

CHEMICAL VARIATION OF FIVE NATURAL EXTRACTS BY NON-POLAR SOLVENT

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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.beta.)-; urs-12-en-3-one; ethanol,2,2-diethoxy-; stigmasta-5,22-dien-3-ol, acetate,(3.beta.)-; 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 enviroins 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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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 ; β -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, H₂O₂ 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 μ l (1 mg/ml) was made in a split less mode using electron impact ionization (EI, 70 eV). The injector and detector temperature were 250°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.

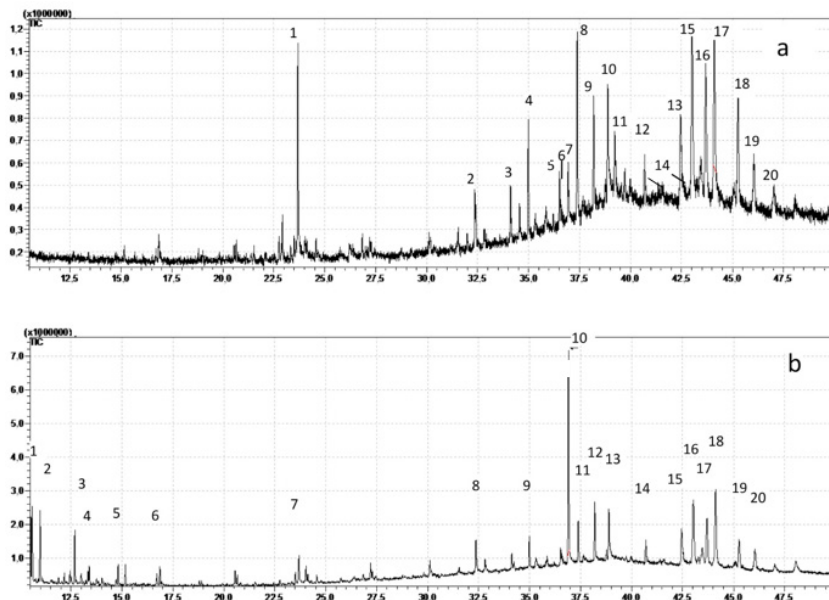


Figure 1: Chromatogram of organic compounds obtain from *Acacia spp.* bark in (a) n-hexane extract (b) petroleum ether extract.

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

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

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).

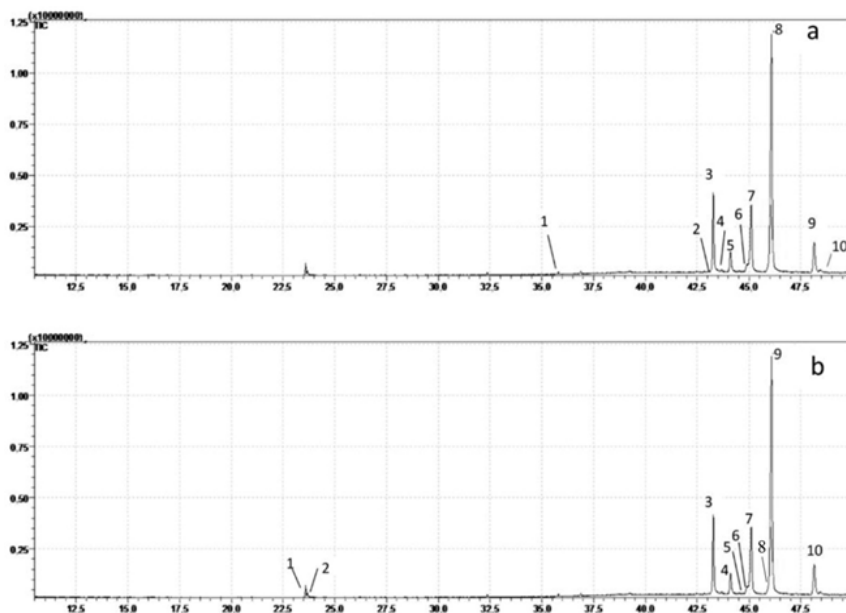


Figure 2: Chromatogram of organic compounds obtain from pulai bark in (a) n-hexane extract (b) petroleum ether extract.

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

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

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-Epoxy-naphth(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).

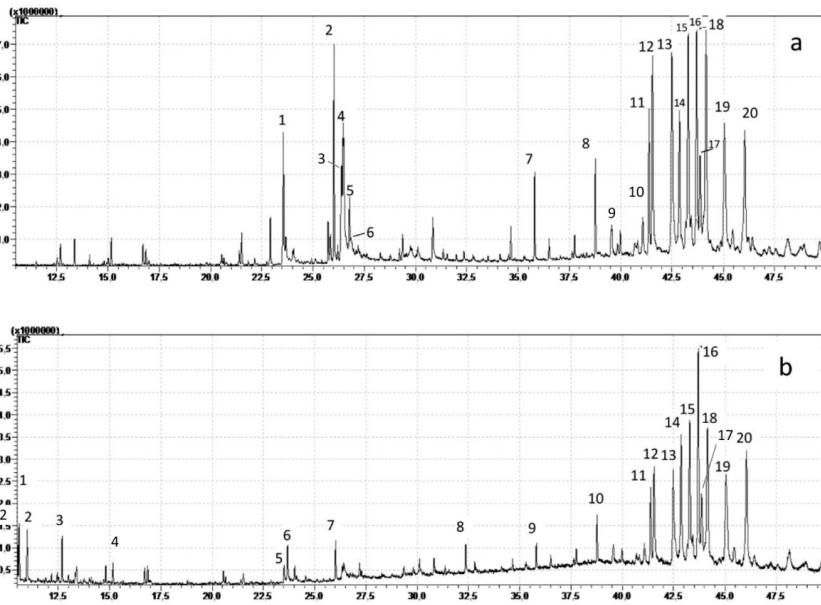


Figure 3: Chromatogram of organic compounds obtain from kumis kucing leaves in (a) n-hexane extract (b) petroleum ether extract.

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

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

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.beta.)-, Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-, .gamma.-Sitosterol, Tetratetracontane, and n-Hexadecanoic acid. Bioactive component of *Cynodon Dactylon* revealed that n-hexadecanoic is used for pesticide and nematocidal and Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)- also have antimicrobial activity (Jebastella and Reginald 2015).

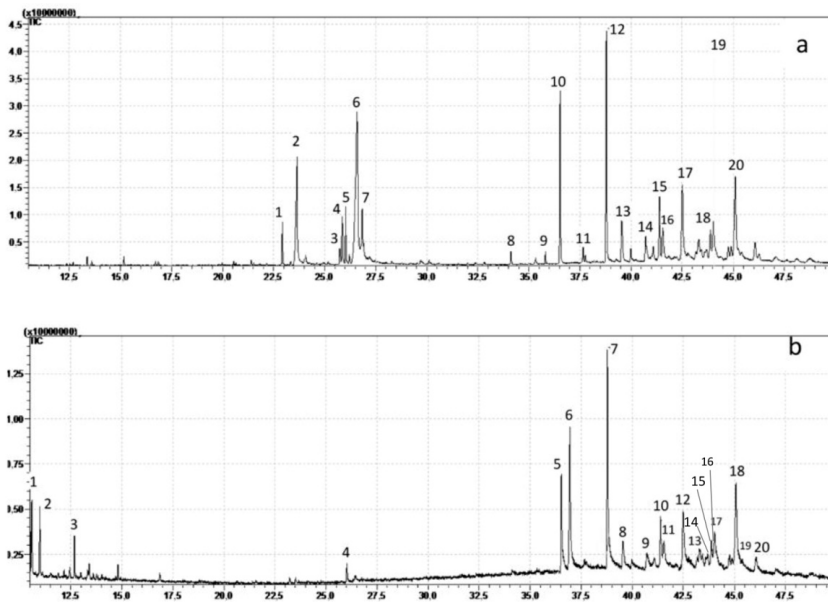


Figure 4: Chromatogram of organic compounds obtain from mimba leaves in (a) n-hexane extract (b) petroleum ether extract.

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

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

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.

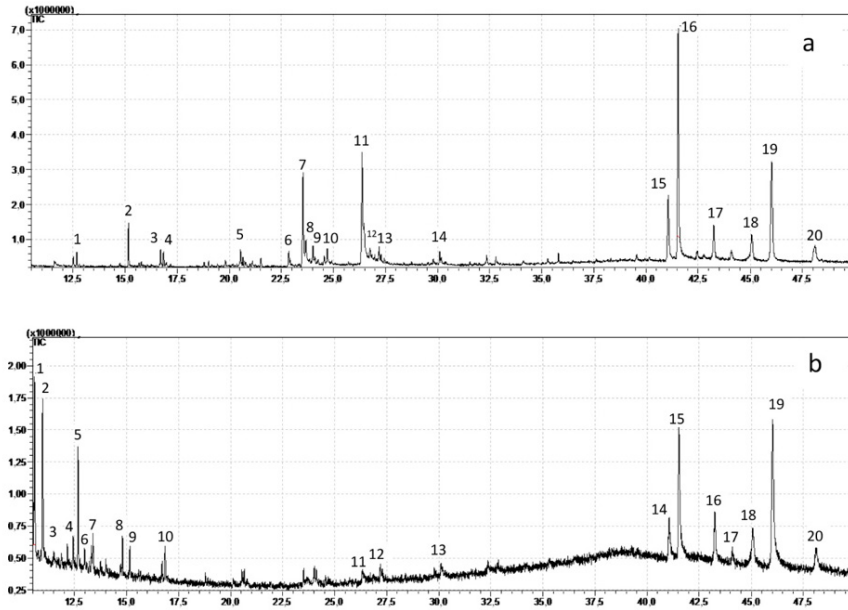


Figure 5: Chromatogram of organic compounds obtain from gadung tubers in (a) n-hexane extract (b) petroleum ether extract.

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

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

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

¹Mutai *et al.* 2009, ²Pedemera *et al.* 2010, ³USDA 2016, ⁴Lucetti *et al.* 2010, ⁵Malik 2016, ⁶Surburg and Panten 2006, ⁷Anburaj *et al.* 2016, ⁸ATSDR 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.beta.), Urs-12-en-3-one, Ethanol, 2,2-diethoxy-, Stigmasta-5,22-dien-3-ol, acetate,(3.beta.)-, 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.

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REFERENCES

Ahmed, S.; Zafar, M.I.; Hussain A. Riaz, M.A.; Shahid, M. 2011. Evaluation of plant extracts on mortality and tunneling activities of subterranean termites in Pakistan. In: Stoytcheva M (ed) *Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment*. InTech: Rijeka, Croatia. ISBN: 978-953-307-457-3. <https://doi.org/10.5772/18999>

Al-Hashemi, Z.S.S.; Hossain, M.A. 2016. Biological activities of different neem leaf crude extracts used locally in Ayurvedic medicine. Pacific science review. *Natural science and engineering* 18:128-131. <http://dx.doi.org/10.1016/j.psr.2016.09.013>

Anburaj, G.; Marimuthu, M.; Rajasudha, V.; Manikandan, R. 2016. Photochemical screening and GC-MS analysis of ethanolic extract of *Tecima stans* (Family: Bignoniaceae) Yellow Bell Flowers. *Journal of*

Pharmacognosy and Phytochemistry 5(4): 172-175. <http://dx.doi.org/10.22271/phyto>

Antwi-Boasiako, C.; Damoah, A. 2010. Investigation of synergistic effect of extracts from *Erythrophleum suaveolens*, *Azadirachta indica*, and *Chromolaena odorata* on the durability of *Antiaris toxicaria*. *Int Biodeter Biodegr* 64: 97-103. <http://dx.doi.org/10.1016/j.ibiod.2009.08.005>

Arora, A.; Meena, S. 2016. Qualitative preliminary phytochemical screening and GC-MS analysis of root of *Sarcostemma viminalis* (L) R. BR., an endangered plant. *International Journal of Pharmaceutical Research and Bio-science* 5(2):89-100. <http://www.ijprbs.com/index.php>

Asgar, S.F.; Habib-Ur, Rehman.; Choudahry, M.I.; Atta-Ur-Rahman. 2011. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of extract (oil) and bioassays of crude extract of *Iris germanica*. *International Journal of Genetics and Molecular Biology* 3(7): 95-100. <http://www.academicjournals.org/ijgmb>

ASTM. 2007. Ether solubility in wood. ASTM D-1108-96. 2007. Annual Book of ASTM Standard.

ATSDR. 2005. Toxicological profile for naphthalene, 1- methylnaphthalene, and 2-methylnaphthalene. Agency for Toxic Substances and Disease Registry. Atlanta, Georgia.

Aziz, A.; Prayitno, T.A.; Hadikusumo, S.A.; Santoso, M. 2013. Uji ekstrak etanol kumis kucing (*Orthosiphon sp.*) sebagai pengawet alami kayu. *Jurnal Ilmu Kehutanan* 7(1). 48-56. <https://doi.org/10.22146/jik.6137>

Azman, I.; Mutalib, S.A.; Yusoff, S.F.M.; Fazry, S.; Noordin, A.; Kumaran, M.; Lazim, A.M. 2015. Novel *Dioscorea hipsida* starch-based hydrogels and their beneficial use as disinfectants. *Journal of Bioactive and Compatible Polymers* 31(1): 42-59. <https://doi.org/10.1177/0883911515597704>

Britto, A.J.; Sheeba, D.H. 2011. *Azadirachta indica* juss – a potential antimicrobial agent. *International Journal of Applied Biological and Pharmaceutical Technology*: 4550–4557. <http://dx.doi.org/10.1016/j.jtusci.2013.05.0031>.

Chattopadhyay, R. 1999. Possible mechanism of anti-hyperglycemic effect of *Azadirachta indica* leaf extract. *Journal of Ethnopharmacology* 67 (3): 373–376. [https://doi.org/10.1016/s0378-8741\(99\)00094-x](https://doi.org/10.1016/s0378-8741(99)00094-x)

Chen, J.; Henderson, G.; Grimm, C.C.; Lloyd, S.W.; Laine, R.A. 1998. Termites fumigate their nests with naphthalene. *Nature* 392: 558-559. <https://doi.org/10.1038/33305>

Chen, P.; Chen, Y.; Yeh, T.; Chang, S. 2014. Mechanism of decay resistance of heartwood extracts from *Acacia confusa* against the brown-rot fungus *Laetiporus sulphureus*. *Wood Sci Technology* 8 (3):451-465. <https://doi.org/10.1007/s00226-014-0615-6>.

Cock, I.E. 2017. Australian *Acacia spp.* extracts as natural food preservatives: Growth inhibition of food spoilage and food poisoning bacteria. *Pharmacognosy Communications* 7(1): 4-15. <https://doi.org/10.5530/pc.2017.1.2>

Daisy, B.H.; Strobel, G.A.; Castillo, U.; Ezra, D.; Sears, J.; Weaver, D.K.; Runyon, J.B. 2002. Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology* 148:3737-3741. <https://doi.org/10.1099/00221287-148-11-3737>

Duan, D.; Bu, C.; Ma, L.; Liu, Y.; Wang, Y.; Shi G. 2012. Identification of acaricidal compounds in inula japonica extracts against *Tetranychus cinnabarinus*. In: Zhu, E.; Sambath, S. (eds). *Information Technology and Agricultural Engineering*. Advances in Intelligent and Soft Computing 134. Springer: Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-27537-1_88

Efevbokhan, V.E.; Hymore, F.K.; Raji, D.; Sanni, S.E. 2015. Alternative solvents for *Moringa oleifera* seeds extraction. *Journal of Applied Sciences* 15(8): 1073-1082. <https://doi.org/10.3923/jas.2015.1073.1082>

Grover, N.; Patni, V. 2013. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L) Kurz. Leaves. *Int J Phar & Pharma Sci* 5(4): 291-295. <http://ijppsjournal.com/index.htm>

- Harborne, J.B.** 1973. Phytochemical methods. Chapman and Hall. <https://catalogue.nla.gov.au/Record/174917>
- Hu, J.B.; Chang, S.S.; Peng, K.Y.; Hu, K.F.; Thevenon, M.** 2015. Bio-susceptibility of shells of *Camellia oleifera* Abel. fruits to fungi and termites. *Int Biodeter Biodegr* 104:219-223. <https://doi.org/10.1016/j.ibiod.2015.06.011>
- Islam, M.; Shams, I.; Ilias, G.N.M.; Hannan, O.** 2009. Protective antifungal effect of neem (*Azadirachta indica*) extracts on mango (*Mangifera indica*) and rain tree (*Albizia saman*) wood. *Int Biodeter Biodegr* 63(2): 241-243. <https://doi.org/10.1016/j.ibiod.2008.07.010>
- Ivanov, I.; Petkova, N.; Tumbarski, J.; Dincheva, I.; Badjakov, I.; Denev, P.; Pavlov, A.** 2018. GC-MS characterization of n-hexane soluble fraction from dandelion (*Taraxacum officinale* Weber ex. F.H. Wigg.) aerial parts and its antioxidant and antimicrobial properties. *Z Naturforsch C* 73(1-2):41-47. <https://doi.org/10.1515/znc-2017-0107>
- Jebastella, J.; Reginald, A.M.** 2015. Bioactive components of *Cynodon dactylon* using ethanol extract. *World J Pharm Sci* 3(12): 2388-2391. <http://www.wjpsonline.org/>
- Kadir, R.; Awang, K.; Khamaruddin, Z.; Soit, Z.** 2015. Chemical composition and termiticidal activities of the heartwood from *Calopyllum inophyllum* L. *Anais da Academia Brasileira de Ciências* 87(2): 743-751. <http://dx.doi.org/10.1590/0001-3765201520140041>
- Karuppasamy, B.; Antony, N.; Veerabahu, R.M.** 2012. GC-MS analysis of *Polycarpaea corymbosa* (L.) Lam whole plant. *Asian Pacific Journal Tropical Biomedicine* 1289-1292. [https://doi.org/10.1016/S2221-1691\(12\)60402-X](https://doi.org/10.1016/S2221-1691(12)60402-X)
- Kumar, S.; Das, G.; Shin, H.; Patra, J.K.** 2017. *Dioscorea* spp. (A Wild Edible Tuber): A study on its ethnopharmacological potential and traditional use by the local people of Similipal Biosphere Reserve, India. *Front Pharmacol* 8: 52. <https://doi.org/10.3389/fphar.2017.00052>
- Lucetti, D.L.; Lucetti, E.C.P.; Bandeira, M.A.M.; Veras, H.N.H.; Silva, A.H.; Leal, L.K.A.M.; Lopes, A.A.; Alves, V.C.C.; Silva, G.S.; Brito, G.A.; Viana, G.B.** 2010. Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated from *Himatanthus drasticus* (Mart.) Plumel. *Journal of Inflammation (London)* 7: 60. <https://doi.org/10.1186/1476-9255-7-60>
- Malik, B.; Pirzadah, T.B.; Tahir, I.; Abdin, M.Z.; Rehman, U.I.R.** 2016. Phytochemical studies on *Cichorium intybus* L. (CHICORY) from Kashmir Himalaya using GC-MS. *Journal of Pharmacy Research* 10(11): 715-726.
- Masendra, M.; Ashitani, T.; Takahashi, K.; Lukmandaru, G.** 2018. Lipophilic extractives of the inner and outer bark from six different *Pinus* species grown in Indonesia. *Journal Forestry Research* (29):1329-1336. <https://doi.org/10.1007/s11676-017-0545-x>
- Mutai, C.; Bii, C.; Vagias, C.; Abatis, D.; Roussis, V.** 2009. Antimicrobial activity of *Acacia mellifera* extracts and lupane triterpenes. *Journal of Ethnopharmacology* 123: 143-148. <https://doi.org/10.1016/j.jep.2009.02.007>
- Old, K.M.; Vercoe, T.K.; Floyd, R.B.; Wingfield, M.J.; Roux, J.; Naser, S.** 2002. *Acacia* spp. *FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm* 20. https://www.biodiversityinternational.org/fileadmin/_migrated/uploads/tx_news/Acacia_spp_829.pdf
- Om, A.D.; Azizan, K.A.; Baharum, S.N.** 2016. Phytochemical screening of ubi gadong (*Dioscorea hip-sida*) by using GC-MS. *Seminar dan konvensyen penyelidikan perikanan*, Fri Batu Maung: Pulau Pinang. <https://www.researchgate.net/publication/307510333>
- Pedemera, A.M.; Guardia, T.; Calderón, C.E.; Rotelli, A.E.; de la Rocha, N.E.; Saad, J.R.; Verrilli, M.A.; Aseff, S.G.; Pelzer, L.E.** 2010. Anti-inflammatory effect of *Acacia visco* extracts in animal models. *Inflammopharmacol* 18: 253-260. <https://doi.org/10.1007/s10787-009-0028-6>
- Prayitno, T.A.; Widyorini, R.** 2016. The adhesion properties of wood preserved with natural preserva-

tives. *Wood Research* 61(2): 197-204. <http://www.woodresearch.sk/articles.php>

Prayitno, T.A.; Widyorini, R.; Lukmandaru, G. 2017. Bonding ability of sengon wood treated with natural extracts. *Wood Research* 62(6):951-960. <http://www.woodresearch.sk/articles.php>

Ragasa, C. Y.; Guardamano, J.D.; Tan, M.C.S.; Ulep, R.A.; Alena, I.A.V. 2016. Chemical constituents of *Dioscorea luzonensis* Schauer. *International Journal of Current Pharmaceutical Review and Research* 7(4): 223-224.

Ramalakshmi, S.; Muthuchelian, K. 2011. Analysis of Bioactive constituents from the ethanolic leaf extract of *Tabebuia rosea* (Bertol.) DC by Gas Chromatography - Mass Spectrometry. *International Journal of ChemTech Research* 3(3): 1054-1059.

Saeed, M.A.; Sabir, A.W. 2002. Irritant potential of triterpenoids from *Ficus carica* leaves. *Fitoterapia* 73(5): 417-420. [https://doi.org/10.1016/S0367-326X\(02\)00127-2](https://doi.org/10.1016/S0367-326X(02)00127-2)

Savi, A.; Calegari, G.C.; Santos, V.A.Q.; Pereira, E.A.; Teixeira, S.D. 2018. Chemical characterization and antioxidant of polysaccharide extracted from *Dioscorea Bulbifera*. *Journal of King Saud University - Science* 32(1):636-642. <https://doi.org/10.1016/j.jksus.2018.09.002>

Se Golpayegani, A.; Thévenon, M.; Gril, J.; Masson, E.; Pourtahmasi, K. 2014. Toxicity potential in the extraneous compounds of white mulberry wood (*Morus alba*). *Maderas-Cienc Tecnol* 16(2): 227-238. <https://doi.org/10.4067/S0718-221X2014005000018>

Şen, S.; Yalcin, M.; Taşçıoğlu, C.; Özbayram, A.K. 2017. Larvicidal activities of some bark and wood extracts against wood-damaging insects. *Maderas-Cienc Tecnol* 19(3): 273-284. <https://doi.org/10.4067/S0718-221X2017005000023>

Singh, M.K.; Gidwani, B.; Gupta, A.; Dhongade, H.; Kaur, C.D.; Kashyap, P.P.; Tripathi, D.K. 2015. A review of the medicinal plants of genus *Orthosiphon* (Lamiaceae). *Int J Biol Chem* 9(6): 318-331. <https://doi.org/10.3923/ijbc.2015.318.331>

Sotannde, O.A.; Yager, G.O.; Zira, B.D.; Usman, A. 2011. Termiticidal effect of neem extracts on the wood of *Khaya senegalensis*. *Research Journal of Forestry* 5(3): 128-138. <https://doi.org/10.3923/rjf.2011.128.138>

Ssemaganda, I.E.; Mugabi, P.; Tumwebaze, S.B. 2011. Effectiveness of selected preservatives in protecting Ugandan grown *Eucalyptus grandis* wood against termite attack. *Maderas-Cienc Tecnol* 13(2): 135-142. <https://doi.org/10.4067/S0718-221X2011000200001>

Subraya, C.K.; Harikiran; Gupta, D. 2012. Antioxidant and anti-inflammatory activity of *Alstonia scholaris* R.Br. stem bark extract. *Free Radicals and Antioxidants* 2(2):55-57. <https://doi.org/10.5530/ax.2012.2.2.9>

Surburg, H.; Panten, J. 2006. *Common fragrance and flavor materials*. WILEY-VCH Verlag GmbH & Co. KGaA: Weinheim. <https://b-ok.asia/book/1003351/5a42ec>.

Syofuna, A.; Banana, A.Y.; Nakabonge, G. 2012. Efficiency of natural wood extractives as wood preservatives against termite attack. *Maderas-Cienc Tecnol* 14(2): 155-163. <https://doi.org/10.4067/S0718-221X2012000200003>

Tascioglu, C.; Yalcin, M.; Troya, T.; Sivrikaya, H. 2012. Termiticidal properties of some wood and bark extracts used as wood preservatives. *Bioresources* 7(3): 2960-2969. <https://doi.org/10.15376/biores.7.3.2960-2969>

USDA. 2016. Dr. Duke's phytochemical and ethnobotanical databases. <http://phytochem.nal.usda.gov/>