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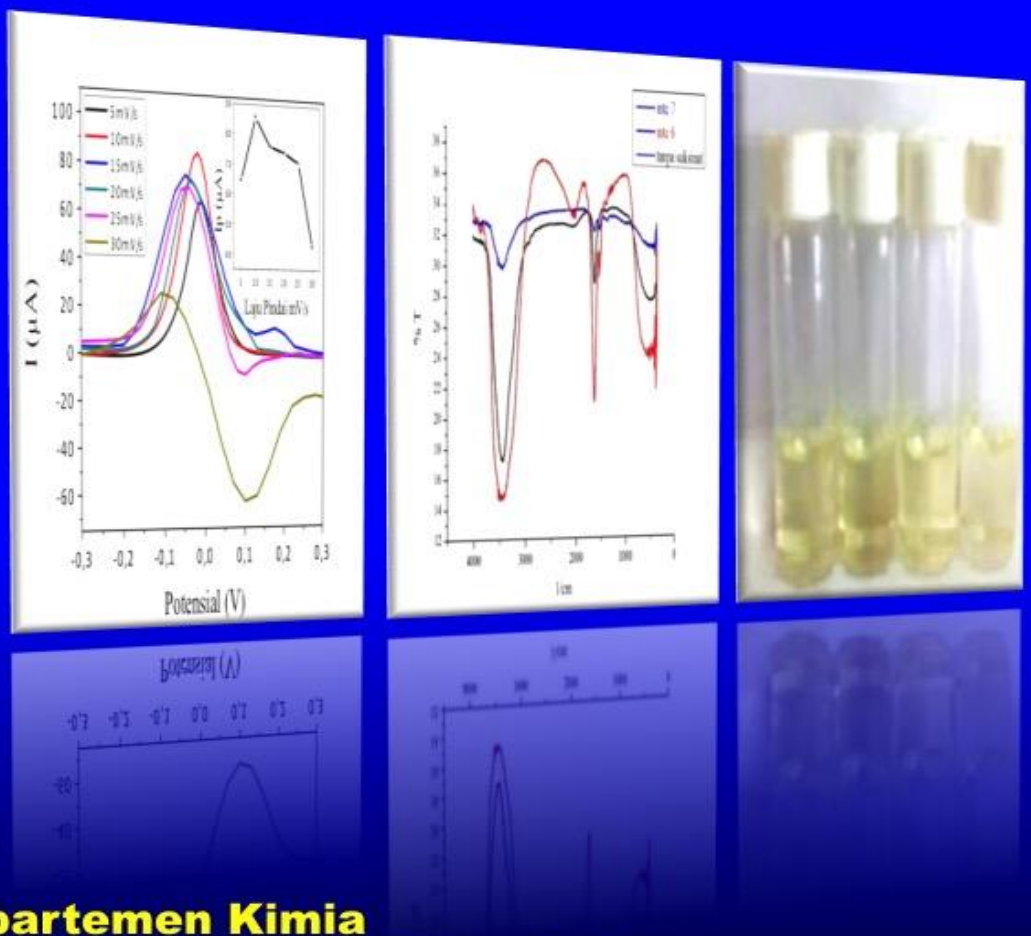

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ISOLATION OF BIOACTIVE COMPOUNDS FROM DICRANACEAE MOSSES

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Abstract

Dicranoloma reflexum and *Dicranella coarctata* are mosses from Dicranaceae family. This study was purposed to identify bioactive compounds contained from both species. *Dicranoloma reflexum* and *Dicranella coarctata* collected form Cangar forest, Batu, East Java. Mosses was rinsed, dried and crushed into powder. Extraction was performed using maceration method with n-hexane, acetic acid, and methanol solvent. Compounds obtained then identified using Gass Chromatography Mass Spectra. Result showed that n-hexane, acetic ethyl, and methanol extract of *Dicranoloma reflexum* contained 61, 16, and 58 compounds respectively. Main component of each extract was 1-octadecene, phenol, and 9-octadecanoic acid. N-hexane, acetic ethyl, and methanol extract of *Dicranella coarctata* contained 5, 38, and 23 compounds respectively. Main component of each extract was thiosulphuric acid, E-15 heptedecenal, and n-hexadecanoic acid.

Keywords : *Dicranaceae, bioactive compounds*

Introduction

Bryophyte or mosses is source for various secondary metabolites (Asakawa, 2008). Mosses from Bryophyte is rich on secondary metabolites, such as terpenoid, flavonoid, and bibenzyles, also other compounds like fatty acid (Asakawa, 2007). With such secondary metabolites contents, many species of Bryophyta possess various biological activities, such as anti-bacteria, anti-fungal, anti-oxidant, and anti-inflammatory properties (Saxena dan Harinder, 2004).

For example Indian mosses *Bryum argenteum* had anti-pyretic, anti-rhynitic, and anti-bacterial properties. Himalayan *Fissidens laxitextus* has been known as an anti-diuretic. *Funaria hygrometrica* from India was also used as medicine for skin disease. *Entodon myurus* was found to be potential as anti-bacterial agent towards *Enterobacter aerogenes* and *Klebsiella pneumoniae* (Alam *et al*., 2015).

Dicranuloma reflexum and *Dicranella coarctata* are mosses species from Bryopsida (Musci) class, Dicranales order, and Dicranaceae family. Both species has different habitat. *Dicranuloma reflexum* is arboreal moss, while *Dicranella coarctata* is terrestrial moss. Up until now, bioactive compounds from both species has yet to be studied. This study was aimed to isolate and identify bioactive compounds from *Dicranuloma reflexum* and *Dicranella coarctata*. This study was expected to give new lights on bioactive compounds contained on both moss species as basic information which then used for evaluating biological activities.

Material and method

Main ingredient used on this study was moss gametophyte of *Dicranoloma reflexum* and *Dicranella coarctata*. Both species was collected from Cangar forest, Batu, Malang, East Java.

Extraction

Moss gametophyte *Dicranoloma reflexum* and *Dicranella coarctata* was rinsed, dried, cut into small pieces, and weighted. Total weigh of *Dicranoloma reflexum* collected was 17.1 g, while *Dicranella coarctata* weighted 4.5 g. Moss was divided into three parts, each weighted 5.7 g and 1.5 g. Each part extracted using n-hexane, acetic ethyl, and methanol. Solvent volume used for extraction of *Dicranoloma reflexum* and *Dicranella coarctata* were 200 ml and 100 ml respectively. Extraction was performed using maceration method. Simplicia soaking was conducted for 4 days and repeated for 4 times. Each extract of *Dicranoloma reflexum* and *Dicranella coarctata* was dried until all solvent had evaporated perfectly.

Bioactive Compound Identification

Each extract obtained was analyzed using Gass Chromatography Mass Spectra.

Results and discussion

Dicranoloma reflexum and *Dicranella coarctata* were mosses lived on arboreal and terrestrial habitat respectively (Fig 1 and 2).

Figure 1. Morphology of *Dicranoloma reflexum* moss.

Figure 2. Morphology of *Dicranella coarctata* moss.

Extract obtained from *Dicranoloma reflexum* and *Dicranella coarctata* were presented on Table 1.

Table 1. Extraction of *Dicranoloma reflexum* and *Dicranella coarctata* using various solvent.

No	Moss species	Simplicia weigh (g)	Solvent volume (ml)	N-hexane extract weigh (g)	Acetic ethyl extract weigh (g)	Methanol extract weigh (g)
1	*Dicanoloma reflexum*	5.7	200	0.1531	0.0851	0.7110
2	*Dicranella coarctata*	1.5	100	0.0063	0.0222	0.0493

GCMS Analysis of Dicranoloma reflexum and Dicranella coarctata Extract

Based on GC-MS analysis, chromatogram profile of n-hexane, acetic ethyl, and methanol of *Dicranoloma reflexum* and *Dicranella coarctata* were presented on Figure 2, 3, 4, 5, and 6.

Result of identification on bioactive compounds from n-hexane, acetic ethyl, and methanol extract were presented on table 2, 3, 4, 5, 6, dan 7. N-hexane, acetic ethyl, and methanol extract was found to contain 61, 16, and 58 compounds respectively (Table 2, 3, and 4). N-hexane, acetic ethyl, and methanol extract of *Dicranella coarctata* contained respectively 5, 38, and 23 compounds (Table 5, 6, and 7).

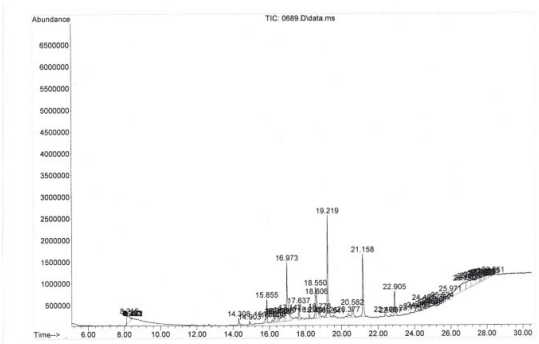

Figure 2. Chromatogram profile of n-hexane extract from *Dicranoloma reflexum*

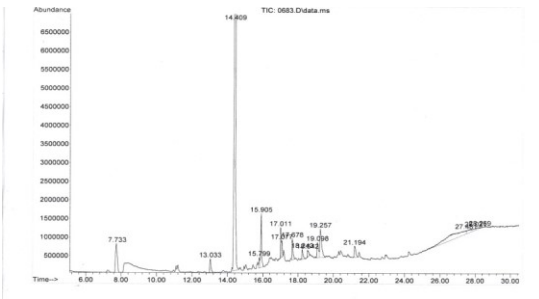

Figure 3. Chromatogram profile of acetic ethyl extract from *Dicranoloma reflexum*

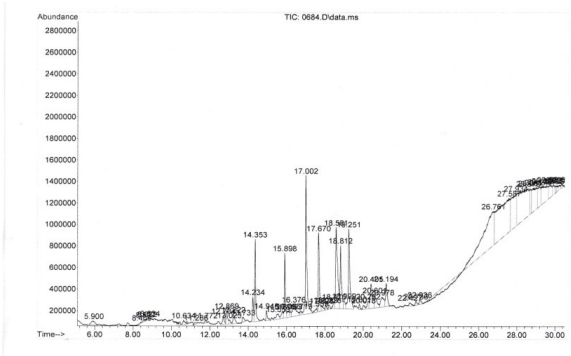

Figure 4. Chromatogram profile of methanol extract from *Dicranoloma reflexum*

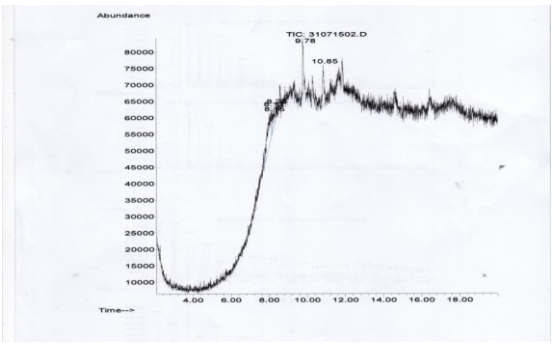

Figure 5. Chromatogram profile of n-hexane extract from *Dicranella coarctata*

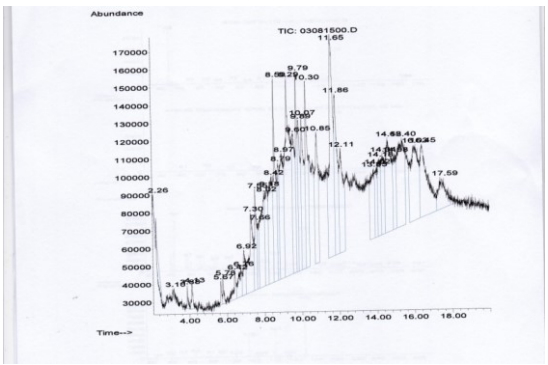

Figure 6. Chromatogram profile of acetic ethyl extract from *Dicranella coarctata*

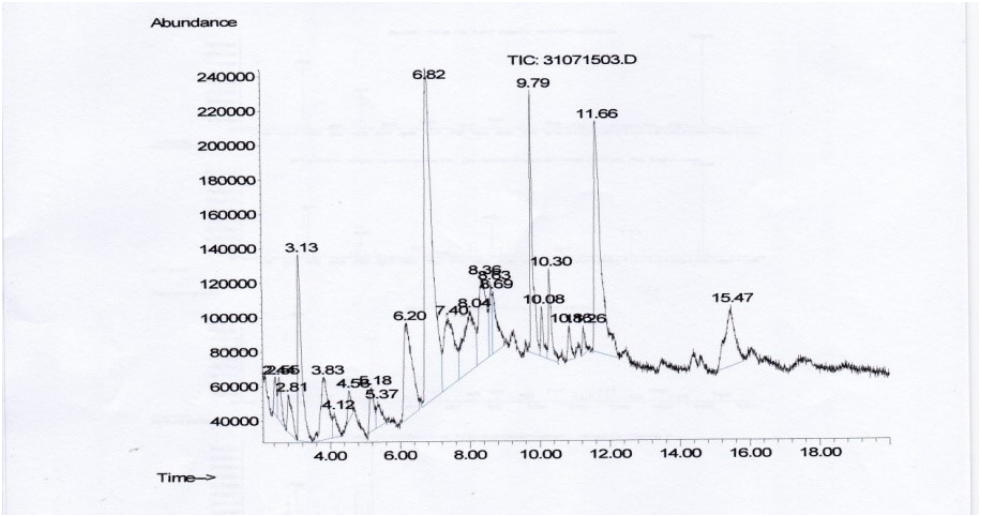

Figure 7. Chromatogram profile of methanol extract from *Dicranella coarctata*

Table 2. Chemical compounds from n-hexane extract of *Dicranoloma reflexum*

Peak	Retention time	Compound name	Areal range (%)
1	8.217	Naphthali Azulene	2.67
2	8.354	Naphthalene	0.21
3	8.383	Naphthalene	0.14
4	8.423	Naphthalene	0.04
5	8.440	Naphthalene	0.03
6	14.311	Pentanoic acid	0.81
7	14.900	5,7-Diamino-1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene	0.36
8	15.764	Heptadecane	0.71
9	15.856	Cyclopentanepropanoic acid	2.51
10	16.147	2',6'-Acetoxylidide	0.48
11	16.268	Trimethylphenylsilane	0.62
12	16.353	Nonyphenol	1.21
13	16.411	4- Nonylphenol	0.89
14	16.479	Nonylphenol	0.47
15	16.497	Nonylphenol	0.68
16	16.714	Nonylphenol	2.05
17	16.851	Phenol	1.33
18	16.971	1-Octadecene	9.95
19	17.143	Eicosane	1.66
20	17.635	Hexahydrofarnesyl acetone	2.08
21	18.202	Nonadecane	0.49
22	18.499	1- Pentadecane	0.26
23	18.551	Pentadecanoic acid	4.17
24	18.608	Hexadecanoic acid	3.70
25	18.774	Hexadecanoic acid	1.18

Continued of Table 2

Peak	Retention time	Compound name	Areal range (%)
26	19.129	Dodecynilsuccinic anhydride 9 Nonadecene	0.38
27	19.220	1- Octadecene	12.53
28	19.541	I-Propyl 14 methyl-Pentadecanoate	0.15
29	20.376	Oleic acid	1.15
30	20.582	Octadecanoic acid	2.10
31	21.160	1- Octadecene	8.25
32	22.402	Eicisanoic acid	0.39
33	22.699	9-Tricosene (Z)-	0.31
34	22.905	9-Tricosene (Z)-	3.89
35	23.775	9-Tricosene (Z)-	0.22
36	24.015	9-Octadecanoic acid	0.30
37	24.204	2-Pyrazoline, 5-hydroxy-3-metyl-5-trifluromethyl1-2-(2-isopropyl-5-methylphenox	0.32
38	24.490	1-nonadecene	1.28
39	24.656	Elaidic acid	0.07
40	24.776	9-Tricosene (Z)-	0.02
41	24.908	Elaidic acid	0.13
42	25.051	9-Octadecenoic acid	0.14
43	25.160	9-Octadecenoic acid	0.07
44	25.360	Elaidic acid	0.35
45	25.526	9-Octadecenoic acid	0.45
46	25.972	Elaidic acid	2.27
47	26.750	9-Octadecenoic acid	10.93
48	26.842	9-Octadecenoic acid	0.68
49	26.996	9-Octadecenoic acid	3.85
50	27.151	2,3- Dihydroxypropyl elaidate	1.02
51	27.340	9-Octadecenoic acid	2.96
52	27.534	Elaidic acid	2.09
53	27.574	Oleic acid	0.54
54	27.620	n-Propyl 9- Octadecanoate	0.41
55	27.689	16-Octadecenoic acid	0.61
56	27.717	n-Propyl 9- Octadecanoate	0.50
57	27.803	9-Octadecenoic acid	0.83
58	27.843	9-Octadecenoic acid	0.26
59	27.889	9-tricosane, (Z)-	0.34
60	28.084	9-Octadecenoic acid	0.98
61	28.358	i-Propyl 9-Octadecenoate	0.55

Table 3. Chemical compounds from acetic ethyl extract of *Dicranoloma reflexum*

Peak	Retention time	Compound name	Areal range (%)
1	7.730	Cyclohexene,3,5,5-timethyl-(CAS)	3.97
2	13.035	Phenol	13.035
3	14.408	Pentan-1,3-Dioldiisobutyrate,2,2,4-Trimethyl	67.78
4	15.798	Heptadecane	0.64
5	15.907	Allyldimethyl(prop-1-ynyl)silane	3.77
6	17.011	1-Octadecene	2.02
7	17.074	1-Octadecene	1.00
8	17.675	2-Pentadecanone,6,10,14-trimethyl-	1.68
9	18.242	Nonadecane	0.92
10	18.545	4-(91Z)-N-hydroxythanimidoyl)2-methylpyridazin-3(2H)-one	0.70
11	19.100	1,2-Benzenedicarboxylic acid, dibutyl ester	1.77
12	19.260	1-Octadecene	3.81
13	21.194	9-Tricosane	1.29
14	27.483	11-Octadecenoic acid	7.81
15	28.020	n-Propyl 9-Octadcenoate	1.41
16	28.267	Erucic acid	0.29

Tabel 4. Chemical compounds from methanol extract of *Dicranoloma reflexum*

Peak	Retention time	Compound name	Areal range (%)
1	5.899	Cycloheptane	0.50
2	8.411	4-Fluorothiophenol	0.06
3	8.594	Azulene	0.14
4	8.680	Naphthalene	0.07
5	8.835	Naphthalene	0.12
6	10.632	1,3-Cyclopentanedione,4-hydroxy-2methyl	0.32
7	11.284	Hexenoic acid	0.15
8	11.770	3-Chloropropionic acid	0.14
9	12.703	2-Furanmethanol	0.45
10	12.869	2,5-Difluorobenzenoic acid	0.71
11	13.023	Heptadecanoic acid	0.21
12	13.224	Phenol	0.66
13	13.733	2(4H)-Benzofuranone,5,6,7,71-tetrahydro-4,4,7a-trimethyl	0.30
14	14.236	2-Tetradecene	0.94
15	14.351	Propanoic acid	2.60
16	14.946	8-(N-(Etfyl) amino)-5,6-dimethoxyquinoline	0.52
17	15.552	Cyclotetradecane	0.58
18	15.781	Cyclopentadecane	0.45
19	15.896	Pentanoic acid	2.01
20	16.096	n-Tetradecane	0.46
21	16.193	1-Tetradecane	0.22
22	16.376	2,6-Diisopropylnaphthalene	1.44
23	16.720	1-Octadecene	0.17

Continued of Table 4

Peak	Retention time	Compound name	Areal range (%)
24	17.000	1-Octadecene	6.32
25	17.561	Cyclohexanone	0.26
26	17.670	2-pentadecanone	2.99
27	17.830	4-((1Z)-(N-Hydroxyethanimydoyl)-2-methylpyridazine-3-(2H)-one	0.31
28	18.047	9-Octadecenoic acid	0.21
29	18.230	2-Hexyldecanol	0.23
30	18.476	11-Octadecenoic acid	0.32
31	18.579	Hexadecenoic acid	5.27
32	18.814	Methyl-3-(3,5-Diterbutyl-4-Hydroxyphenyl)Propionate	2.75
33	18.997	1-Octadecene	0.51
34	18.249	1-Octadecene	4.26
35	18.798	9-Octadecanal	0.21
36	20.16	9-Octadecenoic acid	0.26
37	20.262	Tert-Hexadenanethiol	0.40
38	20.405	9-Octadecenoic acid	2.24
39	20.599	10-Octadecenoic acid	1.07
40	20.977	9-Octadecenoic acid	1.10
41	21.194	1-Heptacosanol	1.29
42	22.430	Thiosulfuric acid	0.24
43	22.739	9-Octadecenoic acid	0.23
44	22.934	9-Octadecenoic acid	0.33
45	26.762	9-Octadecenoic acid	11.06
46	27.586	9-Octadecenoic acid	15.03
47	27.935	9-Octadecenoic acid	5.71
48	28.576	Elaidic acid	10.92
49	28.679	Elaidic acid	1.15
50	29.022	9-Tricosane, (Z)-	3.83
51	29.176	Elaidic acid	1.95
52	29.400	9-Octadecenoic acid	3.19
53	29.680	Elaidic acid	1.35
54	29.926	Elaidic acid	0.64
55	30.029	Elaidic acid	0.24
56	30.098	9-Octadecenoic acid	0.29
57	30.218	9-Tricosane, (Z)-	0.27
58	30.338	9-Octadecenoic acid	0.49

Table 5. Chemical compounds from n-hexane extract of *Dicranella coarctata*

Peak	Retention time	Compound name	Areal range (%)
1	8.12	Thiosulfuric acid	42.52
2	8.15	Tetrahydroxycyclopentadienone	2.72
3	8.26	9-Octadecenoic acid	12.83
4	9.79	Cyclopentane	18.43
5	10.85	9-Octadecenoic acid	23.50

Table 6. Chemical compounds from acetic ethyl extract of *Dicranella coarctata*

Peak	Retention time	Compound name	Areal range (%)
1	2.26	Cyclotetrasiloxane	0.74
2	3.10	2-hexanamine, 4 methyl	0.18
3	3.88	2-hexanamine, 4 methyl	0.18
4	4.13	Naphthalene	0.32
5	5.67	Benzeneethanamine	0.21
6	5.78	Benzeneethanamine	0.23
7	6.42	Benzeneethanamine	0.57
8	6.76	2,4(1H,3G=H)-Pyrimidinedione	0.80
9	6.92	Metraraminol bitartrate	0.92
10	7.30	o-hydroxybiphenyl	2.74
11	7.55	(cis)-2-nonadecene	1.60
12	7.65	Tetrahydroxycyclopentadienone	0.91
13	8.02	2Hydroxymino-N-(P-methoxyphen)	3.25
14	8.18	2Hydroxymino-N-(P-methoxyphen)	2.33
15	8.42	Hexadecanoic acid	3.58
16	8.59	Cyclophenyl 2-Methylenebutanyl	3.94
17	8.80	2Hydroxymino-N-(P-methoxyphen)	1.63
18	8.97	Hydroxymethapyrilene	4.03
19	9.29	E-15-Heptadecenal	8.61
20	9.61	1-Hentetracontanol	3.28
21	9.79	1,2-Cyclohexadediol	2.61
22	9.90	14-BETA-H-Pregna	2.89
23	10.07	Trans pinene	3.62
24	10.30	Citronellyl valerat	3.56
25	10.85	Cyclohexane	3.42
26	11.65	Hexadecanoic acid	7.21
27	11.86	1-Pentadecene	3.85
28	12.11	(trans)-2-nonadecene	3.47
29	13.85	17-Pentatriacontene	2.37
30	14.02	4-Hexenoic acid	1.45
31	14.19	4-Hexenoic acid	1.80
32	14.34	17-Pentatriacontene	2.10
33	14.62	17-Pentatriacontene	4.46
34	14.97	17-Pentatriacontene	1.86
35	15.40	9-Octadecenal	5.15
36	16.03	1-Octadecene	5.05
37	16.45	1-Eiocosene	4.58
38	17.59	Dimer of Coleon F	1.94

Table 7. Chemical compounds from methanol extract of *Dicranella coarctata*

Peak	Retention time	Compound name	Areal range (%)
1	2.44	1,2,3,4-Tetrahydroxybutane	0.88
2	2.56	Pentanal	1.24
3	2.81	N,N'-Dimethylpiperazine	1.51
4	3.13	Cyclopentanone	6.34
5	3.84	Propylamine	4.28
6	4.13	Methyl 2-(phenylsulfonyl)-5-deu	0.90
7	4.55	2-Furancarboxaldehyde,5-(hydro	0.78
8	5.18	N-(2-methoxycarbonylethylidene)	1.61
9	5.37	(E)-4-chloro-2,3-dimethyl	1.04
10	6.20	Isothiazole	6.93
11	6.82	6,6 Dideutero-nonen-1-ol	22.92
12	7.40	Nonanoic acid	6.47
13	8.05	Benzene,1-methyl-2-(2-propenyl	5.85
14	8.36	1H-indene,2,3-dihydro-	5.83
15	8.63	Undecanal	1.57
16	8.69	Hexadecanoic acid	2.42
17	9.80	Neophytadiene	5.73
18	10.08	1-formyl-2,2,6-trimethyl-3,(3-m	1.16
19	10.30	1,8-Nonadiene,2,8-dimethyl-	2.23
20	10.86	14-Beta-H-Pregna	0.89
21	11.26	Methyl-3-(3,5-Diterbutyl-4-Hyd	0.61
22	11.66	n-Hexadecanoic acid	13.62
23	15.48	Cyclopropaneoctanal	5.19

Based on GC-MS analysis, n-hexane extract of *Dicranoloma reflexum* was found to consist 61 peaks, indicating that it contained 61 compounds. Main compound found was 1-octadecene with areal total 12.53% (Table 2). Acetic ethyl extract of *Dicranoloma reflexum* contained 16 compounds, the main compound was phenol with areal range 13.035% (Table 3). Methanol extract of *Dicranoloma reflexum* contained 58 compounds. Main compound found was 9-octadecanoic acid with areal range of 15.03% (Table 4).

1-octadecene was a hydrocarbon compound usually found from *Moringa oleifera*. This compound possessed anti-oxidant, anti-bacterial, and anti-fungal properties towards *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Penicillium aurantio*, *Penicillium griceum*, *Penicillium expansum*, *Penicillium digitatum*, and *Aspergillus niger* (Maruffo *et al.,* 2013). N-hexane extract of *Prunus dome* was also found to contain 1-octadec 91 This compound had anti-bacterial activity towards *Salmonella* and anti-fungal towards *Microsporum canis*. In addition, this compound also had potential as anti-oxidant. Other compound found was phenol with chemical formula of C_6H_5OH and possessed hydroxyl group bound to phenyl ring on its structure. Cowan (1999) reported that phenol compound without hydroxyl group had higher anti-bacterial activity caused of higher affinity towards microbes plasma membrane. Qoumarin and quercetin were phenolic compounds with anti-bacterial activity

towards *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium*, and *Salmonella infantis* (Nitiema *et al*., 2012).

Another compound identified was 9-octadecanoic acid with chemical formula $C_{19}H_36O_2$ and possessed anti-oxidative and anti-carcinogenic biological activities (Syeda et al., 2011 and Hema *et al*., 2011). N-hexadecanoic acid had chemical formula of $C_{16}H_{32}$ and biological properties as anti-fungi, anti-oxidant, hypocholesterolemic, nematicide, and anti-androgenic flavor, haemolitic-5-alpha reductase inhibitor, anti-microbe, and anti-malaria (Hema *et al*., 2011 and Pietro *et al*., 2010). The ethanol extract of leaves of *Indigofera suffruticosa* containing n-hexadecanoic acid (9.83%) and 9-octadecanoic acid (10.21%) (Vijisaral and Arumugam, 2014). The same compound is also found in the ethanol extract of beans, respectively 7.02% and 0.89%. Markkas and Govindharajala (2015) showed that the methanol extract of *Mollugo cerviana* containing hexadecanoic acid and 9-octadecanoic acid. Harkati *et al.* (2012) reported that *Scorzonera undulata* containing hexadecanoic acid (42.2%) and 9-octadecanoic acid (7.7%). Aja *et al*. (2014) reported that the methanol extract of *Moringa oleifera* contains 9-octadecanoic acid is more higher (20.8%) than hexadecanoic acid (1.31%)

Another one compound found was thiosulphuric acid with chemical formula of $C_{20}H_7NO_3S_2$ and 157 molecular weigh. This compound was also identified from methanol extract of *Clerodendron phlomidis* ((Lakshmi dan Viji Stella Bai, 2015). Kumar et al (2011) reported that methanol and acetone extract of *Spirulina platensis* also contained E-15 Heptadecanal. This compound possessed anti-bacterial properties against *Staphylococcus aureus* and *Salmonella typhimurium*.

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