



Maderas. Ciencia y tecnología

ISSN: 0717-3644

ISSN: 0718-221X

Universidad del Bío-Bío

Prayitno, T.A.; Widyorini, R.; Lukmandaru, G.  
Chemical variation of five natural extracts by non-polar solvent  
Maderas. Ciencia y tecnología, vol. 23, 2021  
Universidad del Bío-Bío

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# CHEMICAL VARIATION OF FIVE NATURAL EXTRACTS BY NON-POLAR SOLVENT

T.A. Prayitno<sup>1,\*</sup>, R. Widyorini<sup>1</sup>, G. Lukmandaru<sup>1</sup>

## ABSTRACT

Chemical compounds of wood preservation from plants vary and are not known specific to the species. Chemical analysis of plants is responsible to ensure active compound in natural extracts wood treatment. There are many sources of natural extracts found in Indonesia that were explored for wood preservatives chemicals. They are bark of acacia and alstonia, leaves of orthosiphon and azardirachta and Dioscorea tubers. The present study was aimed at investigating the variation of the chemical constituent of natural extracts material of wood preservative through GC-MS analysis. Five natural extract sources were acacia bark (*Acacia spp.*), pulai bark (*Alstonia scholaris*), kumis kucing leaves (*Orthosiphon spp.*), mimba leaves (*Azardirachta indica*), and gadung tubers (*Dioscorea spp.*). Two non-polar solvents, i.e., n-hexane and petroleum ether were used for five natural source extractions following ASTM soxhlet extraction. The research showed that triterpene and fatty acid derivatives were the major compounds present in five natural extracts. They were lupeol; 7,22-Ergostadienone; Lup-20(29)-en-3-one; Lup-20(29)-en-3-ol, acetate, (3.β.)-; urs-12-en-3-one; ethanol,2,2-diethoxy-; stigmasta-5,22-dien-3-ol, acetate,(3.β.)-; 5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl-; linoleic acid; naphthalene, 1-methyl-. These compounds have been assigned as the possibly responsible to against termites or fungi.

**Keywords:** *Acacia spp.*, *Alstonia scholaris*, *Azardirachta indica*, chemical compounds, *Dioscorea spp.*, *Orthosiphon spp.*, wood preservation.

## INTRODUCTION

The new paradigm of wood preservation is related to the environmental safety. The use of synthetic preservative has long resulted in environs losses. Several synthetic chemicals have been banned recently for wood protection uses by the U.S. Environmental Protection Agency (EPA). Therefore, the search for natural, safe, friendly, and none non-polluting bioactive chemical compounds from plants as an alternative to synthetic preservative becomes essential (Hu *et al.* 2015).

Plant extract contains chemical constituent which has high potency for wood preservatives. Sources of plant extract are found many type such as leaves, barks, wood, tubers, seeds etc. The type of solvent to be used for plant extraction are found many type such as cold water, hot water, ethanol, benzene, ether and n-hexane. Many research have been conducted on natural plant extracts. Extractives of white mulberry heartwood were contained higher hydrocarbons, fatty acids, sterols, and phenols that were toxic against decay termites (Se Golpayegani *et al.* 2014). Fatty acid extracts from *Acacia mollissima*, *Schinopsis lorentzii* and *Pinus brutia* bark were indicated having an insecticide characteristic (Sen *et al.* 2017). The type of solvent has affected the extraction results and affected the resistance of termites (Kadir *et al.* 2015, Syofuna *et al.* 2012). N-hexane fraction from *T. officinale* leaves was detected and reported as antioxidant and antimicrobial (Ivanov *et al.* 2017). Prayitno *et al.* (2017) concluded that natural extracts have influenced the wood adhesion.

*Acacia spp.* belongs to the family Leguminosae and subfamily Mimosoideae. *Acacia* has been planted in more than eighty countries around the world and becomes prominent in tropical and subtropical regions of Asia, Africa, Central, and South America (Old *et al.* 2002). Extract of three species from *Acacia* leaf has po-

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Received: 12.01.2018 Accepted: 17.08.2020

tential in the development of natural food preservatives (Cock 2017). The heartwood of *A. confusa* possesses excellent decay resistance properties (Chen *et al.* 2013). *A. mollissima* bark extract could be used as alternative wood preservatives against *Reticulitermes grassei* termite (Tascioglu *et al.* 2012).

*Orthosiphon* plants are herbaceous shrubs from Lamiaceae family. Singh *et al.* (2015) reviewed that the plant has photochemical, pharmacological, and toxicological properties. *Orthosiphon* extracted with ethanol showed the highest efficiency to be anti-termite with mortality of termite 65% (Aziz *et al.* 2013).

*Alstonia scholaris* is tropical tree belongs to Apocynaceae family. *A. scholaris* stem bark has anti-inflammatory activity on methanolic extract (Subraya *et al.* 2012). Extract of *A. scholaris* leaf has exhibited the activity of causing mortality in termite (Ahmed *et al.* 2011). Padding application of *A. scholaris* extract on teakwood block test gives highest adhesion strength (Prayitno and Widyorini 2016).

*Azadirachta indica* is an evergreen tree native to Southeast Asia. The tree belongs to Meliaceae family and has commercial name neem tree. It has been used medicinally for centuries because of its bioactive compounds. *A. indica* bark extracts could be employed to control fungal stains and molds on easily attacked freshly harvested logs (Antawi-Boasiako and Damoah 2010). Neem seed extract (azadirachtin) offer considerable protection to wood (Ssemaganda *et al.* 2011). Combination neem extract and chlorpyrifos could be efficiently utilized for termite control (Sotande *et al.* 2011).

*Dioscorea hipsida* is a member of Dioscoreaceae family. The plant produces tubers that contain toxic alkaloid (Dioscorides). The dioscorine within the tuber's starch would protect the coated materials from rotting by bacteria or fungi activity (Azman *et al.* 2015). Ragasa *et al.* (2016) reported the dichloromethane extracts of *Dioscorea luzonensis* Schauer were alkyl trans-ferulates ;  $\beta$ -sitosterol and ursolic acid. They were polyphenolic compound group. Kumar *et al.* (2017) reviewed the ethnopharmacological values and traditional use of *Dioscorea spp.* Savi *et al.* (2018) showed the potential antioxidant activity of the polysaccharide extracted from *Dioscorea bulbifera*. They suggested to analyze it by DPPH, ABTS, FRAP, OH radical removal, H<sub>2</sub>O<sub>2</sub> removal and reducing power.

Chemical analysis of natural wood preservation is responsible for the development of wood preservative material. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, etc. (Karuppasamy *et al.* 2012). The combination of the best separation technique (GC) with the best identification technique (MS) made GC-MS an ideal method for qualitative analysis for volatile and semi-volatile bioactive compounds (Grover and Patni 2013). The objective of the present study was to know the variation of the chemical constituent of five natural extract sources potential for preservative material. Furthermore, the isolated matters produced by plants can be used as active principles in natural extracts wood treatment.

## MATERIALS AND METHODS

### Materials

Natural preservatives plant sources namely, akasia bark (*Acacia spp.*), pulai bark (*Alstonia scholaris*), kumis kucing leaves (*Orthosiphon spp.*), mimba leaves (*Azardiachta indica*), and gadung tubers (*Dioscorea spp.*) were collected from Yogyakarta, Indonesia. The site has a temperature range of 26-30°C and humidity range of 90%. Two non-polar solvents were n-hexane and petroleum ether.

### Extraction procedure

Five plant materials were air dried, grinded, and sieved by 40 mesh. Each dry plant sample was extracted with n-hexane and petroleum ether according to ASTM D1108-96 (2007). Two grams plant sample was placed in soxhlet extraction apparatus with 300 cc of solvent.

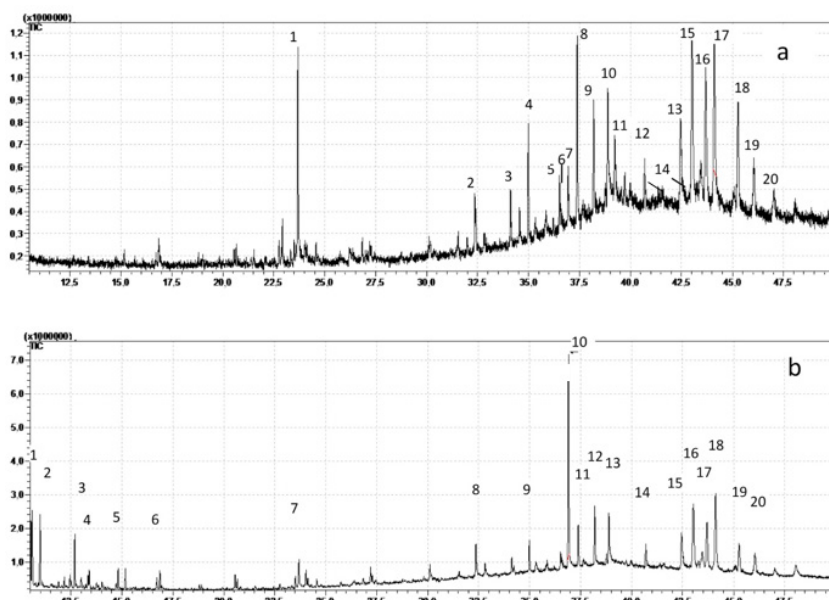
## GC/ MS analysis

Separation and identification were performed on a GC/MS QP 2010 (Shimadzu) with an RTx-5MS column, 30 m (Restek Corp.) Helium was used as the carrier gas. The injection of 1  $\mu$ l (1 mg/ml) was made in a split less mode using electron impact ionization (EI, 70 eV). The injector and detector temperature were 2500°C. The program started at 500°C for 5 minutes, followed by an increase of 40°C/min until 1200°C and remaining at this temperature for 1 minutes. Then, the temperature was set to 3000°C at 60°C/min and maintained for 15 minutes. Compounds were identified by comparison of the mass spectra with those in the NIST 147 libraries. The extractive composition was determined by peak area integration.

## RESULTS AND DISCUSSION

### Chemical component of five natural extracts

In the present study, the constituents of five plant extracts were successively extracted by non-polar organic solvents of n-hexane and petroleum ether. The photochemical screening of *Acacia spp.* showed twenty peaks (Figure 1). The acacia bark extract with n-hexane showed GC peaks at retention times of 23,68 min and 32,36-46,07 min. The acacia bark extract with petroleum ether showed GC peaks at retention times of 10,59-16,86 min, 23,68 min, and 34,98-46,04 min. The compounds separated at 10,5-16,86 min in petroleum ether extract contained mainly hydrocarbon derivatives.



**Figure 1:** Chromatogram of organic compounds obtain from *Acacia spp.* bark in (a) n-hexane extract (b) petroleum ether extract.

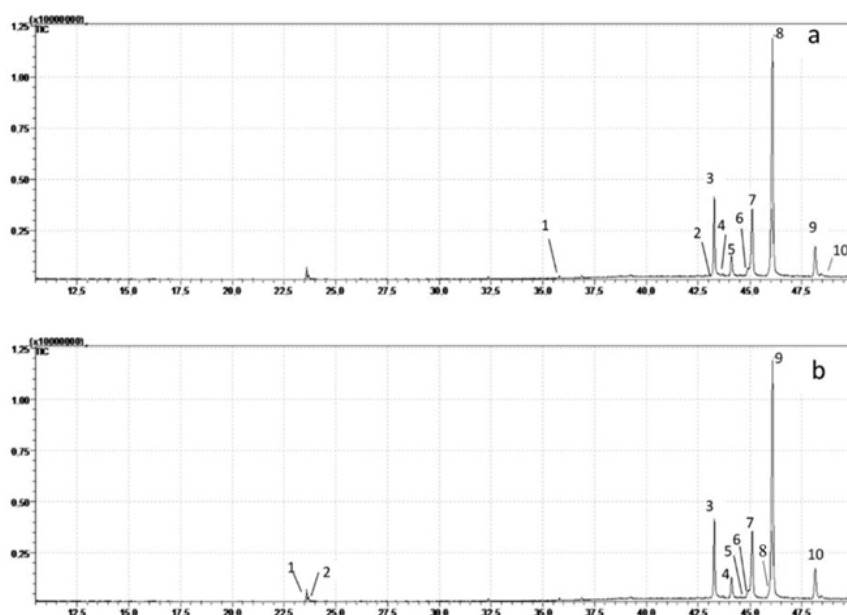
(a) n-hexane extract: Dibutyl phthalate(23,68min)<sup>1</sup>, 1,2-Benzenedicarboxylic acid, diisooctyl ester(32,36min)<sup>2</sup>, Tetracosane, 11-decyl-(34,11min)<sup>3</sup>, 22-Tricosenoic acid(34,98min)<sup>4</sup>, Nonacosane(36,5min)<sup>5</sup>, Pentadecanal(36,58min)<sup>6</sup>, Hexacosanoic acid, methyl ester(36,93min)<sup>7</sup>, Erucic acid(37,37min)<sup>8</sup>, Octadecanal (38,18min)<sup>9</sup>, 13-Tetradecen-1-ol acetate (38,88min)<sup>10</sup>, Octacosanoic acid, methyl ester(39,22min)<sup>11</sup>, Octadecanal(40,69min)<sup>12</sup>, Chondrillasterol(42,46min)<sup>13</sup>, 1-Methyl-3,6-diazahomoadamantan-9-one thiosemicarbazone (42,61min)<sup>14</sup>, 7,22-Ergostadienone(43,01 min)<sup>15</sup>, Lup-20(29)-en-3-one(43,68min)<sup>16</sup>, Lupeol(44,11min)<sup>17</sup>, Germanicol(45,27min)<sup>18</sup>, Lupeol(46,06min)<sup>19</sup>, 7-Hydroxy-6-methyl-oct-3-enoic acid(46,07min)<sup>20</sup>

(b) petroleum ether extract : Naphthalene, 1-methyl-(10,59min)<sup>1</sup>, Naphthalene, 1-methyl-(10,98min)<sup>2</sup>, Tetradecane(12,69min)<sup>3</sup>, Naphthalene, 2,6-dimethyl-(13,4min)<sup>4</sup>, Dodecane, 2-methyl-6-propyl-(14,82min)<sup>5</sup>, Octadecane(16,86min)<sup>6</sup>, Dibutyl phthalate(23,68min)<sup>7</sup>, 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester(32,36min)<sup>8</sup>, Erucic acid(34,98min)<sup>9</sup>, Oxirane, 2,2'-((1-methylethylidene) bis(4,1-phenyleneoxymethylene))bis-(36,9min)<sup>10</sup>, 22-Tricosenoic acid(37,37min)<sup>11</sup>, Octadecanal(38,18min)<sup>12</sup>, 17-Pentatriacontene(38,88min)<sup>13</sup>, Octadecane, 1-(ethenyl-oxy)-(40,69min)<sup>14</sup>, Chondrillasterol(42,45min)<sup>15</sup>, 7,22-Ergostadienone(43,02min)<sup>16</sup>, Lup-20(29)-en-3-one(43,7min)<sup>17</sup>, Lupeol(44,11min)<sup>18</sup>, Germanicol(45,26min)<sup>19</sup>, 7-Hydroxy-6-methyl-oct-3-enoic acid(46,04min)<sup>20</sup>

The data indicate that chemical component of acacia bark that solved with n-hexane was dominated with lupeol from triterpene derivatives, ergostandienon (7,22-ergostadienone) and germanicol (Figure 1). Another chemical is erucic acid from the fatty acid. *Brassica rapa* contained a high concentration of erucic acid and had toxic properties (Harborne 1973).

Acacia bark contains lupeol in both n-hexane and petroleum ether extract. Lupeol is the highest compound in n-hexane extract (16,52%). Lupeol also found in the stem bark of *Acacia mellifera* and *Acacia visco*, and its antimicrobial and anti-inflammatory activities have been already demonstrated (Mutai *et al.* 2009, Pedemera *et al.* 2010). Lupeol is the pentacyclic triterpenes belonging to the lupane family.

Oxirane, 2,2'-((1-methylethylidene)bis(4,1-phenyleneoxymethylene))bis- is the highest compound of *Acacia* bark in petroleum ether extract. Oxirane is epoxide derivative that has a function as an adhesive (Ramalakshmi and Muthuchelian 2011). A minor compound present in *Acacia spp.* bark such as aromatic carbonyls (1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester). 1,2-Benzenedicarboxylic acid, (2-ethylhexyl) ester have antifungal, antitumor, anti-diabetic, anti-cancer, antioxidant, anti-inflammatory, antimicrobial (Syeda *et al.* 2011).



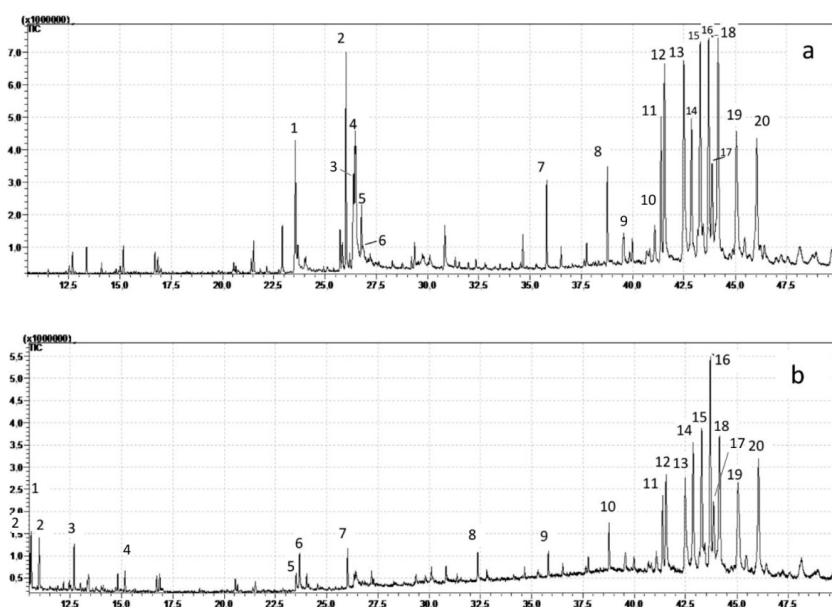
**Figure 2:** Chromatogram of organic compounds obtain from pulai bark in (a) n-hexane extract (b) petroleum ether extract.

(a) n-hexane extract: (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (35,7 min)<sup>1</sup>; 9,19-Cyclolanost-24-en-3-ol, (3.beta.)- (43,1 min)<sup>2</sup>; urs-12-en-3-one (43,3 min)<sup>3</sup>; D-Norandrostane-16-ol, acetate, (5.alpha.,16.beta.)- (43,6 min)<sup>4</sup>; Lupeol (44,1 min)<sup>5</sup>; Lanosta-8,24-dien-3-ol, acetate, (3.beta.)- (44,9 min)<sup>6</sup>; 12-Oleanen-3-yl acetate, (3.alpha.)- (45,1 min)<sup>7</sup>; Lup-20(29)-en-3-ol, acetate, (3.beta.)-; Lup-20(29)-en-3-ol, acetate, (3.beta.)- (46,2 min)<sup>8</sup> (48,2 min)<sup>9</sup> (48,8 min)<sup>10</sup>.

(b) petroleum ether extract: Dibutyl phthalate (23,5)<sup>1</sup>; 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (23,6 min)<sup>2</sup>; urs-12-en-3-one (43,2)<sup>3</sup>; Lupeol (44,1)<sup>4</sup>; Lanosta-8,24-dien-3-ol, acetate, (3.beta.)- (44,8)<sup>5</sup>; Lup-20(29)-en-3-ol, acetate, (3.beta.)- (44,9 min)<sup>6</sup>; urs-12-en-3-one (45,1 min)<sup>7</sup>; trans-Biformene (45,9 min)<sup>8</sup>; 5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl-(46,0 min)<sup>9</sup>; Lup-20(29)-en-3-ol, acetate, (3.beta.)- (48,1 min)<sup>10</sup>.

The GC-MS analysis of pulai bark revealed that triterpene (Lup-20(29)-en-3-ol, acetate, (3.beta.)- (61,76%) is a primary compound of pulai bark in n-hexane extract (Figure 2). This extract also found in *Ficus carica* leaves that showed potent and persistent irritant effects (Saeed and Sabir 2002).

The dominant compound of pulai bark in petroleum ether were 5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl- (56,07%). It was cyclic ethers that used for fragrance agents (Surburg and Panten 2006). Another compound, Lanosta-8, 24-dien-3-ol, acetate, (3.beta.)- have been reported to have an anti-amylase inhibitor, antimicrobial, antidiabetic properties (Arora and Meena 2016).



**Figure 3:** Chromatogram of organic compounds obtain from kumis kucing leaves in (a) n-hexane extract (b) petroleum ether extract.

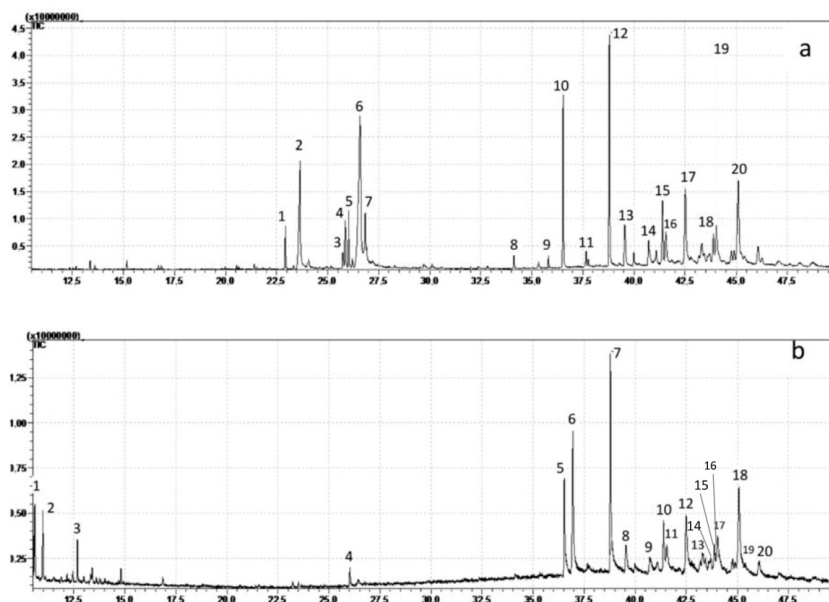
**(a)** n-hexane extract : n-Hexadecanoic acid (23,561 min)<sup>1</sup>; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (26,022 min)<sup>2</sup>; 9,12-Octadecadienoic acid, methyl ester (26,389 min)<sup>3</sup>; Oleic Acid (26,468 min)<sup>4</sup>; 11,14,17-Eicosatrienoic acid, methyl ester (26,5 min)<sup>5</sup>; Octadecanoic acid (26,775 min)<sup>6</sup>; (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (35,803 min)<sup>7</sup>; Tetratetracontane (38,762 min)<sup>8</sup>; Vitamin E acetate (39,564 min)<sup>9</sup>; Ergost-5-en-3-ol, (3.β.)- (41,071 min)<sup>10</sup>; Tetratetracontane (41,38 min)<sup>11</sup>; Stigmasta-5,22-dien-3-ol, acetate, (3.β.)- (41,547 min)<sup>12</sup>; .gamma.-Sitosterol (42,495 min)<sup>13</sup>; urs-12-en-3-one (42,861 min)<sup>14</sup>; urs-12-en-3-one (43,292 min)<sup>15</sup>; urs-12-en-3-one (43,701 min)<sup>16</sup>; 4,22-Cholestadien-3-one (43,872 min)<sup>17</sup>; urs-12-en-3-one (44,161 min)<sup>18</sup>; Stigmast-4-en-3-one (45,056 min)<sup>19</sup>; Urs-12-en-3-ol, acetate, (3.β.)- (46,041 min)<sup>20</sup>

**(b)** petroleum ether extract : Naphthalene, 1-methyl- (10,5 min)<sup>1</sup>; Naphthalene, 1-methyl- (10,9 min)<sup>2</sup>; Tetradecane (12,6 min)<sup>3</sup>; Phenol, 2,4-bis(1,1-dimethylethyl)- (15,1 min)<sup>4</sup>; n-Hexadecanoic acid (23,5 min)<sup>5</sup>; Dibutyl phthalate (23,6 min)<sup>6</sup>; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (26,0 min)<sup>7</sup>; 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (32,3 min)<sup>8</sup>; (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (35,8 min)<sup>9</sup>; Hentriacontane (38,7 min)<sup>10</sup>; Tetratetracontane (41,3 min)<sup>11</sup>; Stigmasta-5,22-dien-3-ol, acetate, (3.β.)- (41,5 min)<sup>12</sup>; .gamma.-Sitosterol (42,4 min)<sup>13</sup>; urs-12-en-3-one (42,8 min)<sup>15</sup>; urs-12-en-3-one (43,2 min)<sup>16</sup>; urs-12-en-3-one (43,6 min)<sup>16</sup>; 4,22-Stigmastadiene-3-one (43,8 min)<sup>17</sup>; urs-12-en-3-one (44,1 min)<sup>18</sup>; Stigmast-4-en-3-one (45,0)<sup>19</sup>; Urs-12-en-3-ol, acetate, (3.β.)- (46,0 min)<sup>20</sup>

Orthosiphon plant is herbaceous that has pharmacological properties and mostly uses as medicinal. The primary compound identified in Orthosiphon leaves in n-hexane, and petroleum ether extract is urs-12-en-3-one from the triterpenoid group (Figure 3). This compound also found in *Inula japonica* that used to be acaricidal compounds (Duan *et al.* 2012). Acaricidal is pesticides that kill the member of arachnid subclass acari, which includes ticks and mites.

Several compounds of kumis kucing leaves that identified in both n-hexane and petroleum ether are Urs-12-en-3-ol, acetate, (3.β.)-, Stigmasta-5,22-dien-3-ol, acetate, (3.β.)-, .gamma.-Sitosterol, Tetratetracontane, and n-Hexadecanoic acid. Bioactive component of *Cynodon Dactylon* revealed that n-hexadecanoic is used for pesticide and nematicide and Stigmasta-5,22-dien-3-ol, acetate, (3.β.)- also have antimicrobial activity (Jebastella and Reginald 2015).





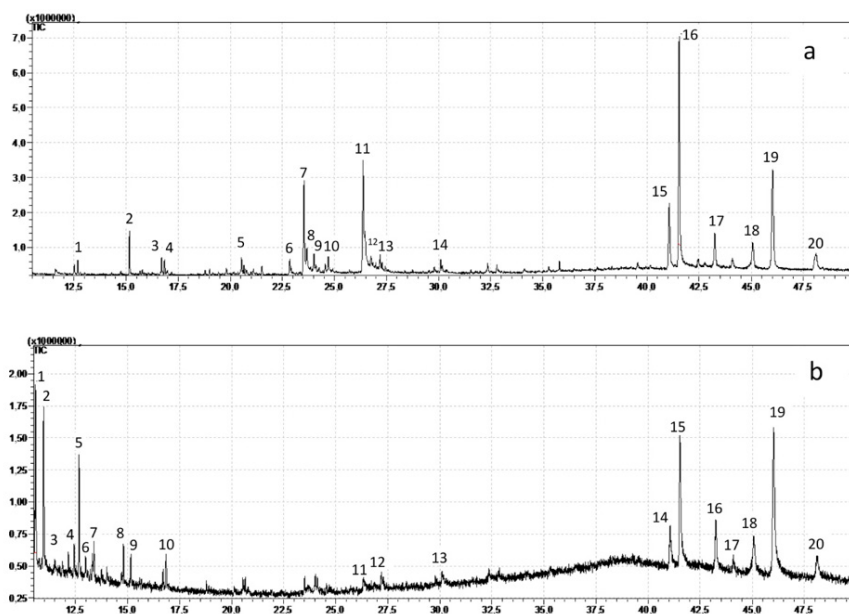
**Figure 4:** Chromatogram of organic compounds obtain from mimba leaves in (a) n-hexane extract (b) petroleum ether extract.

(a) n-hexane extract : 3,9-Dioxa-6-thiaundecane, 2,10-dimethyl- (22,9 min)<sup>1</sup>; 3-Ethylthio-1,2-propanediol (23,6 min)<sup>2</sup>; Triallylphosphine (25,7 min)<sup>3</sup>; 4-Octadecenal (25,8 min)<sup>4</sup>; 1-Dimethyl(ethenyl)silyloxy-2-propene (26,0 min)<sup>5</sup>; Cyclohexanol, 1R-4cis-acetamido-5,6cis-epoxy-2trans,3cis-dimethoxy- (26,5 min)<sup>6</sup>; 1,3,4-Trimethoxy-butan-2-ol (26,8 min)<sup>7</sup>; Ethanol, 2,2-diethoxy- (34,1 min)<sup>8</sup>; Silane, trimethyl((1-methylethylidene)cyclopropyl)- (35,8 min)<sup>9</sup>; Ethanol, 2,2-diethoxy- (36,5 min)<sup>10</sup>; Ethanol, 2,2-diethoxy- (37,6 min)<sup>11</sup>; Ethanol, 2,2-diethoxy- (38,7 min)<sup>12</sup>; 3-Amino-3-(4-amino-6-(dimethylamino)-1,3,5-triazin-2-yl)-1-propanol # (39,5 min)<sup>13</sup>; Allyloxy-dimethyl-silane (40,7 min)<sup>14</sup>; Ethanol, 2,2-diethoxy- (41,3 min)<sup>15</sup>; 6-Ethoxy-6-methyl-2-cyclohexenone (41,5 min)<sup>16</sup>; Allyloxy-dimethyl-silane (42,5 min)<sup>17</sup>; 6-Ethoxy-6-methyl-2-cyclohexenone (43,8 min)<sup>18</sup>; 1-Methyl-1-(prop-2-enyl)-1-silacyclopentane (44,0 min)<sup>19</sup>; Cobalt, (.eta.5-2,4-cyclopentadien-1-yl)((3,4-.eta.)-4,5-diethyl-1,2,2,3-tetramethyl-1-aza-2-sila-5-boracyclopent-3-ene-B5,N1)- (45,1 min)<sup>20</sup>.

(b) petroleum ether extract : 1H-Indole, 1,2-dimethyl-(10,593 min)<sup>1</sup>; 1H-Indole, 2,6-dimethyl-(10,982 min)<sup>2</sup>; Ethanol, 2,2-diethoxy- (12,687 min)<sup>3</sup>; 1-Dimethyl(ethenyl)silyloxy-2-propene (26,021 min)<sup>4</sup>; Ethanol, 2,2-diethoxy- (36,528 min)<sup>5</sup>; 1-Cyclohexyldimethylsilyloxyoctadecane (36,943 min)<sup>6</sup>; Ethanol, 2,2-diethoxy- (38,781 min)<sup>7</sup>; L-Arabinopyranoside, 1-(benzothiazol-2-ylthio)-3,4-O-isopropylidene- (39,541 min)<sup>8</sup>; Methyl 2,5,6-tri-O-acetyl-3-acetamido-3-deoxy-D-altrofuranoside (40,71 min)<sup>9</sup>; Ethanol, 2,2-diethoxy- (41,387 min)<sup>10</sup>; 2-(1-Hydroxy-9a,11a-dimethylhexadecahydrocyclopenta(a)phenanthren-1-yl)-2-methylpropionic acid, ethyl ester (41,548 min)<sup>11</sup>; 2-(((2-Methoxyethoxy)methyl)thio)-1H-imidazole (41,585 min)<sup>12</sup>; Isotridecyl alcohol, trimethylsilyl derivative (42,496 min)<sup>13</sup>; 1-Aza-2-sila-5-boracyclopent-3-ene, 4,5-diethyl-1,2,2-trimethyl-3-(1-methylethenyl)- (43,28 min)<sup>14</sup>; Exo-norbornanol, methyl(pentamethylene)silyl ether (43,696 min)<sup>15</sup>; 1-Dimethyl(ethenyl)silyloxy-2-propene (43,878 min)<sup>16</sup>; 3-O-Methyl-D-glucose (44,03 min)<sup>17</sup>; 1-Dimethyl(ethenyl)silyloxy-2-propene (45,065 min)<sup>18</sup>; Methyl 2,3-diacetamido-2,3-dideoxy-.alpha.-D-glucopyranoside (45,374 min)<sup>19</sup>; 1-Aza-2-sila-5-boracyclopent-3-ene, 4,5-diethyl-1,2,2-trimethyl-3-(1-methylethenyl)- (46,07 min)<sup>20</sup>.

Mimba (*A. indica*) belongs to Meliaceae family is one of the most promising pesticides. The chemical compounds of seeds bark and leaves were isolated and identified as antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal uses (Britto and Sheeba 2011, Chattopadhyay 1999). Mimba extracts alone or mixed with copper sulfate, and boric acid confirmed their antifungal activity was protecting *Mangifera indica* and *Albizia saman* woods (Islam *et al.* 2009).

The major compounds present in mimba leaf extract in n-hexane and petroleum ether were ethanol, 2,2-diethoxy-(Figure 4). It belongs to alcohol derivatives. According to Al-hashemi and Hossain (2016), alkaloids, steroids, flavonoids, tannins, saponins and amino acid were present in all polarities of crude neem leaf extracts except anthraquinone and triterpenoids.



**Figure 5:** Chromatogram of organic compounds obtain from gadung tubers in (a) n-hexane extract (b) petroleum ether extract.

**(a) n-hexane extract :** Tetradecane(12,6 min)<sup>1</sup>; Phenol, 2,4-bis(1,1-dimethylethyl)-(15,1 min)<sup>2</sup>; 3-Hexadecene, (Z)-(16,7 min)<sup>3</sup>; Eicosane(16,8 min)<sup>4</sup>; 1-Heptadecene(20,5 min)<sup>5</sup>; 2-Isopropyl-5-methyl-1-heptanol(22,8 min)<sup>6</sup>; n-Hexadecanoic acid(23,5 min)<sup>7</sup>; Dibutyl phthalate(23,6 min)<sup>8</sup>; 1-Tricosene(24,0 min)<sup>9</sup>; Eicosanoic acid (24,7 min)<sup>10</sup>; 9,12-Octadecadienoic acid (Z,Z)-(26,3 min)<sup>11</sup>; 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(26,4 min)<sup>12</sup>; 1-Nonadecanol (27,191 min)<sup>13</sup>; 1-Tricosene (30,103 min)<sup>14</sup>; Campesterol(41,057 min)<sup>15</sup>; Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-(41,539 min)<sup>16</sup>; urs-12-en-3-one(43,241 min)<sup>17</sup>; Olean-12-ene (45,066 min)<sup>18</sup>; 5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl- (46,02 min)<sup>19</sup>; Lup-20(29)-en-3-ol, acetate, (3.beta.)-(48,099 min)<sup>20</sup>.

**(b) petroleum ether extract :** Naphthalene, 1-methyl-(10,5 min)<sup>1</sup>; Naphthalene, 1-methyl-(10,9 min)<sup>2</sup>; Hexadecane, 2,6,10,14-tetramethyl-(12,17 min)<sup>3</sup>; Biphenyl (12,437 min)<sup>4</sup>; Tetradecane (12,68 min)<sup>5</sup>; Naphthalene, 1,7-dimethyl- (13,317 min)<sup>6</sup>; Naphthalene, 2,6-dimethyl- (13,394 min)<sup>7</sup>; Pentadecane (14,81 min)<sup>8</sup>; Phenol, 2,6-bis(1,1-dimethylethyl)- (15,161 min)<sup>9</sup>; Eicosane, 10-methyl- (16,854 min)<sup>10</sup>; 2-(4-Hydroxybutyl)cyclohexanol (26,333 min)<sup>11</sup>; Trifluoroacetic acid, n-heptadecyl ester (27,185 min)<sup>12</sup>; Pentadec-7-ene, 7-bromomethyl- (30,109 min)<sup>13</sup>; Ergost-7-en-3-ol, (3.beta.)- (41,049 min)<sup>14</sup>; Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)- (41,524 min)<sup>15</sup>; urs-12-en-3-one (43,25 min)<sup>16</sup>; 1,2,4-Triazole, 4-(N-(2-hydroxyethyl)-N-nitro)amino- (44,095 min)<sup>17</sup>; Olean-12-ene (45,056 min)<sup>18</sup>; 5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl- (46,02 min)<sup>19</sup>; Longifolenaldehyde (48,112 min)<sup>20</sup>.

The dominant gadung tubers chemical constituents in n-hexane extract are steroid derivatives (Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)), hydrocarbon (5H-3,5a-Epoxynaphth(2,1-c)oxepin,) and fatty acid (linoleic acid )(Figure 5). Another compound in Gadung tubers n-hexane extract was linoleic acid- (11,9%) which has anti-inflammatory property. N -Hexadecanoic acid (8,96%) was palmitic acid, and it used to antimicrobial, hypocholesterolemic, nematocide, pesticide (Dr. Duke's databases). Hexadecanoic acid also the significant pythoconstituents of gadung in butanol extraction (Om *et al.* 2016).

The primary compounds present in gadung tubers extract in petroleum ether were Naphthalene, 1-methyl- (23,26%). Naphthalene produced by from Magnolia flowers can inhibit natural fungi from proliferating (Chen *et al.* 1998). Muscodor vitigenus fungus also produced naphthalene which purports to be insect repellents (Daisy *et al.* 2002). Naphthalene, 1-methyl- occur naturally in fossil fuels and produced commercially from either coal tar or petroleum, and used for making insecticide carbaryl, leather tanning agents, and dye intermediates (ATSDR 2005).



Variation of five natural extracts

Type of solvent used have effects on the percentage of extraction result. Table 1 summarizes the total percentage extracts and the dominant compound in five plant extracts for the natural preservative. The highest extract percentage is found in pulai barks in both ether (1,97%)and n-hexana solvent (1,91%). The lowest extract is produced by gadung tubers in ether (0,19%) and n-hexane (0,22%). The comparison of the dominant compound of the extracts by different solvent gives a similar figure but different percentage. It is found petroleum ether solvent can detect the compound in lower retention time (Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5). According to Efeovbokhan *et al.* (2015), petroleum ether solvent gave the highest oil yield from Moringa seed followed by hexane then isopropyl alcohol. The comparison of five extracts in terms of the identified compounds, derivatives, and the predicted activity are listed in Table 2. Triterpene derivatives were dominating in the five natural preservatives extract sources. Other compounds are the fatty acid, alcohol, steroid, hydrocarbon, and palmitic acid. It's clear that the most abundant chemical of five natural preservatives extracts by the non-polar solvent is belong to lipophilic constituents. The previous study reported that lipophilic extracts identified from the Pinus bark by GC and GC-MS were composed of four component families i.e., fatty acids and alcohols; monoterpenes and sesquiterpenes; resin acids; steroids and triterpenoids (Masendra *et al.* 2017). By GC-MS analyses, some potential bioactive compounds were detected for the development of natural preservatives from those 5 natural extracts. It is noticed that most of the detected lipophilic compounds here have not been assayed against wood degraders such as destroying-fungi, termites, and marine borers. Therefore three sets of work should be conducted in the near future, i.e., isolation and identification of bioactive compounds from the crude extracts; bioassay of isolated compounds, as well as impregnation and bioassay of bioactive extracts into the solid wood.

Table 1: Percentage extracts and dominant chemical compounds in five natural preservatives extract source.

Percentage extracts, Dominant Compound	N-hexane (%)					Petroleum ether (%)				
	Acacia	Pulai	Kumis kucing	Mimba	Gadung	Acacia	Pulai	Kumis kucing	Mimba	Gadung
Percentage extracts (%)	0,46	1,91	0,93	0,40	0,22	0,82	1,97	0,36	0,35	0,19
Lupeol	16,52	4,54				11,34	4,02			
7,22-Ergostadienone	13,21					8,87				
Lup-20(29)-en-3-one	10,08				2,46	6,34	4,02			
Lup-20(29)-en-3-ol, acetate, (3.beta.)-		61,76			2,46		0,89			
Urs-12-en-3-one		14,66	34,33		4,07		28,78	45,88		5,25
Ethanol, 2,2-diethoxy-				28,32					35,39	
Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-			7,85		27,75			5,86		14,58
5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl-					16,54		56,07			22,16
Linoleic acid			3,33		11,99					
Naphthalene, 1-methyl-						11,97		4,84		23,26
N-Hexadecanoic acid			3,73		8,96			0,58		

**Table 2:** Chemical compounds activity of five natural preservatives extract source.

Compound	Derivatives	Activity
Lupeol	Triterpene	Antimicrobial, antiinflammatory, <sup>1,2</sup>
7,22-Ergostadienone	Triterpene	unknown
Lup-20(29)-en-3-one	Triterpene	Antimalarial, anti-inflammatory <sup>3</sup>
Lup-20(29)-en-3-ol, acetate, (3.β.)-	Triterpene	Anti-inflammatory <sup>4</sup>
urs-12-en-3-one	Triterpene	No activity reported <sup>3</sup>
Ethanol, 2,2-diethoxy-	Alcohol	unknown
Stigmasta-5,22-dien-3-ol, acetate, (3.β.)-	Steroid	Biomarker for the presence of (marine) algal matter in the environment <sup>5</sup>
5H-3,5a-Epoxynaphth(2,1-c)oxepin, dodecahydro-3,8,8,11a-tetramethyl-, linoleic acid	Hydrocarbon	Fragrance agents <sup>6</sup>
	Fatty acid	Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic <sup>5</sup>
Naphthalene, 1-methyl-	Hydrocarbon	Insecticide carbaryl, leather tanning agents, and dye intermediates <sup>8</sup>
N- Hexadecanoic acid	Palmitic acid	Antimicrobial, hypocholesterolemic, nematicide, pesticide <sup>3</sup>

<sup>1</sup>Mutai *et al.* 2009, <sup>2</sup>Pedemera *et al.* 2010, <sup>3</sup>USDA 2016, <sup>4</sup>Lucetti *et al.* 2010, <sup>5</sup>Malik 2016, <sup>6</sup>Surburg and Panten 2006, <sup>7</sup>Anburaj *et al.* 2016, <sup>8</sup>ATSDR 2005.

## CONCLUSIONS

The results of this study shows that five plant sources extracted by n-hexane and petroleum ether gave several chemical compounds which have potential to be natural preservatives. They were Lupeol, 7,22-Ergostadienone, Lup-20(29)-en-3-one, Lup-20(29)-en-3-ol, acetate, (3.β.), Urs-12-en-3-one, Ethanol, 2,2-diethoxy-, Stigmasta-5,22-dien-3-ol, acetate,(3.β.)-, 5H-3,5a-Epoxynaphth(2,1-c)oxepin,dodecahydro-3,8,8,11a-tetramethyl-, linoleic acid, Naphthalene, 1-methyl-, and N- Hexadecanoic acid. Moreover, these results could be useful in the research for isolating and evaluating the compounds against termites or fungi.

## ACKNOWLEDGEMENTS

This research work was carried out within a research project funded by Directorate General of Higher Education, Ministry of Education and Culture, Republic of Indonesia. The project number is LPPM-UGM/1448/LIT/2013. The authors would like to express their sincere appreciation for technical support from Nurmadiana and Masendra.

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