

Identification *Michelia alba* barks extract using Gas Chromatography-Mass Spectrometry (GC-MS) and its antifungal properties to inhibit microbial growth

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Abstract. Swantara IMD, Bawa IGAG, Suprpta DN, Agustina KK, Temaja IGRM. 2020. Identification *Michelia alba* barks extract using Gas Chromatography-Mass Spectrometry (GC-MS) and its antifungal properties to inhibit microbial growth. *Biodiversitas* 21: 1541-1550. Fungicides are substances that inhibit the growth or kill the pathogenic fungi. A substance can be categorized as a fungicide if it shows antifungal activity. This activity is resulting from bioactive compounds derived from the secondary metabolites. *Michelia alba* bark's extract could inhibit the growth of *Curvularia verruculosa*, the cause of leaf spot disease on rice. The purpose of this study was to analyze chemical compounds of bark extract which inhibit the growth of *C. verruculosa*. The extraction of bark metabolites of *M. alba* was done using methanol as the solvent. The inhibitory test of the extract was carried out by the diffusion well method on Potato Dextrose Agar (PDA) medium. Analysis of chemical compounds of the *M. alba* extract was carried out in Gas chromatography-mass spectroscopy. The results of inhibitory test found that the *M. alba* bark extract inhibits the *C. verruculosa* with the inhibition zone as wide as 36 mm in diameter. The majority of chemical compounds identified from the *M. alba* extract consisted of 10 compounds, namely: Hexadecanoic acid, methyl ester; 4H-tomentosine; 4H-tomentosine (compound isomer 2); 3-hydroxy pregn-4-ene-20-one; Tomentosine; 2-hydroxy tomentosine; Tert-butyl-2-aminophenylcarbamol; 2H-cyclohepta [b] furan-2-one, 3,3a, 4,7,8,8a-hexahydro-7-methyl-3-methylene-6-(2-formyl-3-oxobutyl)-; Isoxanthanol and Xanthanol. To conclude, this extract is useful to treat *C. verruculosa* that causes leaf spot disease on rice.

Keywords: Chemical compounds, *Curvularia verruculosa*, fungicides, Gas Chromatography, *Michelia alba*, Spectrometry

INTRODUCTION

White champaca plant (*Michelia alba*) belongs to the family of Magnoliaceae, where almost all parts of the plant parts such as bark, leaves and flowers can be used as medicine (Sinha and Varma 2016). It has believed can cure the symptom of fever, irregular menstruation, bronchitis, cough, vaginal discharge, inflammation, and urinary tract infections. The bark, leaves, and flowers of the tree are also efficacious as expectorants and have diuretic effect, so they can break down kidney stones, and prevent and cure bad breath (Subcharoen 1999).

The application of white champaca, *M. alba* plants as fungicides has never been reported, but plants from the same genus, that produces yellow flowers (*Michelia champaca* L.) have been known to have the potentials as fungicides. Mangang and Chhetry (2012) found that 5% of the yellow-flowered *M. champaca* plant extract was able to inhibit 49.54% growth of the mycelia of *Rhizoctonia solani* that cause root rot in beans (*Phaseolus vulgaris*). Furthermore, Kumar et al. (2011) reported that *M. champaca* crude extract showed high antifungal activity against the fungus *Candida albicans*. Pawar (2015) found

that *M. champaca* plant root extract was able to inhibit the growth of pathogenic *Curvularia lunata* in seeds with an inhibition diameter zone of 15 mm. Plant extract can be categorized as an antifungal if the extract can inhibit fungal growth (Semangun 2006), which indicated by bioactive content of the compounds derived from the secondary metabolites.

Michelia alba plants have been known to contain many terpenoids, alkaloids, and steroid compounds. It has been reported that the flower of white champaca contained monoterpenes and sesquiterpenes (Sanimah et al. 2008), the roots, leaves, flowers and stems contained sesquiterpenes and triterpenes (terpenoid groups), aporphine and oxoaporphine (alkaloid group), and the β -sitosterol and stigmasterol (steroid group) (Huang 2008). The monoterpene compounds found on white champaca plants were α -myrcene, (S)-limonene, eucalyptol, linalool, (R)-fenchone, and camphor (Shang et al. 2002). Sanimah et al. (2008) reported that 33 compounds contained in white champaca flowers scattered in the isoprenoid group which reached 30-50% of the total volatile compounds, the rest in the form of fatty acid derivatives, benzenoid, phenylpropanoid and hydrocarbon compounds. These

compounds include monoterpenoids, consisted of α -myrcene, linalool, dihydrocarveol, eugenol methyl ether, and sesquiterpenoid, consisted of germacrene D; caryophyllene and cadina-3,9-diene.

Other compounds that are also found in white champaca flowers were butanoic acid-2methyl-methyl ester; methyl benzoate and 1-ethenyl-1-methyl-2,4-bis (1-methyl phenyl)-cyclohexane. Other researchers found that white champaca plants contain monoterpenoid linalool, indole alkaloids and phenylethyl alcohol as the main components (Punjee et al. 2009). Bawa (2011) reported that the n-hexane extract of white champaca flower contained 6 major components including 5-(2-propenyl)-1,3-benzoxol; 1-ethenyl-1-methyl-2,4-bis (1-methyletenil)-cyclohexane; 3-methyl-2-phenyl ethyl butanoic; 9,12-octadecadienoic; tricosan; and breakdown.

The methanol extract of *M. alba* leaves was found to contain one new chlorophyll known as michephyll A (Lee et al. 2014; Huang 2008), in addition to chlorophyllpheophytin-a and aristophyll-C. Twenty-six other compounds were found, including seven alkaloids from the aporphines group namely (-)-anonaine, (-)-ushinsunine, (-)-norushinsunine, (-)-N-formylanonaine, (-)-N-acetylanonaine, (-)-oliveroline, (+)-nornuciferine; three alkaloids from the oxoaporphines group, namely lysicamine, liriodenine, oxoxylopinine; 4 sesquiterpenes those were michelenolide, costunolide, 11,13-dehydrol-anuginolide, (+)-cyperone; 2 lignin (syringaresinol and (+)-epiyangambin), one amid (N-trans-feruloyltyramine), 3 benzenoids (p-hydroxybenzaldehyde, p-hydroxybenzoic acid, methylparabene), 1 triterpenoid (ficaprenol-10); 2 steroid (β -sitosterol and stigmasterol), and 3 aliphatic compound (palmitic acid, steric acid, linoleate acid) (Huang 2008).

The extract of *M. alba* bark was found contained 19 compounds, including 6 alkaloids from aporphines groups (-)-anonaine, (-)-ushinsunine, (-)-norushinsunine, (-)-N-formylanonaine, (-)-roemerine, (-)-asimilobine); 2 oxoaporphines (liriodenine and oxoxylopinine); one lignan (+)-syringaresinol); 1 amida (N-trans-feruloyltyramine); 6 benzenoids (p-hydroxybenzaldehyde, p-anisaldehyde, veratraldehyde, 3,4,5-trimethoxybenzoic, 3,4-dimethoxybenzoic acid, eugenol); 1 triterpenoid: ficaprenol-10); 2 steroid (β -sitosterol and stigmasterol) (Huang 2008).

Krisdiana (2010) stated that the essential oils of wet white champaca flowers contained 30 compounds. The 10 major compounds were 3,7-dimethyl-1,6-octadien-3-ol; myristicin; 1-ethenyl-1-methyl-2,4-bis (1-methyletenyl)-siclohexana; etyl-2-methylbutiric; 1,2-dimetoxi-4-(2-propenil)-benzene; 5-(2-propenyl)-1,3-benzodioxol; 1,2,4a,5,6,8a-hexahidro-4,7-dimethyl-1-(1-methylethyl)-naphthalene; 3,7-dimethyl-1,3,7octatriena and 3,7-dimethyl-1,3,6-octatriena, whereas the dried flowers was indentified to have 61 compounds, 5 major compounds includes trans-isocroweacin; 5-(2-propenyl)-1,3-benzodioxol; 1-ethenyl-1-methyl-2,4-bis (1-methyletenyl)-siclohexane; 1-methyl-4-(5-methyl-1-methylen-4-hexenyl)siclohexene; and β -selinene. High number of chemical compounds were found in various parts of white champaca plant, but there is no study has been conducted on the inhibitory effect of the compounds as fungicidal against

Curvularia verruculosa. The purpose of this study was to analyze chemical compounds of bark extract which inhibit the growth of *C. verruculosa*.

MATERIALS AND METHODS

Ethical statement

This research was not using any vertebrate as a sample.

Materials

The material used in this research was isolate of *C. verruculosa*; bark of white champaca (*M. alba*), methanol p.a. (E Brand), Potato Dextrose Agar (PDA) as media.

Instruments

The instrument used in this study was Gas Chromatography-Mass Spectrometry (GCMS-QP2010S Shimadzu Corporation, Japan 2017); AGILENT DB-1 (CrossbondR 100% dimethylpolysiloxane) Column with the Length of 30 meters; ID: 0.25 mm; Film thickness of 0.2 μ m; Helium Carrier gas; Ionizing of EI 70 Ev.

Procedure

The extraction

Samples of white champaca bark were washed in clean water, cut into small pieces, then air-dried at room temperature. Dried samples were blended into powder (100 mesh), then 1000 grams of the sample were macerated three times in 2000 mL methanol for 24 hours, then filtered. The filtrate was combined and evaporated in a rotary vacuum evaporator Buchi Rotavapor R-114 type, so that crude methanol extract was obtained.

The identification

The identification of white champaca bark extract compounds following the procedure of the GC-MS working standard. 20 μ L sample added to 5 mL volumetric flask and diluted with methanol to the mark. Take 1.5 mL of solution and put it in vials and 1 μ L injected into a GC-MS tool with an injection temperature of 250°C, pressure 102.6 kPa, column flow 1.11 mL/minute.

The antifungal activity test

Antifungal activity test of crude methanol extract of white champaca bark (*M. alba*) on *C. verruculosa* was carried out by diffusion well method. Five Petri dish, 9 cm in diameter were filled with 200 μ L of *C. verruculosa* fungal culture which had been finely chopped and dissolved in sterile water, then each Petri dish was added with 10 mL melted Potato Dextrose Agar (PDA) in the temperature of 45°C, vortex horizontally in order to mix the PDA media and *C. verruculosa* evenly, then left to condensed.

Two well using a cork borer (5 mm in diameter) were made in each sample. Each diffusion well is filled with 20 μ L of crude extract of white champaca bark. This culture was placed in a dark place at room temperature (27°C-32°C). Observations were made by measuring the diameter of the inhibition zone formed around the diffusion well.

RESULTS AND DISCUSSION

Identification of fungicidal compounds

The chromatogram of GC-MS analysis on fungicidal effect of *C. alba* bark extract is shown in Figure 1.

Identification of compounds in white champaca bark using gas chromatography has resulted in 10 peaks, which indicated that 10 compounds were detected. The analysis of each peak of mass spectra is described below.

Peak 1 (retention time: 11.140 minutes; abundance 6.07%)

The mass spectra of compound 1 (Figure 2) has similarities with the hexadecanoic acid compound, methyl ester with a molecular weight of 270 grams/mol in the NIST08.LIB Library.

The percentage similarity to compound 1 with the database reaches 95%. The high percentage similarity value of compound 1 to the database reinforces the notion that compound 1 is a hexadecanoic acid, methyl ester compound with the structure as in Figure 3.

Peak 2 (retention time: 12.06 minutes; abundance 2.48 %)

Figure 4 shows the specimen of compound mass 2. This compound has similarities with spathulenol compounds with a molecular weight of 220 gram/mol in the Library NIST08.LIB.

The percentage of similarity of compound 2 to the database is 76%. The appearance of the ion peak at m/z 232 causes the fragmentation pattern of compound 2 to be incompatible with the fragmentation pattern of spathulenol compounds, because this compound has a molecular ion (M^+) at m/z 220, which often appears as ($M^+ - CH_3$) at m/z 205. Based on the fragmentation pattern, compound 2 was more likely to be similar to the fragmentation pattern of the tomentosine compound, which has a molecular weight of

248g/mol, this compound undergoes a hydrogenation reaction, so that it appears as a molecular ion (M^+) at m/z 250. The results of fragmentation analysis of compound 2 are presented in Table 1.

Based on the fragmentation's results of compound 2 (Table 1), it is confirmed that compound 2 was a hydrogenated or reduced tomentosine compound (the ketone group in the butyl chain was reduced to an alcohol group). The initial phase of fragmentation began with the release of water (H_2O), so that the peak fragment appeared at m/z 232, followed by the release of the methyl group (CH_3), resulted in the peak of the fragment appears at m/z 217.

The literature search showed that compound 2 was a 4H-tomentosinee compound with the molecular formula $C_{15}H_{22}O_3$, which was also included in the group of lactone sesquiterpenes (Mustapha et al. 2016; Pawar 2015; Picman 1986). This compound has a molecular weight of 250,322 grams/mol with a structural formula as in Figure 5.

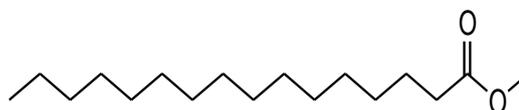


Figure 3. Hexadecanoic acid, methyl ester

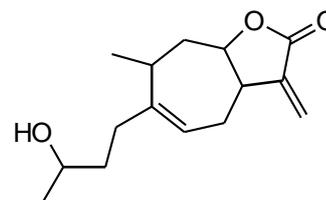


Figure 5. 4H-tomentosinee

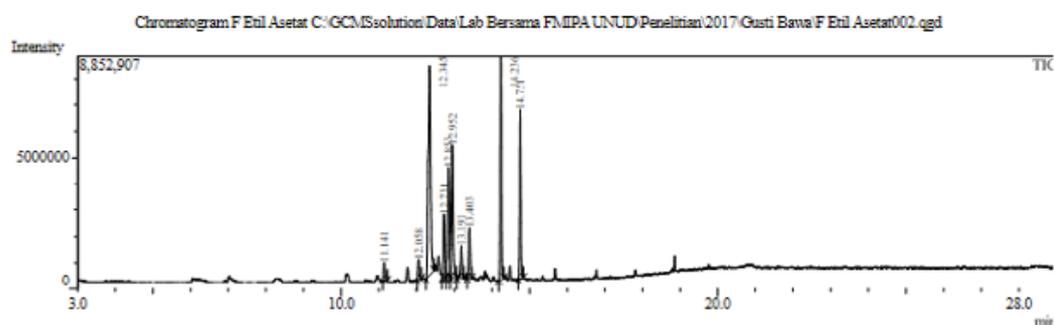


Figure 1. Compounds in white champaca bark analyzed in gas chromatography.

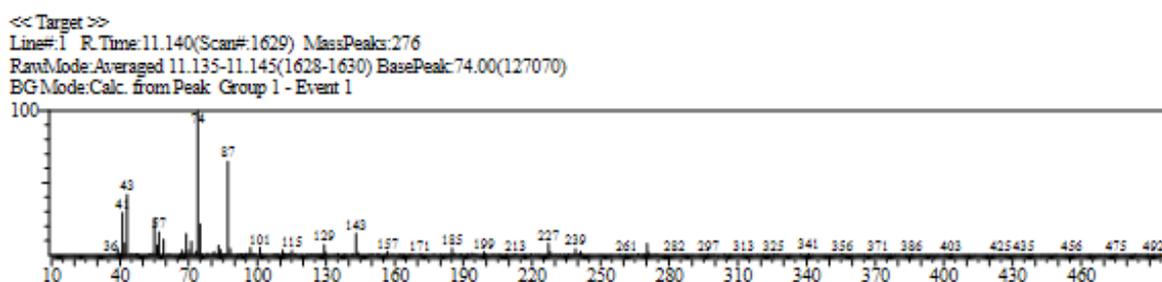


Figure 2. Mass spectra of compound 1

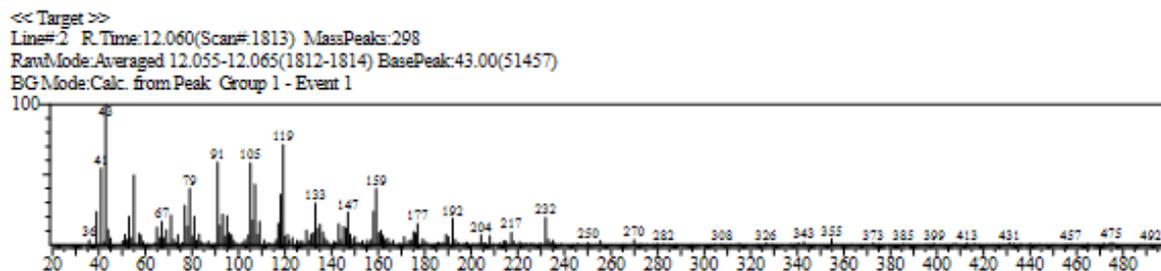


Figure 4. Mass spectra of compound 2.

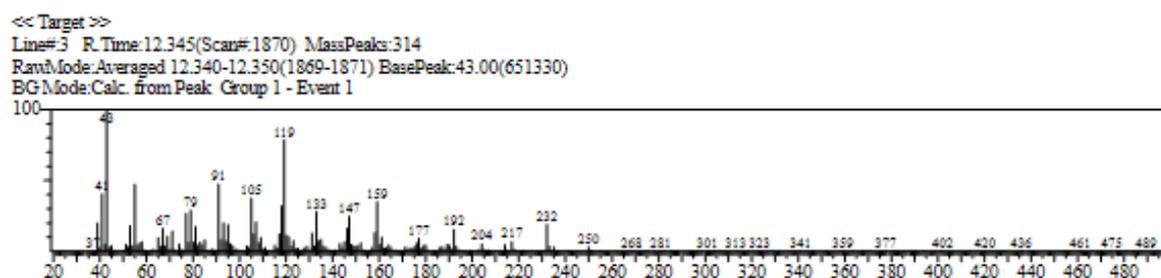


Figure 6. Mass spectra of compound 3

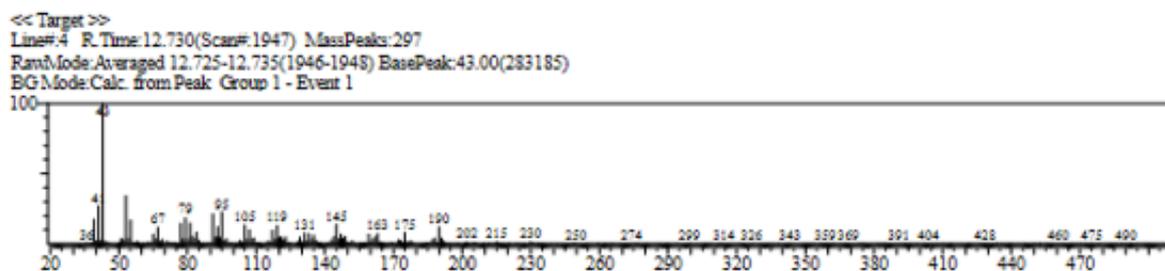


Figure 7. Mass spectra of compound 4

Table 1. Fragmentation pattern of compound 2

m/z	Fragmentation	Fragment lost	Fragment
250	(M ⁺)	-	C ₁₅ H ₂₂ O ₃
232	(M ⁺)-18	H ₂ O	C ₁₅ H ₂₀ O ₂
217	(M ⁺)-18-15	CH ₃	C ₁₄ H ₁₇ O ₂
192	(M ⁺)-18-15-25	C ₂ H	C ₁₂ H ₁₆ O ₂
177	(M ⁺)-18-15-25-15	CH ₃	C ₁₁ H ₁₃ O ₂
133	(M ⁺)-18-15-25-15-44	CO ₂	C ₁₀ H ₁₃
119	(M ⁺)-18-15-25-15-44-14	CH ₂	C ₉ H ₁₁
105	(M ⁺)-18-15-25-15-44-14-14	CH ₂	C ₈ H ₉
91	(M ⁺)-18-15-25-15-44-14-14-14	CH ₂	C ₇ H ₇
67	(M ⁺)-18-15-25-15-44-14-14-14-24	C ₂	C ₅ H ₇
43	(M ⁺)-18-15-25-15-44-14-14-14-24-24	C ₂	C ₃ H ₇

Peak 3 (retention time: 12.345 minutes; abundance 2.26 %)

The mass spectra of compound 3 (Figure 6) has similarity with spathulenol compounds with a molecular weight of 220 gram/mol in the NIST08.LIB Library. The similarity of this compound 3 to the database was 82%.

Figure 6 shows that the fragmentation pattern of compound 3 is very similar to the fragmentation pattern of compound 2 with close retention time, therefore it can be assumed that compound 3 is the isomer of compound 2.

The presence of compound 2 (4H-tomentosinee) as a derivative of the tomentosine compound can have two forms of diastereomers in the form of cis and trans, and 2 forms of enantiomer in the form R and S in position 3 of the butyl group. Based on the fragment pattern, it can be assumed that compound 3 is one isomer of compound 2.

Peak 4 (retention time: 12.730 minutes; abundance 11.98 %)

Figure 7 shows the mass spectra of compound 4. This compound has similarities to pregnenolone or 3-hydroxy-pregn-4-ene-20-one compounds with a molecular weight of 316 gram/mol in the NIST08.LIB Library.

The percentage of similarity of compound 4 to the database was 78%. Fragmentation analysis showed that molecular ion (M⁺) of compound 4 did not appear at m/z 316. The peak fragment shown at m/z 299 was the molecular ion peak after compound 4 loses the hydroxyl

(OH) group. Compound 4 has a base peak at m/z 43. The results of the fragmentation analysis are presented in Table 2.

Based on fragmentation analysis in Table 2, it supports the notion that compound 4 was a pregnenolone compound, which has the molecular formula $C_{21}H_{32}O_2$ and the molecular weight of 316 gram/mol. The structural formula is shown in Figure 8.

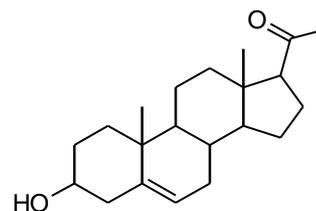


Figure 8. Pregnenolone 3-hydroxy pregn-4-ene-20-one

Peak 5 (retention time: 12.855 minutes; abundance 12.51 %)

Comparing the spectra of compound mass 5 (Figure 9) with the Library of NIST08.LIB shows that the spectra have similarities to tomentosine compounds (Figure 10) with molecular weights of 248 grams mol.

The similarity of compound 5 to the database was 79%. The molecular ion (M^+) compound 5 appeared at m/z 248. The peak fragment at m/z 230 was the peak of the dehydrated compound 5 ($M^+ - H_2O$), then the methyl group ($M^+ - H_2O - CH_3$) was released, so that the peak fragment appeared at m/z 215. Compound 5 has a base peak at m/z 43. The results of the fragmentation analysis are presented in Table 3.

The fragmentation analysis of compound 5 (Table 3) supports the notion that this was a tomentosine compound, which has the molecular formula of $C_{15}H_{20}O_3$ and molecular weight of 248 grams/mol. The structural formula of is shown in Figure 10.

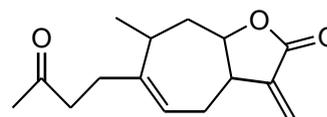


Figure 10. Tomentosine

Table 2. The fragmentation patterns of compound 4

m/z	Fragmentation	Fragment lost	Fragment
316	(M)	-	$C_{21}H_{32}O_2$
299	(M^+) = (M-17)	OH	$C_{21}H_{31}O$
274	(M^+)-25	C_2H	$C_{19}H_{30}O$
230	(M^+)-25-44	C_3H_8	$C_{16}H_{22}O$
215	(M^+)-25-44-15	CH_3	$C_{15}H_{19}O$
190	(M^+)-25-44-15-25	C_2H	$C_{13}H_{18}O$
175	(M^+)-25-44-15-25-15	CH_3	$C_{12}H_{15}O$
145	(M^+)-25-44-15-25-15-30	H_2CO	$C_{11}H_{13}$
119	(M^+)-25-44-15-25-15-30-26	H_2C_2	C_9H_{11}
79	(M^+)-25-44-15-25-15-30-26-40	C_3H_4	C_6H_7
43	(M^+)-25-44-15-25-15-30-26-40-36	C_3	C_3H_7

Peak 6 (retention time: 12.950 minutes; abundance 18.06 %)

The mass spectra of compound 6 are presented in Figure 11. This compound has similarities with the caryophyllene oxide with a molecular weight of 220 grams/mol in the Library of NIST08.LIB.

The similarity of compound 6 to the database was 78%. The fragmentation pattern shows that compound 6 was less likely similar to the caryophyllene oxide, because the fragment peak appeared at m/z 232. Molecular ion (M^+) appeared at m/z 264, with peak base at m/z 43. Fragmentation pattern of compound 6 was more comparable to the fragmentation pattern of 4H-tomentosinee. The peak of the fragment at m/z 250 was the peak of the compound fragment 6 after releasing the methylene group (CH_2). The results of the compound 6 fragmentation analysis are presented in Table 4.

Table 3. Fragmentation pattern of compound 5

m/z	Fragmentation	Fragment lost	Fragment
248	(M^+)	-	$C_{15}H_{20}O_3$
230	(M^+)-18	H_2O	$C_{15}H_{18}O_2$
215	(M^+)-18-15	CH_3	$C_{14}H_{15}O_2$
190	(M^+)-18-15-25	C_2H	$C_{12}H_{14}O_2$
175	(M^+)-18-15-25-15	CH_3	$C_{11}H_{11}O_2$
131	(M^+)-18-15-25-15-44	CO_2	$C_{10}H_{11}$
105	(M^+)-18-15-25-15-44-26	C_2H_2	C_8H_9
91	(M^+)-18-15-25-15-44-26-14	CH_2	C_7H_7
67	(M^+)-18-15-25-15-44-26-14-24	C_2	C_5H_7
43	(M^+)-18-15-25-15-44-26-14-24-24	C_2	C_3H_7

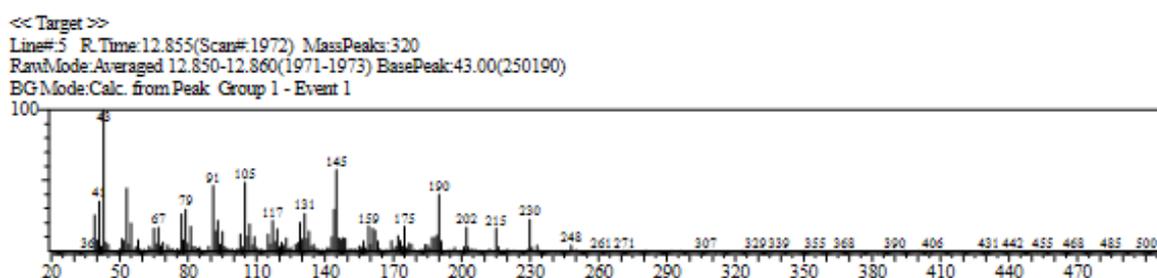


Figure 9. Mass spectra of compound 5

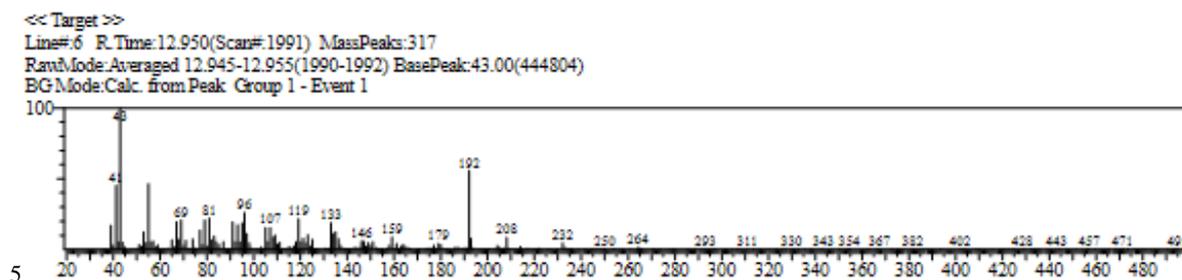


Figure 11. Mass spectra of compound 6

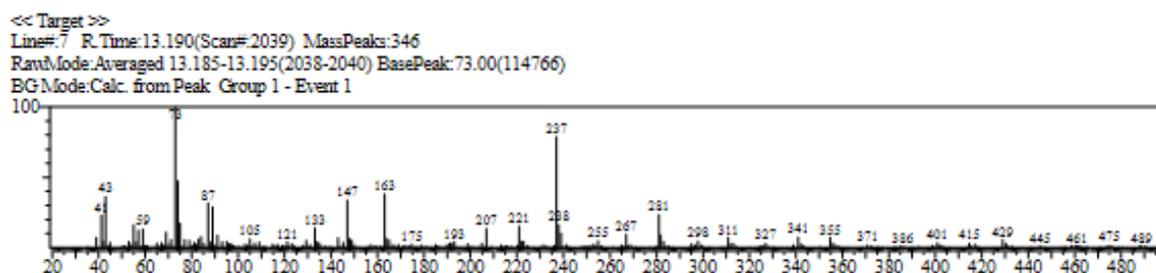


Figure 13. Mass spectra of compound 7

Table 4. Fragmentation pattern of compound 6

m/z	Fragmentation	Fragment lost	Fragment
264	(M ⁺)	-	C ₁₅ H ₂₀ O ₄
250	(M ⁺)-14	CH ₂	C ₁₄ H ₁₈ O ₄
232	(M ⁺)-14-18	H ₂ O	C ₁₄ H ₁₆ O ₃
192	(M ⁺)-14-18-40	C ₂ O	C ₁₂ H ₁₆ O ₂
146	(M ⁺)-14-18-40-46	HCO ₂ H	C ₁₁ H ₁₄
119	(M ⁺)-14-18-40-46-27	C ₂ H ₃	C ₉ H ₁₁
105	(M ⁺)-14-18-40-46-27-14	CH ₂	C ₈ H ₉
81	(M ⁺)-14-18-40-46-27-14-24	C ₂	C ₆ H ₉
67	(M ⁺)-14-18-40-46-27-14-24-14	CH ₂	C ₅ H ₇
43	(M ⁺)-14-18-40-46-27-14-24-14-24	C ₂	C ₃ H ₇

Table 5. Fragmentation pattern of compound 7

m/z	Fragmentation	Fragment lost	Fragment
355	(M ⁺ +1)	-	C ₁₇ H ₃₄ N ₂ O ₂ Si ₂
298	(M ⁺ +1)-56	C ₄ H ₈	C ₁₃ H ₂₆ N ₂ O ₂ Si ₂
281	(M ⁺ +1)-56-17	OH	C ₁₃ H ₂₅ N ₂ Osi ₂
163	(M ⁺ +1)-56-17-118	C ₄ H ₁₂ ONSi	C ₉ H ₁₃ Nsi
73	(M ⁺ +1)-56-17-118-89	C ₃ H ₁₁ Nsi	C ₆ H ₂

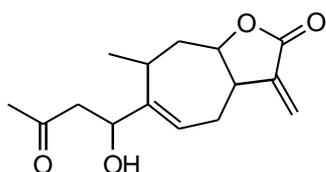


Figure 12. 2-hydroxy tomentosine

Based on the results of fragmentation analysis of compound 6 (Table 4) support that compound 6 was a 2-hydroxy tomentosine that has the molecular formula of C₁₅H₂₀O₄ and molecular weight of 264 grams/mol. The structural formula of compound 6 is presented in Figure 12.

Peak 7 (retention time: 13.190 minutes; abundance 3.29 %)

Mass spectra of compound 7 (Figure 13) has similarities to the tert-butyl-2-aminophenylcarbamate with a molecular weight of 352 grams/mol in the NIST08.LIB Library.

The similarity of compound 7 to the database was 59%. The fragmentation pattern of compound 7 shows that compound 7 was a result of the reduced of tert-butyl-2-aminophenylcarbamate compound, with a molecular weight of 354 grams/mol. However, the mass spectra of molecular ions of compound 7 appear as (M⁺+1) at m/z 355. The results of fragmentation analysis of compound 7 are presented in Table 5.

The results of fragmentation analysis of compound 7 (Table 5) found that the compound was likely to tert-butyl-2-aminophenylcarbamate, as a result of the reduction of tert-butyl-2-aminophenylcarbamate compound, with the molecular formula of C₁₇H₃₄N₂O₂Si₂, molecular weight of 354 grams/mol and structural formula as shown in Figure 14.

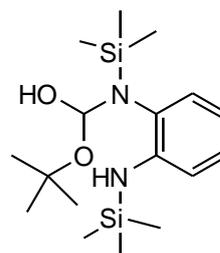


Figure 14. Tert-butyl-2-aminophenylcarbamate

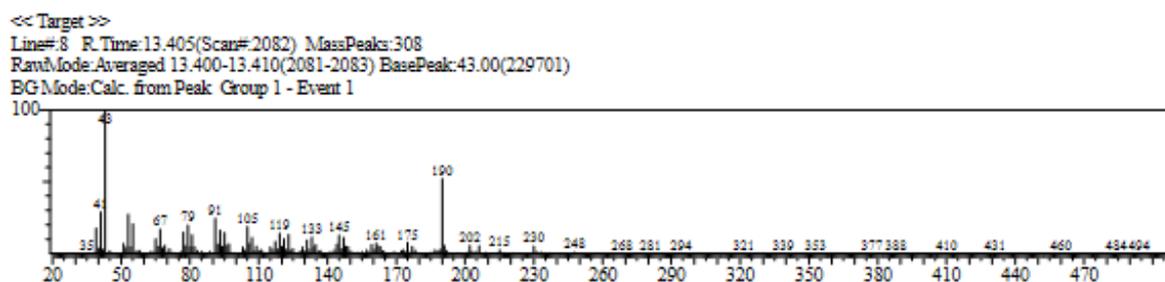


Figure 15. Mass spectra of compound 8

Table 6. Fragmentation pattern of compound 8

m/z	Fragmentation	Fragment lost	Fragment
294	M ⁺	-	C ₁₆ H ₂₂ O ₅
248	(M ⁺)-46	CH ₂ O ₂	C ₁₅ H ₂₀ O ₃
230	(M ⁺)-18	H ₂ O	C ₁₅ H ₁₈ O ₂
215	(M ⁺)-18-15	CH ₃	C ₁₄ H ₁₅ O ₂
202	(M ⁺)-18-15-13	CH	C ₁₃ H ₁₄ O ₂
190	(M ⁺)-18-15-13-12	C	C ₁₂ H ₁₄ O ₂
145	(M ⁺)-18-15-13-12-45	CO ₂ H	C ₁₁ H ₁₃
119	(M ⁺)-18-15-13-12-45-26	C ₂ H ₂	C ₉ H ₁₁
91	(M ⁺)-18-15-13-12-45-26-28	C ₂ H ₄	C ₇ H ₇
43	(M ⁺)-18-15-13-12-45-26-28-48	C ₄	C ₃ H ₇

Table 7. Fragmentation pattern of compound 9

m/z	Fragmentation	Fragment lost	Fragment
308	(M ⁺)	-	C ₁₇ H ₂₄ O ₅
250	(M ⁺)-58	C ₂ H ₂ O ₂	C ₁₅ H ₂₂ O ₃
232	(M ⁺)-58-18	H ₂ O	C ₁₅ H ₂₀ O ₂
217	(M ⁺)-58-18-15	CH ₃	C ₁₄ H ₁₇ O ₂
192	(M ⁺)-58-18-15-25	C ₂ H	C ₁₂ H ₁₆ O ₂
177	(M ⁺)-58-18-15-25-15	CH ₃	C ₁₁ H ₁₃ O ₂
133	(M ⁺)-58-18-15-25-15-44	CO ₂	C ₁₀ H ₁₃
107	(M ⁺)-58-18-15-25-15-44-26	C ₂ H ₂	C ₈ H ₁₁
81	(M ⁺)-58-18-15-25-15-44-26-26	C ₂ H ₂	C ₆ H ₉
67	(M ⁺)-58-18-15-25-15-44-26-26-14	CH ₂	C ₅ H ₇
43	(M ⁺)-58-18-15-25-15-44-26-26-14-24	C ₂	C ₃ H ₇

Peak 8 (retention time: 13.405 minutes; abundance 8.33 %)

Figure 15 shows the mass spectra of compound 8. This compound has similarities to tomentosine, with a molecular weight of 248 grams/mol (NIST08.LIB Library).

The similarity of compound 8 to the database was 77%. Based on the fragmentation pattern, compound 8 was very similar to the fragmentation pattern of the tomentosine, it can be assumed that compound 8 was one of the derivatives of tomentosine. The molecular ion of compound 8 appears as (M⁺) at m/z 294, which is the result of a formylation reaction of the tomentosine in position 2 of the butyl group, with molecular formula of C₁₆H₂₂O₅. The results of the fragmentation analysis of compound 8 are shown in Table 6.

The fragmentation analysis of compound 8 (Table 6) supports the notion that compound 8 was a 2H-cyclohepta[b]furan-2-one, 3,3a, 4,7,8,8a-hexahydro-7-

methyl-3-methylene-6-(2-formyl-3-oxobutyl)-which has a molecular weight of 294 grams/mol, with the molecular formula of C₁₆H₂₂O₅ and the structural formula as shown in Figure 16.

Peak 9 (retention time: 14.235 minutes; abundance 20.81 %)

Spectra of compound 9 (Figure 17) has similarities to the isoaromadendrene epoxide compound, with molecular weight of 220 gram/mol in Library NIST08.LIB.

The percentage similarity of compound 9 to the database was 85%. The fragmentation pattern of compound 9 did not follow the fragmentation pattern of isoaromadendrene epoxide. This can be seen from the absence of molecular ion (M⁺) isoaromadendrene epoxide compound at m/z 220 and the emergence of fragmentation peaks at m/z 232. The pattern of fragmentation of compound 9 was more similar to the pattern of 4H-tomentosinee (Figure 3), with molecular weight of 250 gram/mol. Therefore, it can be assumed that compound 9 was a derivative compound of 4H-tomentosinee. The molecular ion compound 9 was thought to appear as (M⁺) at m/z 308. The results of the fragmentation analysis of compound 9 are presented in Table 7.

The fragmentation analysis of compound 9 is shown in Table 7. It is assumed that the compound 9 was an isoxanthanol, which has the molecular formula of C₁₇H₂₄O₅ and a molecular weight of 308 grams/mol. The structural formula of compound 8 is presented in Figure 18.

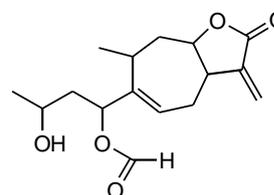


Figure 16. 2H-cyclohepta[b]furan-2-one, 3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-6-(2-formyl-3-oxobutyl)

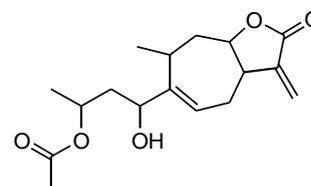


Figure 18. Isoxanthanol

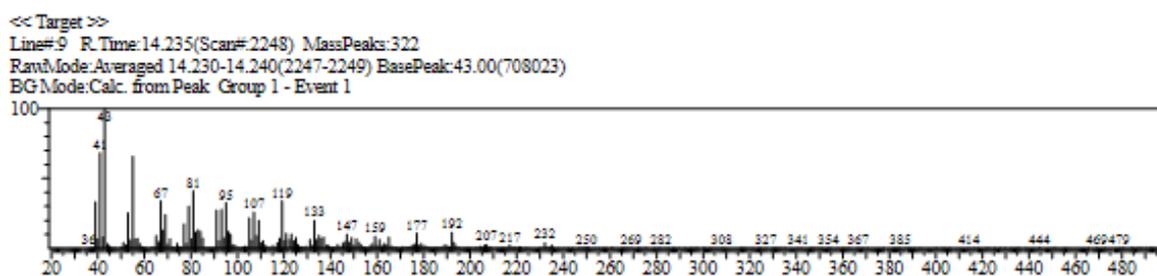


Figure 17. Mass spectra of compound 9

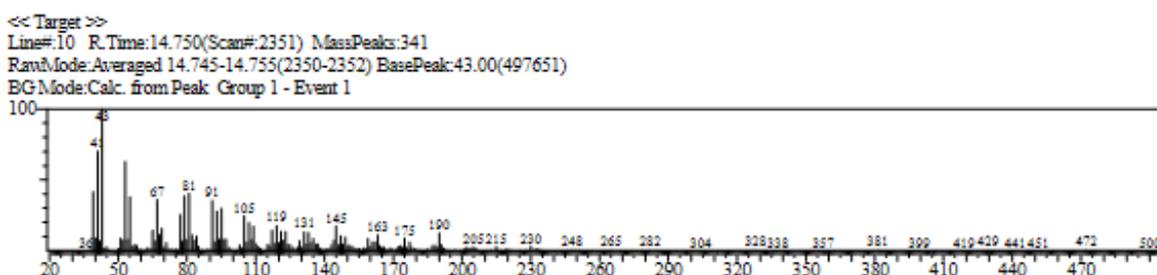


Figure 19. Mass spectra of compound 10

Table 8. Fragmentation pattern of compound 10.

m/z	Fragmentation	Fragment lost	Fragment
308	M	-	C ₁₇ H ₂₄ O ₅
248	(M ⁺) = M-60	C ₂ H ₄ O ₂	C ₁₅ H ₂₀ O ₃
230	(M ⁺)-18	H ₂ O	C ₁₅ H ₁₈ O ₂
215	(M ⁺)-18-15	CH ₃	C ₁₄ H ₁₅ O ₂
190	(M ⁺)-18-15-25	C ₂ H	C ₁₂ H ₁₄ O ₂
175	(M ⁺)-18-15-25-15	CH ₃	C ₁₁ H ₁₁ O ₂
131	(M ⁺)-18-15-25-15-44	CO ₂	C ₁₀ H ₁₁
105	(M ⁺)-18-15-25-15-44-26	C ₂ H ₂	C ₈ H ₉
91	(M ⁺)-18-15-25-15-44-26-14	CH ₂	C ₇ H ₇
67	(M ⁺)-18-15-25-15-44-26-14-24	C ₂	C ₅ H ₇
43	(M ⁺)-18-15-25-15-44-26-14-24-24	C ₂	C ₃ H ₇

Table 9. Chemical compound content from methanol extracted barks of *C. alba*.

Compounds	Groups	Abundance (%)
Hexadecanoic acid, methyl ester	Fatty acid esters	6.07
4H-tomentosine	Terpenoids	2.48
4H-tomentosine (isomer from compound 2)	Terpenoids	2.26
3-hydroxy pregn-4-ene-20-one	Steroids	11.98
Tomentosine	Terpenoids	12.51
2-hydroxy tomentosine	Terpenoids	18.06
Tert-butyl-2-aminophenylcarbamol	Fatty acid esters	3.29
2H-cyclohepta[b]furan-2-one,	Terpenoids	8.33
3,3a,4,7,8a-hexahydro-7-methyl-3-methylene-6-(2-formyl-3-oxobutyl)-		
Isoxanthanol	Terpenoids	20.81
Xanthanol	Terpenoids	14.20

Peak 10 (retention time: 14.750 minutes; abundance 14.20 %)

The mass spectra of compound 10 are presented in Figure 19. This compound has similarities with the compound ledene oxide with a molecular weight of 220 gram/mol (NIST 08. Library).

The percentage similarity of compound 10 to the database was 83%. The fragmentation pattern of compound 10 did not follow the fragmentation pattern of the ledene oxide compound. This can be seen from the absence of the molecular ion (M⁺) of the compound at m/z 220. The similarity of fragmentation pattern of compound 10 close to the fragmentation pattern of the tomentosine compound, with a molecular weight of 248 grams/mol. Therefore, it can be presumed that compound 10 was a derivative of tomentosine. The ion fragment at m/z 248 was thought to be the top of the compound fragment 10, after releasing the acetic acid (CH₃COOH). As a result, compound 10 has molecular ions at m/z 308, which did not appear on the chromatogram. The results of fragmentation analysis of compound 10 are presented in Table 8.

The results of fragmentation analysis of compound 10 (Table 8) showed that the compound was assumed as xanthanol, which has the molecular formula of C₁₇H₂₄O₅ and a molecular weight of 308 grams/mol. The structural formula is shown in Figure 20.

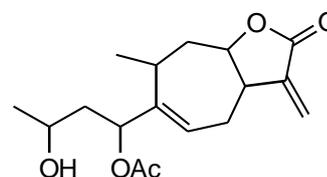


Figure 20. Xanthanol

The abundance chemical compound, the group and the abundance of chemical compound extracted from *C. alba* are presented in Table 9. It was shown that the majority of 10 group compounds was terpenoid (7 out of 10 compounds), 2 belonged to the group of fatty acid and 1 steroid.

Discussion

The compounds of hexadecanoic acid, methyl ester (compound 1) and Tert-butyl-2-aminophenylcarbamol (compound 7) belong to the group of fatty acid ester compounds.

The hexadecanoic acid, methyl ester compounds were also found in non-polar extracts of *Albizia adianthifolia* (Schumach) and *Pterocarpus sangolensis* (DC), which were found functioning as antibacterial (Mustapha et al. 2016). Similar compounds were also found in the ethanol extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms, which have antioxidant activity, as hemolytic, hypocholesterolemic, flavor, nematicide, anti-androgenic (Sudha et al. 2013; Tyagi and Agarwal 2017), pesticide, hemolytic, and 5-Alpha reductase inhibitor (Sudha et al. 2013; Venkatesan and Sarada 2017).

Chandrasekaran et al. (2008) stated that the compound of hexadecanoic acid and methyl ester were found as antioxidants, anticancer, 5- α -reductase inhibitors, antimicrobial, hemolytic and antifibrinolytic activities, which were extracted from *Boswellia ovalifoliolata*. Fatty acid methyl ester compounds are found in four halophytic plants, those were from *Arthrocnemum mindicum*, *Salicornia brachiata*, *Suaeda maritima*, and *Suaeda monoica* which belong to the *Chenopodiaceae* family. These compounds were identified as antibacterial and antifungal.

The compounds of H-tomentosine (compounds 2 and 3); Tomentosine (compound 5); 2-hydroxy tomentosine (compound 6); 2H-cyclohepta [b] furan-2-one, 3,3a, 4,7,8,8-hexahydro-7-methyl-3-methylene-6-(2-formyl-3-oxobutyl)-(compound 8); Isoxanthanol (compound 9) and Xanthanol (compound 10) are belong to the lactone sesquiterpenic derivative compounds (Rozenblat et al. 2008).

Lactone sesquiterpenic compounds were found in Asteraceae (Compositae) which belonged to the angiosperm family. This compound has cytotoxic, anti-tumourgenic, anti-bacterial and anti-fungal activity (Picman 1986). In addition, the lactone sesquiterpenic compound was found in the genus of *Centaurea* L. (Asteraceae) has antimicrobial, antioxidant, cytotoxic, anti-inflammatory activity (Sokovic et al. 2017). Four new sesquiterpene lactones, those were 8 α -(2'Z-tigloyloxy)-hirsutinolide, 8 α -(2'Z-tigloyloxy)-hirsutinolide-13-O-acetate, 8 α -(4-hydroxytigloyloxy)-hirsutinolide, and 8 α -hydroxy-13-O-tigloyl-hirsutinolide, isolated from leaves and stems of *Vernonia cinerea* extracted in chloroform-methanol. These compounds were found to impede the growth of glioblastoma U251MG breast cancer cells and MDA-MB-231 (Youn et al. 2014). Sesquiterpene lactones were also found contained in the *Artemisia* genus, which functioning as antitumor, anti-inflammatory, analgesic,

anti-ulcer, antibacterial, antifungal, antiviral, antiparasitic, and as insect deterrent (Ivanescu et al. 2015).

The compounds of 3-hydroxy pregn-4-ene-20-one or pregnenolone are steroid hormone, including progesterogens, androgens, estrogens, glucocorticoids, and mineralocorticoids. In addition, pregnenolone is biologically active in itself, acting as a neurosteroid (Henderson et al. 1950; Marx et al. 2011). Chepkirui et al. (2018) reported that he had successfully isolated 5 previously unknown pregnenolone compounds from Kenyan basidiomycete *Fomitiporia aethiopica*. The compound was named trivial aethiopinolones A-E. This compound shows have cytotoxic effect on various human cancer cells.

Crude methanol extract of white champaca bark at a concentration of 1.0% is able to perforate the *C. verruculosa* fungal cell membrane which results in nutrient leakage, which causes the fungus to stunt its growth and even die. In addition, at a concentration of 2.0%, the extract was able to plasmolysis fungal cells so that the fungal hyphae became shrunk and eventually the fungus died (Bawa 2019). More details Bawa (2019) explains the application of white champaca bark extract in a greenhouse shows that at a concentration of 1.5% can reduce the intensity of leaf spot disease in Cihang rice plants caused by *C. verruculosa* fungus to 62.68% and increase production yields at 60.62% (Bring 2019).

In summary, based on the research, it can be concluded that the methanol extract of white champaca (*M. alba*) bark actively inhibited the growth of *C. verruculosa* the cause of leaf spot disease on rice. The extract containing 10 chemical compounds consisting of 2 fatty acid ester compounds; 8 secondary metabolites (7 terpenoid groups, and 1 steroid group). Field testing is needed to use the formula of crude champaca whitebark extract to control leaf spot disease in rice plants.

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REFERENCES

- Bawa IGAG. 2011. Antioxidant and antifungal activity of *Michelia alba* flowers. *J Chem* 5 (1): 43-50.
- Bawa IGAG. 2019. Senyawa Aktif dan Aktivitas Fungisida Ekstrak Kulit Kayu Cempaka Putih (*Michelia alba*) Terhadap Jamur *Curvularia verruculosa* Penyebab Penyakit Bercak Daun pada Tanaman Padi (*Oryza sativa* L.). [Disertasi]. Fakultas Pertanian Universitas Udayana, Bali. [Indonesian]

- Chandrasekaran M, Kannathasan K, Venkatesalu V. 2008. Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. *Z Naturforsch C* 63 (5-6): 331-336.
- Chepkirui C, Sum W, Cheng T, Matasyoh JC, Decock C, Stadler M. 2018. Aethiopinolones A-E, New pregnenolone type steroids from the East African Basidiomycete *Fomitiporiaaethiopica*. *Molecules* 23: 369-378.
- Henderson E, Weinberg M, Wright WA. 1950. Pregnenolone. *J Clin Endocrinol Metab* 10 (4): 455-474.
- Huang LY. 2008. *Magnolia* Chemical Composition and Cytotoxicity Studies. <http://fedetd.mis.nsysu.edu.tw/FED-db/cgi-bin/FED-search>.
- Ivanescu B, Miron A, Corciova A. 2015. Sesquiterpene lactones from *Artemisia* Genus: Biological activities and methods of analysis. *J Anal Methods Chem* 2015: 1-21.
- Krisdiana D. 2010. Isolation, characterization, identification of components and test of essential oil activity of *Michelia alba* flowers Against *Escherichia coli* and *Staphylococcus aureus*. <http://karyailmiah.um.ac.id/index.php/kimia/article/view/6168>. [Indonesian]
- Kumar VR, Kumar S, Shashidhara S, Anitha S, Manjula M. 2011. Antioxidant and antimicrobial activities of various extracts of *Michelia champaca* Linn flowers. *Deccan J Pharmacol* 2 (1): 28-39.
- Lee CH, Chen HL, Li HT, Chao WY, Chen C. 2014. Review on pharmacological activities of *Michelia alba*. *Intl J Pharm Therapeutics* 5 (4): 289-292.
- Mangang HC, Chetry GKN. 2012. Antifungal properties of certain plant extract against *Rhizoctonia solani* causing root rot of french bean in organic soil of Manipur. *Intl J Sci Reas Pub* 2 (5): 1-4.
- Marx C E, Bradfor DW, Hamer RM. 2011. Pregnenolone as a novel therapeutic candidate in schizophrenia: emerging preclinical and clinical evidence. *Neurosci* 191: 78-90.
- Mustapha N, Abubakar, Runner RTM. 2016. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). *Medicines* 3 (3): 3-9.
- Pawar BT. 2015. Antifungal activity of some latex against seed-borne pathogenic fungi. *J Biol Chem Research* 32 (1): 183-187.
- Picman, AK. 1986. Biological activities of sesquiterpene lactones. *Biochem System Ecol* 14 (3): 255-281.
- Punjee P, Dilokkunanant U, Sukkatta U, Vajrodaya S, Haruethaitanasan V, Pitpiangchan P, Rakhaworn P. 2009. Scented extracts and essential oil extraction from *Michelia alba* D.C. *Kasetsart J Nat Sci* 43: 197-203.
- Rozenblat S, Grossman S, Bergman M, Gottlieb H, Cohen Y, Dovrat S. 2008. Induction of G2/M arrest and apoptosis by sesquiterpene lactones in human melanoma cell lines. *Biochem Pharmacol* 75 (2):369-382.
- Sanimah S, Suri R, Nor-Azizun R, Hazniza A, Radzali M, Rusli I, Hassan MD. 2008. Volatile compounds of essential oil from different stages of *Michelia alba* flower development. *J Trop Agric Food Sci* 36 (1): 109-119.
- Semangun H. 2006. Food Crop Diseases in Indonesia. Gadjah Mada University Press, Yogyakarta. [Indonesian]
- Shang C, Hu Y, Deng C, Hu K. 2002. Rapid determination of volatile constituents of *Michelia alba* flowers by Gas Chromatography-Mass Spectrometry with Solid-Phase Microextraction. *J Chromatogr* 942: 283-288.
- Sinha R, Varma R. 2016. *Michelia champaca* L. (Swarna Champa): A Review. *Intl J Enh Res Sci Technol Eng* 5 (8): 78-82.
- Sokovic M, Ciric A, Glamoclija J, Skaltsa H. 2017. Biological activities of sesquiterpene lactones isolated from the Genus *Centaurea* L. (Asteraceae). *Curr Pharm* 23 (19): 2767-2786.
- Subcharoen P. 1999. Herbal and Thai Culture: Part III, Aromatic Plants, The Institute of Thai Traditional Medicine Printing, Bangkok.
- Sudha T, Chidambarampillai S, Mohan VR. 2013. GC-MS analysis of bioactive components of aerial parts of *Fluggea leucopyrus* Willd. (Euphorbiaceae). *J Appl Pharm Sci* 3 (05): 126-130.
- Tyagi T Agarwal M. 2017. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms. *J Pharm Phytochem* 6 (1): 195-206.
- Venkatesan G, Sarada NC. 2017. GC-MS analysis of bioactive compounds from bark and leaf of *Boswellia ovalifoliolata*. *Asian J Chem* 30 (1): 15-19.
- Youn UJ, Miklossy G, Chai X, Wongwiwatthananut S, Toyama O, Songsak T, Turkso J, Chang LC. 2014. Bioactive sesquiterpene lactones and other compounds isolated from *Vernonia cinerea*. *Fitoterapia* 93: 194-200.