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GCMS analysis of bioactive compounds in n-hexane, ethyl acetate, and methanol extract of *Piper betle* L. var. nigra.

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Black betel (*Piper betle* L. var nigra) is one of endemic Piperaceae species in Indonesia. So far, few information was available about its bioactive compounds content. Thus, this study was designed to identify bioactive compounds contained in n-hexane, ethyl acetate, and methanol extract of *P. betle* L. var. Nigra leaves. Symplicia of black betel was extracted from the leaves using three types of solvent, before being analyzed of its compound types and amounts using Gas Chromatography Mass Spectra (GCMS). Result showed that three main components found in n-hexane extract were 1-pentene (22.57%), 3-butenoic acid (18.29%), and furan (16.83%). Three main components of ethyl acetate extract were acetic acid (58.35%), methylamine (21.5%), and acetic acid (15.29%). In the other hand, methanol extract contained three main components of methylamine (85.08%), acetic acid (4.78%), and pyridine (0.21%).

Keywords: ***Piper betle*** **L. var Nigra, GCMS**

INTRODUCTION

Piper is the largest genus comprising the Piperaceae family, consisting of 1000-2000 species distributed in tropical and subtropical area (Santos et al., 2001; Guimarales et al., 2006; Ghosh et al., 2014). Plants in Piperaceae contained various secondary metabolites, such as monoterpene, diterpene, phenylpropanoids, sesquiterpenes, alkaloids,amides, lignans, neolignans, steroids, piperolides, flavones, chalcones, and dihydrochalcones (Santos et al., 2014; Roser et al., 2001; Assis et al., 2013).

Previous study on secondary metabolites of *Piper hymenophyllum*collected from Nallamala forest, Eastern Ghats, India resulted in compounds with bioactivity able to inhibit growth of *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* (Ratnam et al., 2015). Ethnopharmacological study of *Piper hispidum* from Central America and South America resulted in its potential as antiseptic and medicine for skin ulcers (Flores et al., 2008). Other *Piper* species, *Piper glabratum*, contained benzoic acid which could function as antiparasite, antiseptic, and medicine for skin ulcers (Flores et al., 2008; Braga et al., 2001; Svetaz et al., 2010). Meanwhile methanol extract of Piper nigrum collected from Karayar forest, Tamil-Nadu, was found to have larvicide effect against Aedes aegepty larvae (Escaline et al., 2015).

Secondary metabolites derived from *Piper longum*, *Piper betle*, and *Piper cubeba* had bioactivity as insecticide against fleas and mosquitos (Lee, 2005). Leaf extract of *Piper betle* was found to have high level of antioxidant activity (Battacharya et al., 2005), and potential to treat hypertension, heart, and respiratory disease (Nalina and Rahim, 2007). In the other hand,

essential oil extracted from *Piper cubataonum* possessed anti-proliferative activity (Santos et al., 2014).

Black betel is one member of Piperaceae family known to have a number of benefits and prospects for further development. So far, few information is available on the bioactive compounds content of this plant. Thus, this study was aimed to identify bioactive compounds of n-hexane, ethyl acetate, and methanol extracts of black betel leaf.

MATERIALS AND METHODS

Plant Materials and Extraction

Leaves of *Piper betle* L. var nigra were collected from Flower Market, Kayoon Surabaya. Leaves were first washed and air-dried. Next, black betel leaves were blended into powder. As much as 88.5 g of leaf powder was divided into 3 parts of 29.5 g respectively. Each part was then macerated by dissolving it into either n-hexane, ethyl acetate, or methanol. Maceration was performed for three days, repeated three times. Each step of maceration used 500 ml of solvent, thus total of 1500 ml of respective solvent was used.

GCMS Analysis

Each extract was analyzed for content compound types and amounts using Gas Chromatography Mass Spectra (GCMS) method. Identification of compounds in the extract was performed using GCMS (Agilent 6890 A). As much as 1 μl of each extract sample was injected. GC detector used was MSD Agilent 5973 Inert. GC condition applied was flow acceleration of 1.3 ml/minute, temperature 2800^{0}C, detector AUX temperature 2800^{0}C, MS Source 2300^{0}C, MS Quadrupole 1500^{0}C. 5% Phenyl metal siloxane was used as stationary phase in analysis while helium ultrapure gas was used as mobile phase. Column type was capillary HP 5 with column size of 30 μm x 320 μm x 0.25 μm.

RESULTS

Result of GCMS analysis of black betel leaves n-hexane, ethyl acetate, and methanol extracts was presented in Figure 1, 2, and 3, and Table 1, 2, and 3 respectively. GC analysis of n-hexane extract showed 53 peaks indicated that it contained about 53 compounds. Chromatogram profile of black betel leaves n-hexane extract was presented in Fig.1, while compounds identified from it were listed in Table 1.

Table1. Chemical compounds identified from black betel leaves n-hexaneextract

Peak	RT	Area (%)	Phytocomponent
1	1.11	8.10	Methylamine
2	1.19	0.02	Pentane
3	1.24	0.06	Butane
4	1.30	16.83	Furan
5	1.33	18.29	3-Butenoic acid
6	1.36	16.02	2-Methylpropenoic acid
7	1.37	14.13	1-Hexene
8	1.47	22.57	1-Pentene
9	1.56	0.06	Pentane
10	1.61	3.21	Cyclohexane
11	1.65	0.09	3-methylhexane
12	1.71	0.02	Tridecane
13	1.73	0.01	Cyclopentane
14	1.79	0.02	Heptane
15	1.88	0.00	Furan
16	1.97	0.02	Cyclohexane
17	2.02	0.02	2-Pentanol
18	2.21	0.02	3-Pentanol
19	2.39	0.01	Methylbenzene
20	2.46	0.01	2-Penten
21	2.60	0.01	3-Hexanone
22	2.66	0.08	Cyclopentanol
23	2.72	0.02	3-Hexanol
24	2.79	0.02	2-Hexanol
25	3.56	0.02	Cyclopentanol
26	3.66	0.01	Cyclopentanone
27	4.78	0.01	1-Butanamine
28	5.50	0.01	Silane
29	5.99	0.01	3-pentanol
30	6.15	0.02	1,3-Cyclohexadiene
31	7.16	0.01	3-butenyl pentyl
32	7.29	0.02	Pyridine
33	7.73	0.01	Propane
34	7.95	0.00	3-Butenamide
35	8.39	0.00	Benzene
36	8.55	0.01	Z-Ocimene
37	9.05	0.00	Methyl ester
38	9.21	0.00	1,3-Dioxolane
39	9.93	0.00	Dipropylamine
40	10.04	0.02	Myrcene
41	10.38	0.00	1-Methylamino-propylamine
42	11.78	0.00	3-butenamide
43	13.20	0.00	Oxetane
44	13.28	0.00	Piperazine
45	14.99	0.01	1,3-Cyclopentadiene
46	15.98	0.00	1-Bromoadamantane
47	16.20	0.00	Benzoin
48	16.73	0.00	Homarine
49	17.04	0.00	Benzyl alcohol
50	20.68	0.03	Tridecanoic acid
51	22.38	0.04	Cyclohexane
52	22.45	0.07	Cyclohexene
53	22.72	0.01	2-Aminoacetamide hydrochloride

Three main components contained were 1-

pentene (22.57%), 3-butenoic acid (18.29%), and furan (16.83%) (Table 1).Based on GC analysis, ethyl acetate extract of black betel leaves resulted in 36 peaks, indicating that about 36 chemical compounds was contained in it. Chromatogram profile of this extract was presented in Fig 2, while compounds were listed in Table 2. This extract contained three main components of acetic acid (58.35%), methylamine (21.15%), and acetic acid (15.29%) (Table 2).

Table 2. Chemical compounds identified from black betel leaves ethyl acetate extract

Peak	RT	Area (%)	Phyto component
1	1.11	21.15	Methylamine-D2
2	1.16	1.84	Ethanol
3	1.24	0.05	Acetic acid
4	1.29	0.10	Pentane
5	1.32	0.15	Pentane
6	1.36	0.16	Hexane
7	1.42	58.35	Acetic acid
8	1.47	15.29	Acetic acid
9	1.50	2.05	Acetic acid
10	1.61	0.06	Acetic acid
11	1.78	0.00	Ethanol
12	1.88	0.42	Propanoic acid
13	2.11	0.02	2-Pentanone
14	4.01	0.01	Aethylbenzol
15	4.21	0.03	p-Xylene
16	4.89	0.01	p-Xylene
17	6.15	0.06	Cyclopropene
18	7.29	0.07	Pyridine
19	1.74	0.00	Methylester
20	8.56	0.01	1,4-Pentadiene
21	10.04	0.02	Pyridine
22	12.48	0.00	Acetic acid
23	13.20	0.00	1-Propanaminium
24	14.40	0.00	Methyl L-alaninate
25	14.59	0.01	Urea
26	14.99	0.03	7-Methylenenorcarane
27	15.12	0.00	Amphetamine
28	15.78	0.00	Benzeneethanol
29	15.98	0.02	Pyridine
30	16.09	0.00	1-Propanol
31	16.20	0.01	Hydroxylamine
32	16.74	0.01	Cyclopropane
33	17.04	0.01	Delta6-bicyclo
34	19.78	0.01	5-hydroxylinalol
35	20.04	0.00	Propanamide
36	20.23	0.01	2,4-Hexadiene

Based on GC analysis, from methanol extract of black betel leaves, 58 peaks were able to be identified, representing about 58 types of chemical compound. Chromatogram profile of methanol extract was presented in Fig. 3, while compounds were listed in Table 3. Three main components identified form this extract including methylamine (85.08%), acetic acid (4.78%), and pyridine (0.21%) (Table 3).

Table 3. Chemical compounds identified from black betel leaves methanol extract

Peak	RT	Area (%)	Phytocomponent
1	1.03	0.13	Carbonic acid
2	1,05	8.52	Methyloamina
3	1.21	85.08	Methylamine
4	1.25	0.19	Ethyl alcohol
5	1.29	0.05	Ethylamine
6	1.35	0.13	Boric acid
7	1.39	4.78	Acetic acid
8	1.54	0.03	Formic acid
9	1.73	0.01	Semicarbazide
10	1.86	0.01	Diisopropylamine
11	1.97	0.00	Acetic acid
12	2.29	0.03	2,2-Dimethoxybutane
13	2.35	0.04	2,2-Dimethoxybutane
14	6.13	0.16	2-Picoline
15	7.29	0.21	Pyridine
16	7.76	0.01	Myrcene
17	7.94	0.01	Undecane
18	8.57	0.03	Cyclobutane
19	10.05	0.06	Myrcene
20	10.48	0.03	Hydrazine
21	13.20	0.01	2-Dodecanone
22	13.86	0.01	1-Methylene-2-vinylcyclopentane
23	14.40	0.02	1,2-Dimethylbenzene
24	14.59	0.02	Cyclopentene
25	14.91	0.00	Ethyl oxamate
26	14.99	0.08	3-Methylene
27	15.11	0.01	Propanenitrile
28	15.43	0.01	7-Methylene
29	15.71	0.01	7-Methylenenorcarane
30	15.78	0.01	p-Xylene
31	15.94	0.01	1H-Pyrrole
32	15.98	0.04	Pyridine
33	16.09	0.01	1-Hydroxymethyl-2-methyl-4-cyclohexene
34	16.21	0.02	para-Xylene
35	16.29	0.01	Cycloprophylcarbinol
36	16.38	0.01	Pyridine
37	16.67	0.01	Urea
38	16.73	0.03	Myrcene
39	17.04	0.03	Cyclopentane
40	17.83	0.01	Tetrahydro-
41	18.91	0.01	Propanedioic acid
42	19.78	0.01	2,7-Octadiene
43	19.86	0.00	3-Piperidinol
44	20.23	0.01	Piperazine
45	20.63	0.01	Propanamide
46	20.68	0.01	Diazene
47	21.02	0.01	Tetradecane
48	21.64	0.02	Methylimidazole
49	21.76	0.00	Carbamic acid
50	22.27	0.01	Dispermine
51	22.39	0.02	1,2-Epoxy-1-vinylcyclododecene
52	22.45	0.02	1,3-Cyclooctadiene
53	22.59	0.01	Ho-trienol
54	22.78	0.00	Cyclopentane
55	22.84	0.00	4-Allylimidazole
56	23.32	0.00	Dioxolan
57	23.33	0.00	Formamide
58	25.58	0.01	Propanamide

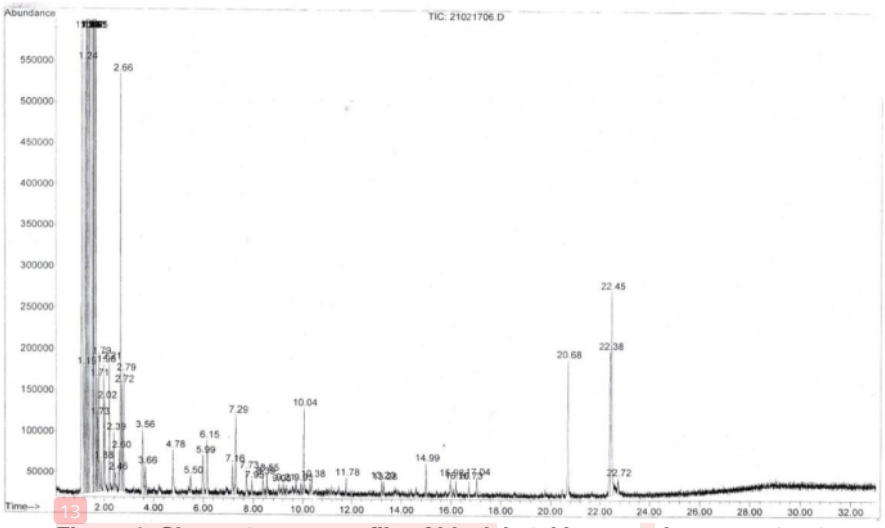

Figure 1. Chromatogram profile of black betel leaves n-hexane extract

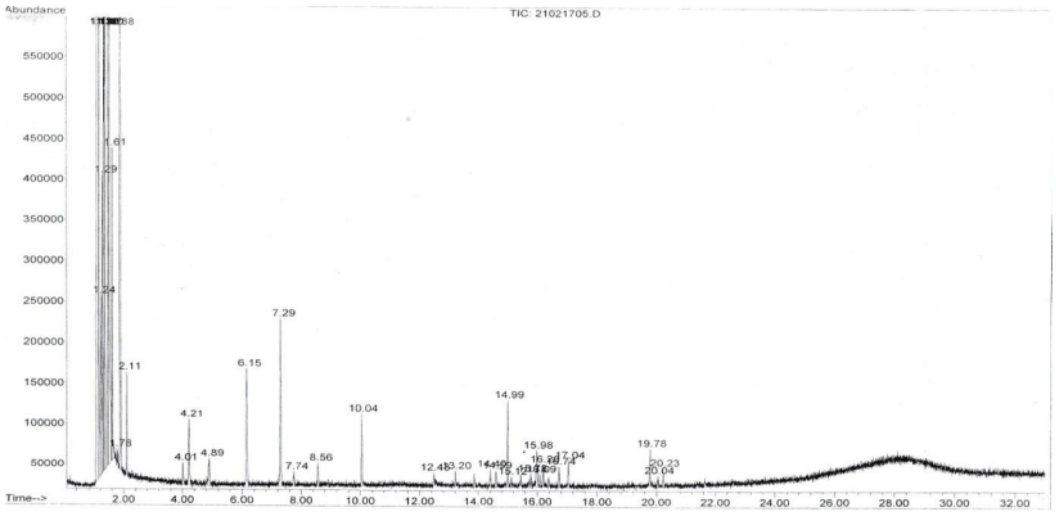

Figure 2. Chromatogram profile of black betel leaves ethyl acetate extract

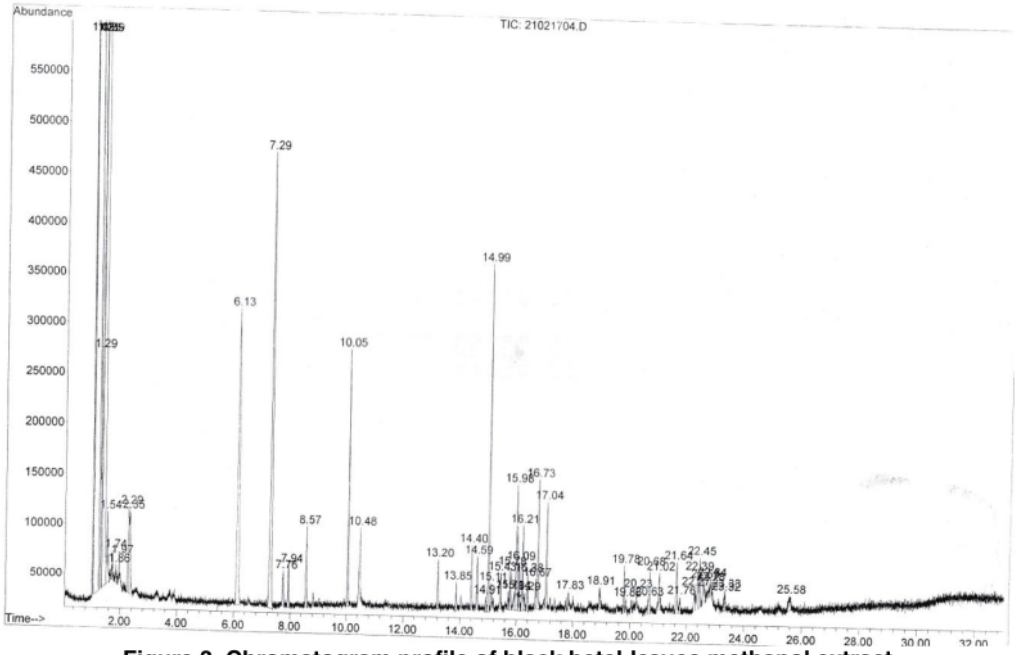

Figure 3. Chromatogram profile of black betel leaves methanol extract

DISCUSSION

Based on result of analysis, compounds contained in the three extract of Piper betle were different from compounds found on other species of Piperaceae family.*Piper longum* contained piplartine (first amide) that was found to possess various pharmacological activities (Bezzera et al., 2013). Amide isolated from *Piper* species including piperine, piperiline, pellitorine, pipercide and guinensine which had insecticide property (Kwute et al., 2013). *Piper arboretum* and *Piper divaricatum* contained safrole, while *Piper caldense*, *Piper marginatum*, *Piper tuberculatum*, *Piper aduncum* were found to contain dillapiole (Paz et al., 2017).

N-hexane extract of *Piper betle* L. var nigra contained 1-pentene, 3-butenoic acid, and furan. This was different from n-hexane extract of *Piper betle* leaf stalk collected from Kolkata, India. The extract was found to contain 3 main components of dodecanoic acid, hexadecanoic acid, and tetradecanoic acid (Dwivedi et al., 2010). 1-pentene belong to alkene or olefin group, which is unsaturated hydrocarbon and volatile compounds (Oleivera et al., 2009). 1-pentenehad also been found from *Ficuscarica*leaf extract (Oleivera et al., 2010). This volatile compound had several biological functions. Volatile compounds isolated from *Mindium laevigatum* had antioxidative, anti-microbial, and cytotoxic properties (Ebrahimabadi et al., 2016). Other plants containing volatile compounds which could function as antioxidant including *Chamerion angustifolium*(Kaskoniene et al., 2016); *Myrtus communis* (Serreli et al., 2017); *bee pollen* (Fatrcova-Sramkova et al., 2015).

In addition, some volatile compounds had also been found to have antimicrobial property (Singh, 2011; Fialho et al., 2011). Several kind of plants which contained volatile compounds and had antimicrobial property were Muscodor albus (Strobel et al., 2001); Mansoa difficilis (Guilhon et al., 2012); *Pinusdensiflora* (Park and Lee, 2012). Other compound contained in n-hexane extract was 3-butenoic acid. Butenoic acid possessed antiproliferative activity (Todorovic et al., 2013). Another compound extracted was furan, which was an organic compound, belong to aromatic heterocyclic which have ring structure with 1 O atom and 4 C atoms. Furan was colorless, volatile, and toxic (Anupam et al., 2011). Biological function of furan including antibacteria, (Choi, 2008), anticonvulsan (Abdel-Wahab, 2009), antinociceptive (Abdel-Wahab, 2009), antifungi (Abdel Aziz, 2009), antitumor, and antiviral (Galal, 2009).

Compared to other *Piper* species, compounds extracted in previous studies was clearly different

from in this study. *Piper hispidum* extract contained 29.0% monoterpene component, while main component of *Piper cernuum* were β-elemene (11.6%) and epicubebol (13.1%). The man components of *Piper glabratum* were β-caryophyllene (14.6%) and longiborneol (12.0%).*Piper hispidum* also contained khusimene (12.1%) and γ-cadinene (13.2%) (Assis et al., 2013). Composition of essential oil derived from Piper hymenophyllum consisted of E-phytol (21.87%), dihydroterpineol (17.42%) and α-terpineol (13.93%) (Ratnam et al., 2015).

Acetic acid is one of the simplest form of carboxylic acids, which have antibacterial property against *Pseudomonas aeruginosa*, *Acinetobacterbaumannii*, *Pseudomonas mirabilis*, *Staphylococcus aureus*, and *Klebsiella pneumonia* (Halstead et al., 2015). Meanwhile methylamine is an organic compound with chemical formula of CH_3NH_2. This compound had activity as anti-microbe (Patel et al., 2016; El Wahab, 2012). Methanol extract of *Piper nigrum* leaves was found to have 3 main components; thymol (20.77%), elemene (10.42%), and octadecanoic acid (6.98%) (Escaline et al., 2015).

One of the compounds contained in black betel methanol extract was pyridine. Pyridine is a heterocyclic compound with shape of simple aromatic ring and chemical formula of C_5H_5N. This compound was found to have important role in drug production. Pyridine had a wide variety of biological activity, such as anti-inflammatory (Pettus et al., 2013), analgesic (Sladowska, 1998), anti hipersensitive (Hoffmann, 1983), antihistaminic (Gore, 2008), antidiabetic (Mylari, 1998), anticancer (Kaizerman, 2010.), antimicrobial (Elassar, 2008), and central nervous system activities (Mitchinson et al., 2006)

CONCLUSION

Main components contained in n-hexane of *Piper betle* L. var Nigra were 1-pentene (22.57%), 3-butenoic acid (18.29%), and furan (16.83%). Ethyl acetate extract had three main components of acetic acid (58.35%), methylamine (21.5%), and acetic acid (15.29%). Methanol extract of black betel leaves contained three main components of methylamine (85.08%), acetic acid (4.78%), and pyridine (0.21%).

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

JUN and NMZ designed and performed the experiments and also write the manuscript. LIS designed experiments and reviewed the manuscript. All authors read and approved the final version.

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