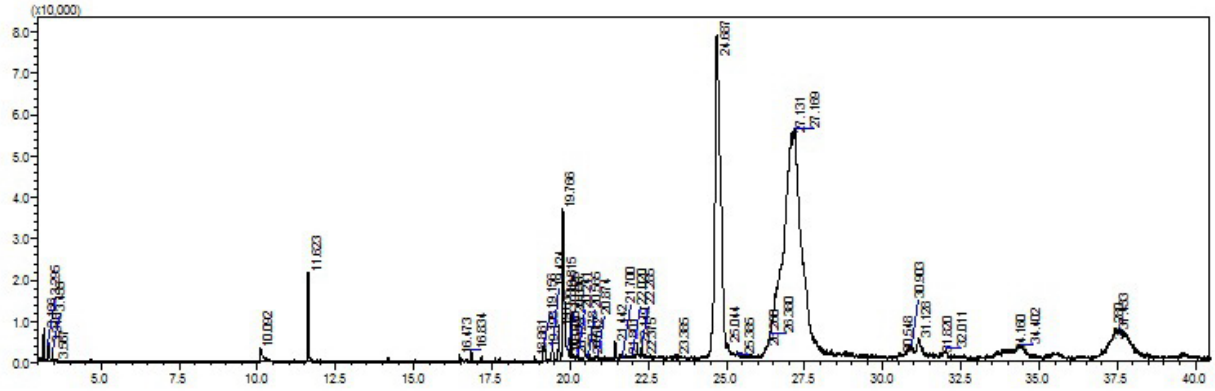

Fig. 1. GC chromatogram of ethyl acetate fraction of *C. tomentosa* (Blume) root

Table 2. Chemical composition of ethyl acetate fraction of *C. tomentosa* (Blume) root

Peak	Retention Time	Peak Area %	Base peak m/z	Molecule weight	Molecule Formula	Compounds
1	3.166	0.49	55.00	112	C_8H_{16}	Cyclohexane, 1,3-dimethyl-
2	3.294	0.20	55.05	112	C_8H_{16}	Cyclopentane, 1-ethyl-3-methyl-
3	3.348	0.22	55.05	112	C_8H_{16}	Cyclopentane, 1-ethyl-2-methyl-, cis-
4	3.455	0.12	55.05	112	C_8H_{16}	Cyclohexane, 1,3-dimethyl-, trans-
5	3.567	0.07	55.00	97	C_5H_7NO	Acetamide, N-2-propynyl-
6	10.092	0.38	105.00	382	$C_{20}H_{18}N_2O_6$	Cyclobutane-1,1-dicarboxamide, N,N'-di-benzoyloxy-
7	11.623	1.24	69.05	154	$C_{10}H_{18}O$	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-
8	16.473	0.08	148.95	270	$C_{13}H_{15}ClO_4$	Phthalic acid, 2-chloropropyl ethyl ester
9	16.834	0.16	139.00	284	$C_{18}H_{36}O_2$	cis-9,10-Epoxyoctadecan-1-ol
10	18.861	0.09	124.00	334	$C_{21}H_{34}O_3$	Myristic acid, 4-methoxyphenyl ester
11	19.156	0.31	55.00	138	$C_{10}H_{18}$	Cyclooctane, ethenyl-
12	19.195	0.24	123.00	164	C10H16Si	Diallyldivinylsilane
13	19.424	0.43	97.00	138	$C_9H_{14}O$	2,6-Heptadienal, 2,4-dimethyl-
14	19.638	0.72	148.95	356	$C_{22}H_{28}O_4$	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
15	19.766	2.88	55.05	238	$C_{16}H_{30}O$	7-Hexadecyn-1-ol
16	19.815	1.14	133.00	186	$C_{11}H_{11}NO_2$	4-isoquinolinol, 3-ethoxy-
17	19.910	0.15	80.00	95	CH_5NO_2S	Methane sulfonamide
18	19.925	0.16	95.00	166	$C_{11}H_{18}O$	4-(1,2-Dimethyl-cyclopent-2-enyl)-butan-2-one
19	19.975	0.07	95.00	110	C_8H_{14}	1,4-Pentadiene, 2,3,4-trimethyl-
20	20.036	0.76	69.00	168	$C_{10}H_{16}O_2$	Cyclopropanecarboxylic acid, 3-(3-butenyl)-2,2-dimethyl-
21	20.128	0.12	57.00	92	C_4H_9Cl	Propane, 2-chloro-2-methyl-
22	20.241	0.14	91.00	370	$C_{25}H_{38}O_2$	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester
23	20.478	0.24	69.05	156	$C_{10}H_{20}O$	Cyclopentaneethanol, .beta.,2,3-trimethyl-
24	20.565	0.09	57.00	296	$C_{10}H_{11}F_7O_2$	3,5-Octanedione, 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-
25	20.642	0.10	148.95	418	$C_{21}H_{23}BrO_4$	Phthalic acid, 4-bromophenyl heptyl ester
26	20.874	0.14	57.00	254	$C_9H_{19}I$	Nonane, 1-iodo-
27	21.442	0.25	206.90	163	$C_8H_5NO_3$	1H-Isoindole-1,3(2H)-dione, 2-hydroxy-
28	21.700	0.09	69.00	207	$C_{10}H_9NO_4$	Cyclopropanecarboxylic acid, 4-nitrophenyl ester
29	21.801	0.16	67.00	194	$C_{12}H_{18}O_2$	1,6-Bis(2-propyn-1-yloxy)hexan
30	22.020	0.10	57.00	240	$C_8H_{17}I$	Octane, 1-iodo-
31	22.149	0.28	189.00	204	$C_{14}H_{20}O$	Lilial
32	22.285	0.33	71.00	152	$C_{11}H_{20}$	4-t-Pentylcyclohexene
33	22.375	0.10	221.90	128	$C_8H_{16}O$	Cyclohexanol, 3,5-dimethyl-
34	23.385	0.10	71.00	250	$C_{12}H_{26}O_3S$	Sulfurous acid, nonyl 2-propyl ester
35	24.687	26.31	69.05	410	$C_{30}H_{50}$	Squalene
36	25.044	0.17	151.00	236	$C_{15}H_{12}N_2O$	1,3-Dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one
37	25.385	0.07	91.00	358	$C_{26}H_{46}$	Benzene, (1-propylheptadecyl)-
38	26.260	0.17	67.00	202	$C_9H_{15}Br$	Cyclohexane, (2-bromocyclopropyl)-, trans-
39	26.380	0.44	96.10	410	$C_{30}H_{50}$	Olean-12-ene
40	27.131	32.08	146.00	246	$C_{17}H_{26}O$	Ethanone, 1-(1,3,4,4a,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalen-8-yl)-
41	27.169	24.94	105.05	246	$C_{30}H_{50}O$	Lupeol
42	30.548	0.14	81.00	156	$C_9H_{16}O_2$	Acetic acid, trans-4-methylcyclohexyl ester
43	30.903	0.74	67.10	218	$C_4H_6N_6O_5$	Furazan-3,4-diamine, N,N'-dimethyl-N,N'-dinitro-
44	31.128	1.23	224.95	254	$C_{15}H_{10}O_4$	9,10-Anthracenedione, 1-hydroxy-2-(hydroxymethyl)-
45	31. 820	0.15	133.00	244	$C_{12}H_{14}ClFO_2$	6-Chlorohexanoic acid, 3-fluorophenyl ester
46	32.011	0.33	148.95	390	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate
47	34.160	0.11	133.00	252	$C_{15}H_{16}N_4$	Bicyclo[2.2.1]heptane, 2,5-diphenyl-1,2,4,5-tetraaza-
48	34.402	0.61	69.10	362	$C_{21}H_{30}O_3S$	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-
49	37.280	0.12	79.00	150	$C_{10}H_{14}O$	5,6-Epoxy-2,2-dimethyloct-7-ene-3-yne
50	37.433	0.24	149.10	426	$C_{30}H_{50}O$	alpha.-Amyrin

GC-MS results on the ethyl acetate fraction obtained major compounds are Ethanone, 1-(1,3,4,4a,5,6,7-hexahydro-2,5,5-trimethyl-2H-2,4a-ethanonaphthalen-8-ol)-(40), Squalene (35), Lupeol (41), 7-Hexadecyn-1-ol (15), 2,6-Octadient-1-ol, 3,7-dimethyl-, (Z) - (7),), 9,10 -Anthracenedione, 1-hydroxy-2- (hydroxymethyl) - (44), dan 4- isoquinoline, 3-ethoxy- (16). The (44) is a phenolic compound, and the (16) is an aromatic alkaloid, which is hydroxyl group substituted, both of these compounds have antioxidant potential. Characteristics of potent antioxidant compounds have one or more aromatic rings with one or more −OH groups capable of donating H (Brewer 2011, Erwin et al. 2018, Kartika et al. 2019).

In addition, other minor aromatic (phenolic) compounds can also be potential as antioxidants such as Cyclobutane-1,1-dicarboxamide, N,N'-di-benzoyloxy- (6), Phthalic acid, 2-chloropropyl ethyl ester (8). Myristic acid, 4-methoxyphenyl ester (10), 9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester (22). Phthalic acid, 4-bromophenyl heptyl esrter (25), 1H-Isoindole-1,3(2H)-dione, 2-hydroxy- (27), Cyclopropanecarboxylic acid, 4-nitrophenyl ester (28), Lilial (31), 1,3-Dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (36), 6-Chlorohexanoic acid, 3-fluorophenyl ester (45), Bis(2-ethylhexyl) phthalate (46), Bicyclo[2.2.1]heptane, 2,5-diphenyl-1,2,4,5-tetraaza-(47), 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)- (48).

CONCLUSION

The ethyl acetate fraction was the most active extract compared to the others for the toxicity test and antioxidant activity test with LC_{50} and IC_{50} values of 123.83 µg / mL and 31.160 µg/mL, respectively. The content profile of the chemical compounds of ethyl acetate fraction shows various types of secondary metabolites. Several phenolic or aromatic compounds have been identified. The compounds may have antioxidant properties.

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