

Chemical Investigation of Methanol Extracts of *Swietenia mahagoni* Leaves and Its Antioxidant Activity

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Abstract

Swietenia mahagoni is among the species of trees used by the Indonesian Government for the purpose of afforestation and timber production through Perhutani Enterprise. The common use of this species as wood products has prompted investigating the chemical properties of its leaves. Based on this background, this study aimed at investigating the methanol extracts of both 2- and 3-year old *S. mahagoni* leaves extractives together with its antioxidants and phenols contents. The antioxidant activity was conducted through the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, while the total phenolic and flavonoid content were measured through Folin-Ciocalteu and AlCl_3 methods, respectively. Additionally, 24 compounds were characterized by the GC-MS, and were grouped into phenolics, fatty acids and hydrocarbons, and terpenoids. The total phenolic and flavonoid contents in both 2 and 3 years old leaves of *S. mahagoni* ranged from 36.4 ± 0.84 to 42.0 ± 0.18 mg GAE/g dried extract and from 2.24 ± 0.15 to 18.55 ± 1.05 mg QE/g dried extract, respectively. Also, the antioxidant values were $66.45 \pm 1.85\%$ and $77.59 \pm 11.23\%$, respectively. Based on the results, the antioxidant activity of *S. mahagoni* leaves was indicated as a result of the presence of α -tocopherol and α -tocopherolquinone in the leaves extracts.

Keywords: *S. mahagoni*, Antioxidant, Total phenolic content, Total flavonoid content, DPPH

Introduction

Swietenia mahagoni is a tree species introduced to Indonesia from Jamaica (Panda *et al.* 2010; Rahman *et al.* 2014). The presence of this species in this country has enhanced the characterization and identification of some of its properties. The leaves of *S. mahagoni* is a potential source of antioxidant agents, which are the source of phenolic compounds extracted with polar solvent such as methanol (Roy *et al.* 2009; Al-Radahe *et al.* 2012; Roy *et al.* 2014). In order to discover the antioxidants sources from these leaves, there is need to investigate the total phenolic and flavonoid content of its methanol extracts due to the positive correlation between its antioxidant and phenol contents.

This species is used for the purpose of afforestation in Indonesia due to its beneficial roles in terms of ecology, as well as a source of foreign exchange. This species consistently provides evergreen leaves throughout the year, which positively impact timber production, thereby contributing to the economy development of the country. The trees have been extensively used as home building materials, ornamental purposes, and protection in sloppy areas. According Jøker (2000), its wood is excellent for producing quality timber, with density within the range of 560-850 kg/m³ and 15% moisture content, making it a good potential for usage in large scale timber production.

Previous studies on the chemical compositions of the leaves of *S. mahagoni* characterized cyclomahogenol, swiemahogins A, and swiemahogins B as its componenta (Chakraborty *et al.* 1971; Chen *et al.* 2007; Naveen *et al.* 2014). Also, there were reports of some bioactivities from the leaves and seeds such as antifeedant (Mostafa *et al.* 2012), depressant (Rahman *et al.* 2010), antimicrobial (Sharma *et*

al. 2011), and antidiabetic (Sathish *et al.* 2010). Therefore, this study aimed at chemically analysing the methanol extracts of *S. mahagoni* leaves, its antioxidants activities and the phenols contents using the Gas Chromatography-Mass Spectrometry (GC-MS) in order to support its utilization in Indonesia.

Materials and Methods

Sample collection and extraction

The leaves sample, about 2 and 3 years old, of *S. mahagoni* were obtained from Temanggung, Central Java, Indonesia (Perhutani). These green leaves were collected from 3 different and then cut into small pieces. The actual fresh sample required (15 g) was extracted using *n*-hexane for three days at room temperature. The sample was then filtered using filter paper and evaporated using a rotary evaporator. The dried extract was then weighed to obtain the amount of *n*-hexane extract as a percentage of the fresh leaf sample. Subsequently, another sample was extracted with methanol using the former protocol.

Total phenol content (TPC)

The Folin-Ciocalteu method was used to determine the TPC of the sample. This involved the mixture of 2.5 ml of Folin-Ciocalteu phenol reagent (10 times dilution) and 0.5 ml of sample (1000 ppm) and incubated for 2 min at room temperature. Further, 2 ml of 7.5% aqueous sodium carbonate was added to the solution and then incubated for 30 min, also at room temperature. The sample absorbance was read at 765 nm and the results of TPC were expressed

as gallic acid equivalents (mg GAE/g extract). The TPC measurement was conducted in three replications.

Total flavonoid content (TFC)

The aluminium chloride (AlCl₃) method, according to Brighente *et al.* (2007), was used to determine the sample TFC. This involved reaction between 2 ml of 1000 ppm sample and 2 ml of 2% AlCl₃.6H₂O solution. This was allowed to stand for 1 h at 20 °C and the sample absorbance was read at 415 nm. The results from three replications were expressed in quercetin equivalents (mg QE/g extract).

GC-MS analysis

Prior to being subjected to GC-MS, the sample was methylated based on the method proposed by Jantan *et al.* (1999). This involved the mixing together of 1 ml 1000 ppm sample with 100 µl of tetramethyl ammonium hydroxide (TMAH). Then, 1 µl of the sample was injected to the GC-MS machine in three replications and data collected with a GCMS-QP 2010 (Shimadzu, Japan). The GC condition: Rtx-5MS capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan); detection temperature of 285 °C; column temperature from 70 °C (2 min) to 290 °C at 5 °C/min; injection temperature of 200 °C; acquisition mass range of 50-500 amu using helium as the gas carrier. Finally, the mass spectra of the sample was compared with the NIST library and the peak relative method was used for quantification of the compound. In addition, the minimum similarity index mass spectra between library and sample was 60%.

DPPH scavenging activity

The antioxidant activity assay was conducted in thrice measurement. This involved mixing 0.1 ml extract in methanol with 1000 ppm sample and then allowed to react with 3 ml of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH), and then incubated for 30 min. Then, its absorbance was read at 517 nm and the antioxidant activity calculated using equation (1):

$$\text{DPPH scavenged (\%)} = 100 \times (\text{Ao}-\text{A1}) / \text{Ao} \quad (1)$$

where Ao is absorbance of blank and A1 is absorbance of sample.

Statistical analysis

The results from the 2- and 3-year old leaves were subjected to independent t-test analysis with 95% confidence interval using SPSS 20 (IBM, USA). All assays were conducted in triplicates.

Chemicals

The following chemicals, TMAH, DPPH and gallic acid were purchased from Sigma-Aldrich (Germany).

Results and Discussion

Extractive content

The amount of methanol extract of *S. mahagoni* leaves ranged from 5.6 ± 0.36 to 7.0 ± 0.96 % (means ± standard deviation). Also, based on t-test, there was no significant difference in the amount of methanol extract between the 2- and 3-year-old leaves as *p* > 0.81, as shown in Table 1.

Table 1. Independent t-test between ages of *S. mahagoni* leaves extracts

Parameter	T-test for equality of means	
	Sig. (2-tailed)	df
Extractive content	0.81ns	4
Total phenolic content	<0.01*	4
Total flavonoid content	<0.01*	4
DPPH radical scavenging activity	0.17ns	4
Phenolics group constituent	0.97ns	4
Fatty acids and hydrocarbons group constituent	0.63ns	4
Terpenoids group constituent	0.62ns	4

ns=not significant; *=significant at 0.05 level

Theoretically, polar solvents such as methanol dissolve phenolic compounds (Fengel and Wegener 1989; Sjöström 1993). However, in this experiment, the use of methanol was to identify the chemical properties of *S. mahagoni* leaves, as well as its antioxidant activity. Also, the amounts of methanol extract in this result were lower compared to the 90% ethanol extract of *S. mahagoni* and *S. macrophylla* leaves from plants cultivated at the Zoological Garden, Giza, Egypt progeny (Mousa *et al.* 2014) and *Azadirachta indica* leaves from Botanical Survey of India (Alok *et al.* 2011). However, it was higher than the value of 80% aqueous ethanol extract (1.58 to 6.2 %) from *Azadirachta indica* from Kogi State, Nigeria (Raphael 2012).

Total phenol and flavonoid content

The total phenolic and flavonoid contents of the methanol extracts ranged from 36.4 ± 0.84 to 42.0 ± 0.18 mg GAE/g and 2.24 ± 0.15 to 18.55 ± 1.05 mg QE/g respectively of both dried extracts (means ± standard deviation). The t-test was conducted to observe the effects of tree ages and results showed that it has significant effects on the TPC and TFC of methanol extract. The TPC in 3-year-old leaves was over two folds higher than the 2-year-old samples. Similarly, the TFC of the 3-year-old was nine times compared with the 2-year-old leaves. This showed that the 3-year-old sample is a potential source of phenolic molecules for antioxidant, antimicrobial and antifungal activities.

This study also revealed that the extractive contents of the 2- and 3-years old leaves were not significantly different but the phenols contents differed significantly. In addition, the TPC of the leaves was lower compared with the methanol extract of *S. mahagoni* seeds at 55 mg GAE /g dried extract

(Salleh *et al.* 2014). Therefore, in comparison, the TFC of *S. mahagoni* leaves in this experiment was in a lower concentration.

GC-MS analysis

The analysis of methanol extracts from the leaves of *S. mahagoni* using GC-MS is shown in Table 2 and the chromatogram in Fig. 1. Based on Table 1, there was no significant difference in the concentration of phenolics, fatty acids and hydrocarbons, and terpenoids in both the 2- and 3-year-old leaves. The leaves constituents are these three substances; phenolics, fatty acids and hydrocarbons, and

terpenoids. These were dominated by fatty acids and hydrocarbons as well as terpenoids, while phenolics were found in low quantity. In addition, the ratio between terpenoids - fatty acids and hydrocarbons was almost 1:1 and the predominant compounds in fatty acids and hydrocarbons were palmitic and linolenic acid. However, squalene and α -tocopherol were the main components of terpenoids while 1-pyrrolidinebutanoic acid 4-methoxyphenyl ester was the highest in phenolics. Several components in this experiment showed a comparatively low similarity index level which suggests possibility assignments for other components. Therefore, standard components should be used to confirm those actual constituents in further works.

Table 2. GC-MS results of methanol extracts of *S. mahagoni* leaves

No	Ret. time (min)	Constituents	Concentration (% of dried extract) \pm std. dev		Similarity index (%)
			2 years	3 years	
		Phenolics	4.32 \pm 0.91	4.39 \pm 2.98	
1	19.11	Disophenol	0.64 \pm 0.31	1.79 \pm 1.83	71
2	40.64	1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester	1.92 \pm 0.73	1.54 \pm 0.66	62
3	40.90	1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester isomer	1.76 \pm 0.15	1.06 \pm 0.93	62
		Fatty acids and hydrocarbons	47.15 \pm 4.08	44.65 \pm 7.25	
4	25.11	4-Undecene, 7-methyl	0.33 \pm 0.33	0.62 \pm 0.05	76
5	25.34	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	2.57 \pm 0.36	2.72 \pm 1.18	79
6	25.85	1-Hexadecyne	3.08 \pm 0.80	4.07 \pm 0.79	89
7	25.99	Hexahydropseudoionone	0.84 \pm 0.40	0.48 \pm 0.42	89
8	26.29	Phytol, acetate	0.41 \pm 0.10	0.51 \pm 0.13	79
9	26.61	1-Octadecyne	1.04 \pm 0.28	1.41 \pm 0.16	86
10	27.40	Palmitic acid, methyl ester	5.76 \pm 0.43	5.17 \pm 0.91	94
11	28.15	Palmitic acid	13.35 \pm 2.7	7.31 \pm 6.46	91
12	30.20	Linolelaidic acid, methyl ester	2.30 \pm 1.46	2.58 \pm 0.78	92
13	30.29	12-Octadecenoic acid, methyl ester	1.69 \pm 1.62	2.16 \pm 0.17	93
14	30.32	Hexadecatrienoic acid, methyl ester	3.94 \pm 0.88	3.50 \pm 0.23	72
15	30.67	Stearic acid, methyl ester	1.43 \pm 0.63	1.49 \pm 0.42	89
16	30.93	Methyl 13,14-octadecadienoate	1.41 \pm 1.18	1.62 \pm 1.46	60
17	31.06	Linolenic acid	7.72 \pm 1.17	10.09 \pm 1.41	91
18	34.15	trimethyltridecyl) dihydro-2(3H)-furanone	0.78 \pm 0.38	0.46 \pm 0.40	84
19	43.62	1-Chloroeicosane	0.51 \pm 0.57	0.47 \pm 0.44	79
		Terpenoids	39.67 \pm 5.14	42.48 \pm 7.49	
20	40.22	Squalene	19.42 \pm 5.55	21.77 \pm 5.87	95
21	40.51	α -Farnesene	2.82 \pm 3.47	1.67 \pm 0.83	68
22	42.17	Stigmast-5-en-3-ol, oleate	0.60 \pm 0.11	0.45 \pm 0.16	60
23	44.68	α -Tocopherol	9.93 \pm 1.34	12.74 \pm 5.04	90
24	44.75	α -Tocopherolquinone	6.90 \pm 0.12	5.85 \pm 1.67	65

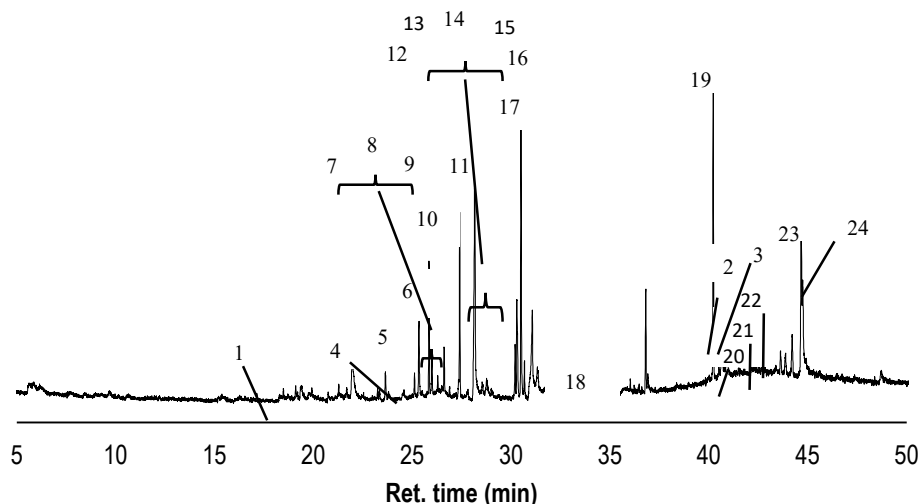


Figure 1. Chromatogram of GC-MS of methanol extracts of *S. mahagoni* leaves; 1. Disophenol, 2. 1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester, 3. 1-Pyrrolidinebutanoic acid 4-methoxyphenyl ester isomer, 4. 4-Undecene, 7-methyl, 5. 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol, 6. 1-Hexadecyne, 7. Hexahydropseudoionone, 8. Phytol, acetate, 9. 1-Octadecyne, 10. Palmitic acid, methyl ester, 11. Palmitic acid, 12. Linolelaidic acid, methyl ester, 13. 12-Octadecenoic acid, methyl ester, 14. Hexadecatrienoic acid, methyl ester, 15. Stearic acid, methyl ester, 16. Methyl 13,14-octadecadienoate, 17. Linolenic acid, 18. trimethyltridecyl)dihydro-2(3H)-furanone, 19. 1-Chloroeicosane, 20. Squalene, 21. α -Farnesene, 22. Stigmast-5-en-3-ol, oleate, 23. α -Tocopherol, 24. α -Tocopherolquinone

Antioxidant activity

The antioxidant activity of methanol extracts from the leaves of *S. mahagoni* is shown in Fig. 2. The concentration of methanol extracts from each of 2- and 3-year-old leaves was 1000 ppm, with antioxidant values of $66.45 \pm 1.85\%$ and $77.59 \pm 11.23\%$ respectively (means \pm standard deviation). For the purpose of comparison, the positive controls of gallic acid standard with concentration of 250 ppm resulted to antioxidant value of 93.14%. Based on the GC-MS results, the antioxidant activity of *S. mahagoni* leaves might be affected by the presence of these three compounds (phenolics, fatty acids and hydrocarbons, and terpenoids). Also, since the phenolic components of the leaves were in low concentration as shown in Table 2, this might be the cause of the low antioxidative activity values.

The antioxidant activity of *S. mahagoni* leaves was higher than previous study on other species of *Tylophora asthmatica* (Malathi *et al.* 2012), and *Actinodaphne madraspatana* (Suneetha *et al.* 2014), *Sesbania grandiflora* (Roy *et al.* 2014) and *Odina woodier* (Valli and Jeyalakshmi 2012) leaves. Based on the GC-MS analysis, the constituents of the leaf extracts such as phenolics, fatty acids and hydrocarbons, and terpenoids, might be responsible for the leaf's antioxidant activity. However, an earlier study by Mousa *et al.* (2014) showed that phenolic compounds and terpenoids were absent, but fatty acids and hydrocarbons were detected in *S. mahagoni* leaves. To a large extent, the presence of phenolic compounds and terpenoids was due to methylation process during the GC-MS experiment, as some

compounds were methylated in order to be detected by the GC detector. Further analysis of the fatty acids and hydrocarbons, showed the presence of palmitic and stearic acid in the leaves of *S. mahagoni* (Mousa *et al.* 2014). The study also showed that palmitic and linoleic acid were its major compounds, which was a little bit different from the results of this study, where the dominant compounds from fatty acid group were palmitic with linolenic acid.

In this study, linolenic acid was detected in the 2- and 3-year old leaves at $7.72 \pm 1.17\%$ and $10.09 \pm 1.41\%$, respectively. Additionally, the contribution of unsaturated fatty acids to the antioxidant activity of the leaves was higher than that of phenolic compounds. However, there is still of last group of the constituents of *S. mahagoni* i.e terpenoids, with a concentration almost similar to fatty acids and hydrocarbons. Thus, the presence of these compounds in *S. mahagoni* leaves have considerable effect on its antioxidant activity. The terpenoids was dominated by squalene, followed by α -tocopherol and α -tocopherolquinone, these compounds were suggested to have strong antioxidant activity (Liebler and Burr 2000). In comparison, α -tocopherol also was contained in leaf of *Abutilon indicum* (Ramasubramaniraja 2011). Furthermore, the antioxidant activity of these leaves might be linked with the phenols content. There was increase in the total phenolic and flavonoid content from the 2-year old leaves to the 3-year. The same increase was also observed in their antioxidant levels.

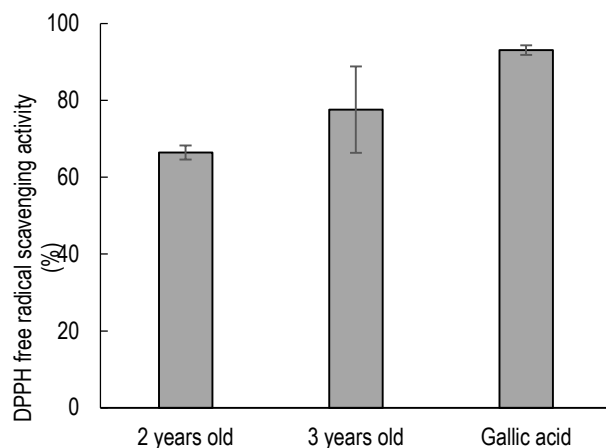


Figure 2. Antioxidant activity of methanol extracts of *S. mahagoni* leaves

Conclusions

Based on the results, the extractive content of the 3-year-old was lower than the 2-year-old leaves. However, the phenols contents and antioxidant activity were more in the 3-year compared with the 2-year old leaves. Additionally, 24 compounds were characterized by GC-MS, and were grouped into phenolics, fatty acids and hydrocarbons, and terpenoids. This study established that the antioxidant activity of the *S. mahagoni* leaves was as a result of its phenols contents. Furthermore, the antioxidant activity in the leaves were strongly affected by the presence of α -tocopherol and its derivative (α -tocopherolquinone), as the high level of these compounds make the leaves a potential source antioxidant agents and vitamin E.

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