Antifungal and Antioxidant Activities of Lipophilic Compounds from *Swietenia mahagoni* (l.) Jacq. Leaves

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Abstract

The investigation on the *n*-hexane lipophilic extractives of *Swietenia mahagoni,* alongside the antifungal and antioxidant properties was conducted. The leaf sample was collected from 2- and 3-years-old trees in *Perhutani* enterprise of Temanggung, Central Java, Indonesia. In addition, the antifungal activity was tested using the white-rot of *Phanerochaete chrysosporium,* while the antioxidant property involved the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The results show antifungal of 28.6% and 37.5% growth inhibition in 2- and 3 -years-old leaf, respectively, while 21.7% and 25.4% antioxidant activity were also recorded. Furthermore, the GC-MS results showed the domination of *S. mahagoni* leaf by fatty acids and hydo-carbons, while the terpenoids and steroids were in minor quantities. These components possibly exhibit growth inhibitory and antioxidant effects against *P. chrysosporium* and DPPH radicals, respectively.

Keywords: *S. mahagoni*, leaves, lipophilics, antifungal, antioxidant, hydrocarbons

Introduction

Swietenia mahagoni (L.) Jacq. is a member of the Meliaceae family, which is native to Jamaica, Hispaniola, The Bahamas, Southern Florida and Cuba. This species has been introduced and utilized in plantations located in South East Asian countries, including Indonesia (Panda *et al.* 2010; Rahman *et al.* 2014), where it was chosen as a species for afforestation, through *Perhutani* (state own enterprise). Furthermore, *S. mahagoni* has good characteristics as a fancy wood with beautiful dark red color and the timber has been utilized for home building and furnitures.

Various parts of the *S. mahagoni* have been utilized traditionally in the remediation of various disease, including diabetes, diarrhea, malaria, and hypertension. Previous researchers have also identified a tetracyclic triterpene namely cyclomahogenol (Chakraborty *et al*. 1971), alongside two limonoids, termed swiemahogins A and B (Chen *et al*.; Naveen *et al*. 2014), as well as fatty acids and hydrocarbons in the plant leaves (Mousa *et al*. 2014)

Essential fatty acids are one of the dominant lipophilic components of the leaves, possibly extracted using nonpolar solvents, e.g., *n*-hexane. Moreover, the interaction between antifungal compounds and fungi are assumed to occur following a either direct or indirect connection between the fatty acid component and cell membrane (Avis and Bélanger 2001). Conversely, some edible plants, including *Urtica dioica*, *Cardamine hirsuta*, *Cichorium intybus* and others containing carotenoids and tocopherols were reported as good sources of lipophilic antioxidant (Burton and Traber 1990; Lagouri 2015).

S. mahagoni is one of the potent sources of allelopahty, which inhibits the growth and development after a plant to plant interaction (Gniazdowska and Bogatek 2005). These bioactive compounds also tend to play roles against other organisms, e.g., fungi, resulting from the ability to neutralize free radicals as an antioxidant agent. However, there are limited reports focused on evaluating the antioxidant and antifungal activity of lipophilic components of *S. mahagoni* leaf. This research is, therefore, aimed at investigating the *n*-hexane extracts of *S. mahagoni* leaf, and also the possible investigate the antifungal and antioxidant activities.

Materials and Methods

Sample Collection and Extraction

The young leaf of *S. mahagoni* selected from the 2 and 3 years-old trees were collected from *Perhutani* Enterprise in Temanggung, Central Java, Indonesia. A total of three individual trees were selected for each age, and the green leaf were cut into small pieces of 15 g weight. These were successively macerated in *n*-hexane for three days at room temperature, followed by the evaporation of solutions to dryness, using a rotary evaporator machine, and the output was then weighed. The yield of extractives was calculated on the basis of fresh leaf sample.

Antifungal Activity

The antifungal test required the use of *Phanerochaete chrysosporium* (white-rot), and prior to assay, the fungi strain was incubated on a PDA medium petri dish at 25±1 °C in order to ensure that most of the plate surfaces are covered. Therefore, the growth inhibitory capacity of *S. mahagoni n*-hexane extracts against *P. chrysosporium* was conducted according to Lukmandaru (2013), where the 1000 ppm concentration sample was prepared, and 300 µl of extract was spread on the surface of 20 ml PDA medium in a Petri-dish (diameter of 9 cm) with final concentration of

12.3 mg/cm^2. Therefore, the solution was dried at a room temperature in 1 h prior to inoculation, and a blank experiment was performed on the sample without extract. These tests were conducted in three replications, while the steroid, β-sitosterol, isolated from *P. merkusii* bark was adopted as the positive control. The growth inhibition capacity was evaluated using equation 1 as follows:

$$\text{Growth inhibition (\%)} = \frac{A0-A1}{A0}\ x100 \quad (1)$$

Where A = π x (d/2)^2, d = diameter of sample growth, A_0 is growth inhibition of blank and A_1 is growth inhibition of sample.

Determination of DPPH Radicals Scavenging Activity or Antioxidant Activity

The antioxidant activity was evaluated by reacting 0.1 ml extract in dimethyl sulfoxide (DMSO) with a concentration of 1000 ppm and 3 ml of DPPH (1,1-diphenyl-2-picrylhydrazyl) 0.004% in methanol. The reaction product stood for 30 minutes at ambient temperature, and the sample absorbance was read at 517 nm, using the UV-Vis spectrophotometer (model SP-3000 Nano, Optima, Tokyo, Japan). Furthermore, the antioxidant activity was calculated using equation 2:

$$\text{DPPH scavenged (\%)} = \frac{A0-A1}{A0}\ x100 \quad (2)$$

where A_0 and A_1 represent the absorbance of blank and sample, respectively (Baba and Malik 2015).

GC-MS Analysis

The GC-mass spectrometry (GC-MS) data were obtained using a GCMS-QP 2010 (Shimadzu, Japan), after 1 µl of sample (1 mg/ml) was directly injected to the machine, under the following condition: Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan); temperature of column ranging from 100 °C (1 min) to 320 °C at 5 °C/min; injection at 250 °C; and detection at 320 °C; acquisition mass range of 50-700 amu (?), using helium as the carrier gas. The sample mass spectrum was compared to data collected at the NIST library, while the peak relative method was applied for the quantification of individual substances.

Statistical Analysis

The results from two different ages (2 and 3 years-old) were analysed with the independent t-test (95% confidence level), using SPSS 25 (IBM, USA).

Chemicals

Gallic acid (97.5%), quercetin (≥95%), 1,1-diphenyl-2-picrylhydrazyl, and potato dextrose agar (PDA) were purchased from Sigma Aldrich (Germany).

Results and Discussion

Extractives Content

The *n*-hexane leaf extraction of *S. mahagoni* yielded 0.9% and 0.7% for the 2- and 3-years-old leaf, respectively, based on the fresh sample. Therefore, Independent t-test was performed to ascertain the effects of aging on the yields, indicating the amount of *n*-hexane extract affected by age of trees ($p < 0.02$) (Table 1).

In comparison, the yield was lower using *n*-hexane than petroleum ether for both *S. mahagoni* and *S. macrophylla* leaves from Egypt, with values ranging from 1.9 to 2.1 % (Mousa *et al*. 2014). The polarity index between *n*-hexane and petroleum ether are about similar (0.1). This indicates that the lower result of yield extractives than study reported by Mousa *et al.* (2014) is might due to different cite of sample rather than the use of the solvent. Further, the yield and composition of extractives tend to also vary with respect to age (Pereira 1988) and species (Wilkes 1984). However, the experiment of this study has a comparable similar range of value with others, including the four species of *Terminalia* leaf at 0.74 to 1.89 % (Rakholiya *et al*. 2015).

Table 1. Independent t-test of each parameter

Parameter	t-test for equality of means		
	T	Sig. (2-tailed)	df
n-Hexane extract	0.45	0.02*	4
Fungal growth inhibition	-0.68	0.54ns	4
Radical scavenging activity	-1.28	0.27ns	4
Mono- and sesquiterpenes	1.73	0.16ns	4
Fatty acids and hydrocarbons	-3.12	0.04*	4
Steroid and triterpenoid	0.31	0.77ns	4

ns=not significant, *=significant at 0.05 level

GC-MS Analysis

The GC-MS analysis detected the following lipophilic constituents: monoterpenes-sesquiterpenes, fatty acids-hydrocarbons, and streoids-triterpenoids, as shown in Table 2 and Figure 1. Based on the ages, significant differences were only recognized in the concentration of fatty acids and hydrocarbons group ($p = 0.04$), while mono- and sesquiterpenes, as well as steroids and triterpenoids group were not considerably affected by age ($p > 0.05$) (Table 1). In addition, the 2-years-old samples possessed relatively higher concentrations of mono-sesquiterpenes, including cyclohexanebutanol, perhydroazulene, and blumenol C, and a similar trend was observed with the steroids and triterpenoids. However, the reverse pattern was observed in terms of fatty acid and hydrocarbon content, while octacosane was the most dominant hydrocarbon in all samples, which was followed by tetratetracontane, hentriacontane, and heneicosane. A previous study by Mousa *et al*. (2014) reported on the presence of eicosane, heneicosane, and octacosane in the leaf of *S. mahagoni, S. macrophylla*, and *Trichilia connaroides* (Senthilkumar *et al*. 2012; Hossain *et al*. 2013).

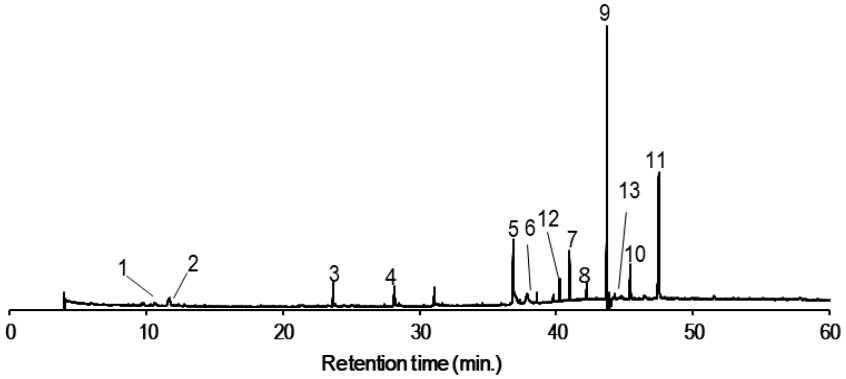

Figure 1. GC-MS chromatogram of *n*-hexane extracts of *S. mahagoni* leaf; 1. Cyclohexanebutanol, 2. Perhydroazulen, 3. Blumenol C, 4. Pentadecanoic acid, 5. Eicosane, 6. 2-methylhexacosane, 7. Heneicosane, 8. 2-methyloctacosane, 9. Octacosane, 10. Hentriacontane, 11. Tetracontane, 12. Squalene, 13. Stigmast-5-en-3-ol, oleate.

With regard to the steroids and triterpenoids present, a comparably higher amount of squalene was detected in the 2-years-old samples. It is assumed that the amount of squalene was changed along with the growth of the age depending on variety either increased or decreased. Squalene is considered as a precursor of sterols and triterpenoids. Hence, the lower amount of squalene signified that squalene has been converted to other phytomolecules. This indicates that squalene at the age of 3 years has been converted to other components so that its content decreased. Related to the presence of squalene in the plants, previous studies have also identified squalene in the leaves of *Dysoxylum mollissimum*, *Trichilia claussenii* and *Dysoxylum gaudichaudianum,* included in the Meliaceae family (Ragasa *et al*. 2013; Ragasa *et al*. 2014; Pupo *et al*. 1996).

Table 2. *n*-hexane extract compounds from *S. mahagoni* leaf

No	Ret. time [minute]	Constituents	Concentration [%]		Similarity index [%]
			2 yr	3 yr	
		Mono- and sesquiterpene	***3.36***	***0.39***	
1	11.58	Cyclohexanebutanol	0.56	0.24	72
2	11.70	Perhydroazulene	1.32	0.16	77
3	23.66	Blumenol C	1.49	tr	92
		fatty acids and hydrocarbons	***87.12***	***94.45***	
4	28.14	Pentadecanoic acid	0.87	3.19	73
5	38.58	Eicosane	0.65	0.22	91
6	39.79	2-methylhexacosane	0.55	0.45	88
7	40.97	Heneicosane	3.77	3.99	95
8	42.23	2-methyloctacosane	1.95	0.69	94
9	43.74	Octacosane	38.63	53.19	95
10	45.41	Hentriacontane	7.97	3.54	96
11	47.51	Tetracontane	32.72	29.17	97
		Steroid and triterpenoid	***3.81***	***3.43***	
12	40.25	Squalene	2.71	2.99	97
13	44.27	Stigmast-5-en-3-ol, oleate	1.10	0.44	54

Antifungal Activity

Figure 2 shows the result of antifungal evaluation, where the leaf of 2 years-old *S. mahagoni* had lower activity than the 3 years leaf. However, no significant differences were observed using t-test ($p > 0.05$) for that fungal measurement between 2 and 3 years leaves, as shown in Table 1. Compared to positive control of β-sitosterol, the both leaves outcomes were in the lower inhibition level. The growth inhibition tendencies were 28.6% and 37.5%, respectively, which was half the activity of the positive control (54.51%).

Older leaves showed higher antifungal activity compared to younger leaves. Similar result was reported from previous research on *Melaleuca leucadendron* leaf oil, where in some concentration oils from 5 years old trees showed lower antifungal activity against *Fusarium oxysporum*, *Thanatephorus cucumeris*, and *Rhizopus oryzae* compared to oils from 10 years old trees (Pujiarti *et al*. 2012). This result might be caused by the higher concentration of certain compounds from the older tree. The appearance of antifungal effect of *S. mahagoni* leaf extracts and β-sitosterol are shown in Figure 3.

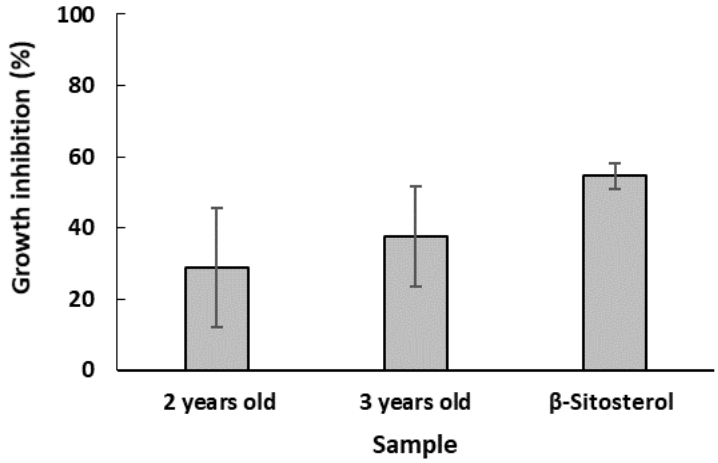

Figure 2. Antifungal activity of *n*-hexane extracts of *S. mahagoni.*

The GC-MS results showed the relatively higher amount of fatty acids and hydrocarbons in both samples, compared to other groups. This phenomenon possibly influences the growth inhibition characteristics of *S. mahagoni n*-hexane extracts against *P. chrysosporium*. In addition, the antifungal performance was attributed to the presence of four hydrocarbon compounds, encompassing octacosane, tetracontante, hentriacontane, and heneicosane. Those constituents were predicted to act as antifungal agents in *Cryptococcus neoformans* (Passos *et al*. 2002), while a prior study has shown the presence of comparably higher hydrocarbon amounts in seeds and leaves of *Caryocar brasiliensis*.

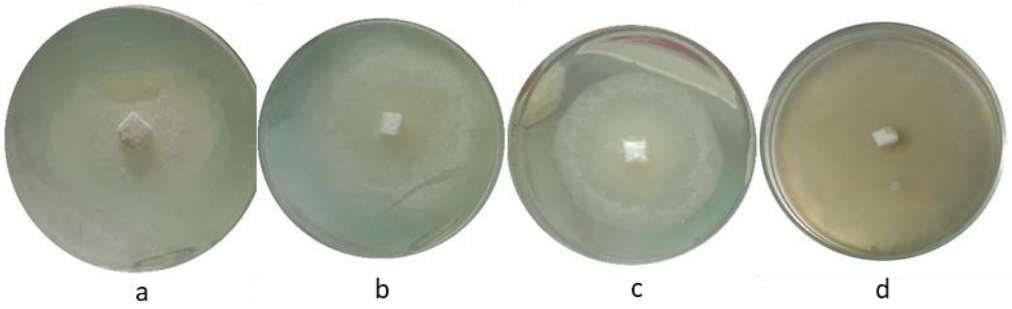

Figure 3. Appearance of growth rate of *P. chrysosporium* against *n*-hexane extracts from 2 -years-old leaf (a), 3 -years-old leaf (b), β-sitosterol (c), and control (d)

In addition, the leaf of *S. mahagoni* also contained low levels of mono- sesquiterpene, triterpenes, and steroids, suggested to confer inhibitory properties against *P. chrysosporium*. In addition, the monoterpene and sesquiterpene presented with hydrocarbon (perhydroazulene) and oxygenated structures (cyclohexanebutanol and blumenol C), and antifungal activity is demonstrated, despite their minor quantity.

Other constituents detected in *S. mahagoni* were related to the triterpenoids and steroids groups, where squalene and stigmast-5-en-3-ol oleate were identified in low concentrations. Furthermore, the *n*-hexane leaf extracts are generally known to contain low molecular terpenes and waxes, which belonged to the lipophilic fraction, and are thus capable of exhibiting antifungal activities (Ribera and Zuñiga 2012). Lukmandaru (2013) reported the inhibitory effect of squalene and triterpene on the mycelium growth of *Cladosporium cladosporiedes,* while cytotoxic activity of triterpenoids, e.g., squalene, extracted from the leaves of *Dysoxylum gaudichaudianum* have previously been reported against colon and breast cancer cells (Ragasa *et al*. 2014).

Antioxidant Activity

Figure 4 shows the results of DPPH test, where the leaf of 2 and 3 years-old leaf demonstrated respective inhibition values of 21.7% and 25.4%. This shows a relatively lower activity in the samples from 2 years-old, although both showed less values compared to standards of galic acid, catechin, and quercetin. In addition, the values obtained were four times lower than the positive controls, and prior comparative research with the seeds (Bera *et al*. 2015), leaves (methanol and aqueous extracts) (Naveen and Urooj 2015), also ascertained poorer antioxidant activity in the *n*-hexane *S. mahagoni* extracts. Conversely, the low inhibition of DPPH was attributed to the low amount of phenyl propanoid or phenolic compounds, being the main contributor during antioxidant assay (Iravani and Zolfaghari 2011). Table 1 shows no significant difference between ages ($p > 0.05$), based on the t-test.

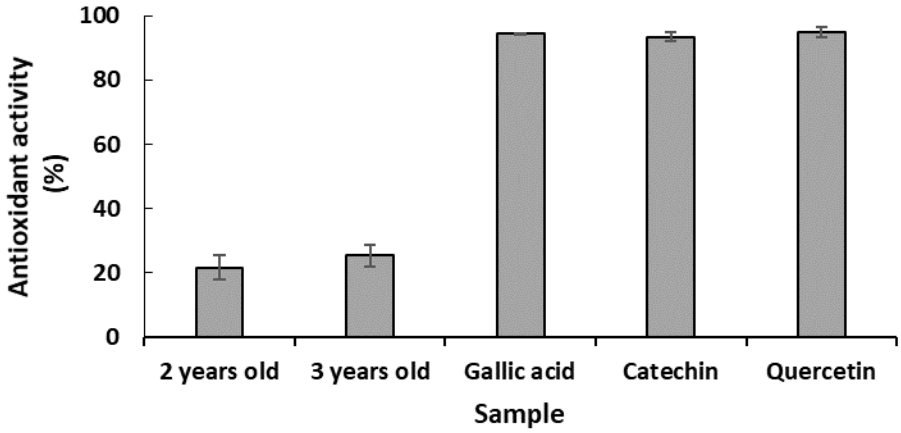

Figure 4. Antioxidant activity of *n*-hexane extracts of *S. mahagoni* leaf.

The *n*-hexane extract constituents of *S. mahagoni* were highly dominated by fatty acids and hydrocarbons, alongside the small quantity of mono-, sesqui, triterpenoids, and steroids. Furthermore, the effective antioxidant component include compounds with the ability to donate proton and stabilize DPPH to DPPH-H, while constituents with hydroxyl structure capably contribute as proton donators, including 1,8-cineole, which is a monoterpene identified in the essential oil of *Eucalyptus globulus* (Luis *et al*. 2015). Table 2 showed some mono-sesquiterpenoids with hydroxyl structural component, including cyclohexanebutanol and blumenol C, which were identified in low concentrations. In addition, pentadecanoic acid of the fatty acids and hydrocarbons groups were also detected, and Figure 5 shows the chemical structure of all compounds. These compounds in Figure 5 are, therefore, assumed to be weak antioxidants, with possible contribution in the *n*-hexane extracts of *S. mahagoni*.

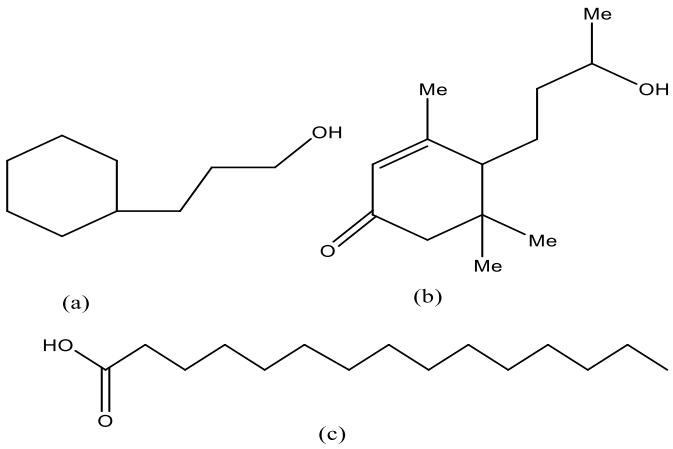

Figure 5. Chemical structure of cyclohexanebutanol (a), blumenol C (b), and pentadacanoic acid (c)

Conclusions

Based on GC-MS performance, the constituents of the *n*-hexane extracts of *S. mahagoni* leaf (2 and 3 years-old) were dominated by fatty acids and hydrocarbons, alongside terpenoids and steroids which were detected in minor quantities. In addition, both ages showed weak growth inhibition capacity againts *P. chrysosporium,* and poor radical scavenging activity againts DPPH. The antifungal activity of *S. mahagoni* was, therefore, suggested to have been influenced by hydrocarbons, including octacosane and tentracontane, while the antioxidant effects were attributed to the terpenoids present, including blumenol C and cyclohexanebutanol.

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