Composition of Seed Oil and Leaf Essential Oil of *Swietenia mahagoni* (L) Jacq (Mahogani) by GC-MS Analysis

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Abstract

Swietenia mahagoni L. has folkloric and scientifically documented medicinally beneficial effects. The present work thus investigates the composition of seed oil and leaf essential oil of the plant. From the GC-MS analysis of the extracted essential oil from the leaf, 4-hydoxy-4-methyl hexa-5-enoic acid (RT 14.42 min), 2, 6, 10-trimethyltetradecane (RT 16.19 min), 3,5-dimethoxycinnamic acid (RT 17.69 min) and methyl 4-(1,5 – dioxolan-2-yl) benzoate (RT 17.69 min) were suggested in the essential oil. For the analysis of the seed oil, the fatty acid glycerides were first converted to fatty acid methyl esters (FAME) with $MeOH/K_2CO_3$ before GC-MS analysis. The average chain length (18 carbons) of the fatty acids in the oil has been estimated from to be 18 carbonsfrom CH_2/CO peak intensity in the IR spectrum of FAME. GC-MS suggests the methyl esters of 2-hydroxy-4-methyl pentanoic acid (RT 14.37 min), (11 E, 14 E)-11,14-eicosadienoic acid (RT 15.80 min) and di- and mono- methyl esters of sebacic acid (RT 15.85 min). Phytochemical tests and proximate analysis of the seed and leaf have also been carried out. The knowledge of the compounds present in the seed oil and leaf essential oil as well as the kinds of phytoconstituents suggested by phytochemical tests in the plant is will contribute to further understanding of the medicinal value of mahogany..

Keywords : *Swietenia mahagoni* L. , seed oil, leaf essential oil, phytoconstituents, GC-MS

1. Introduction

Traditional medicinal plants play a very important role in health care system of Myanmar because most of its population residesin the rural area and they have been using traditional medicine for centuries. The plant kingdom constitutes an invaluable source of chemical products which may be important due to their biological properties and their potential use in medicine. Mahogany is one of such plants. The earliest recorded use of *Swietenia mahagoni* was in 1514. Other records refer to the use of mahogani between 1521 and 1540, when Spanish explorers employed the wood for making canoes and for ship repair work in the West Indies. Mahogani's first major use in Spain and England was for ship building, and during the 18th century it was the chief wood employed in Europe for that purpose. Mahogani is a deciduous, erect tree growing to a height of 10 meters, with a heavy, dark-green, and dense crown. Bark is dark gray and ridged. The bark in younger specimens is smooth and grayish, becoming darker and furrowed with age (Figure 1). In the U.S. mahoganies are semi-deciduous, losing all or most of their leaves over winter or shedding at the flush of new growth in spring (Philippine Medicinal Plants, 2016). The bark contains tannin; limonoids and alkaloids, terpenoids, antraquinones, cardiac glycosides, saponins, phenols, flavonoids and long chain unsaturated acid (Mayur *et al.,* 2011). *Swietenia mahagoni* possessastringent, antipyretic, abortifacient, depurative, anticoagulant, antioxidant, antimicrobial, antidiabetic, antiprotozoal, anthelmintic, cytotoxic, gastroprotective, and hepatoprotective properties (Philippine Medicinal Plants, 2016). Crude methanolic seed extract yielded alkaloids, terpenoids, anthraquinones, cardiac glycosides, saponins, and volatile oils (Geethaa *et al*., 2009).The present work deals with the investigation on the seed oil and the essential oil in the leaf of the plant.

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Figure 1. Photographs of *Swietenia mahagoni* (Mahogani)

2. Materials and Methods

Sample Collection

The leaves and seeds of Mahogani were collected from East Dagon Township, Yangon Region, Myanmar. The collected plant samples were cleaned by washing with water and then were shade dried. The dried sample was ground into coarse powder. The dried powdered sample was stored in air tight container to prevent moisture changes and other contaminations

Preliminary Phytochemical Tests

A few grams of sample powder were subjected to the tests of alkaloids, α-amino acids, anthraquinones, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, starch and tannins by the preliminary phytochemical test according to reported methods(Tin- Wa,1972 and Trease and Evans,1980).

Proximate Analysis

In the present study, some nutritional values such as moisture, ash, protein, fiber, fat, carbohydrate and energy values of *Swietenia mahagoni* (Mahogani) leaves and seeds were determined by AOAC methods (A.O.A.C, 2000).

Qualitative Elemental Analysis

Elemental analysis of *Swietenia mahagoni* (Mahogani) leaves and seedswere done by ED-XRF onEDX-8000.

Extraction of Essential Oil from Mahogani Leaves

The leaf sample was macerated with petroleum ether for two weeks shaking from time to time, filtered and the solvent evaporated. The obtained petroleum ether extract was mixed with some sand and submitted to steam distillation. The essential in the condensed oil-water mixture was then extracted by partition with petroleum ether. The petroleum extract was dried over anhydrous Na_2SO_4. Evaporation of the petroleum ether yields the essential oil for GC-MS analysis.

Extraction of Seed Oil

The seed sample was macerated with petroleum ether for two weeks shaking from time to time, filtered and the solvent evaporated to obtain seed oil. The constituting fatty acids in the

oil were obtained as their methyl esters by transesterification of the glycerides with MeOH/K_2CO_3 and separation on a silica gel column.

Transesterification

Seed oil (5 g), MeOH (1.5 mL) and K_2CO_3(0.3 g) were mixed in a 100 mL round bottom flask. The flask was fitted with a water cooled condenser that returned any vapourized methanol to the reacting mixture. The reaction temperature was maintained at 65ºC using a sand bath, stirring continuously with a magnetic stirrer. After 25 min, 0.2 M A/A (4 mL) was added slowly to the mixture and stirred for a few minutes. The product mixture of fatty acid methyl esters (FAMEs)obtained as upper layer wasremoved using a separating funnel, and then dried over anhydrous $MgSO_4$. The dried FAME mixture (0.2 g) was purified by chromatography on a silica gel (7 g) column (diameter 1.2 cm) with PE : EtOAc (95 : 5) for further analysis by GC-MS. FAME average carbon chain length was also determined by FTIR.

The ratio of the peak intensities of CH_2 and CO, respectively, at 2922 and 1745 cm^{-1} was calculated from the FT IR spectrum of FAME. Using the values thus obtained and the published curve of CH_2/CO ratio versus FAME carbon chain length, the average chain length of carbon in the fatty acids can be determined.

3. Results and Discussion

Phytochemical Test by Using Test Tube Method

The phytochemical tests were done on the leaf and seeds with a view to determine the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins and the absence of flavonoids, starch and tannins . The results were shown in Table 1.

Proximate Analysis of *S. mahagoni* (Mahogani) Leaves and Seeds

Some nutritional values such as moisture, ash, fiber, fat, protein, carbohydrates and energy values of the leaves and seeds of *S. mahagoni* (Mahogani) were summarized in Table 2. Fiber content (41.14 %) is predominant in the leaves sample and the fat content (28.77 %) is predominant in the seed sample.

Table 1. Nutritional Values of *S. mahagoni* (Mahogani) Leaves and Seeds

No.	Nutrients	Content (%)	
		Leaves	Seed
1	Moisture	18.20	7.25
2	Ash	6.54	2.50
3	Protein	8.42	17.49
4	Fiber	41.14	22.23
5	Fat	2.16	28.77
6	Carbohydrate	23.54	21.75
	Energy Value (kcal/100g)	147.28	403.89

Semi-Quantitative Elemental Analysis of the Leaves and Seeds of Mahogani by Energy Dispersive X- Ray Fluorescence

The ED-XRF spectra and data of the leaves and seeds samples were shown in Figure 3 and Table 3. It can be seen that Ca (70.943 % and 45.431 %) and K (21.137 % and 86.871%) in leaf and seed, respectively.

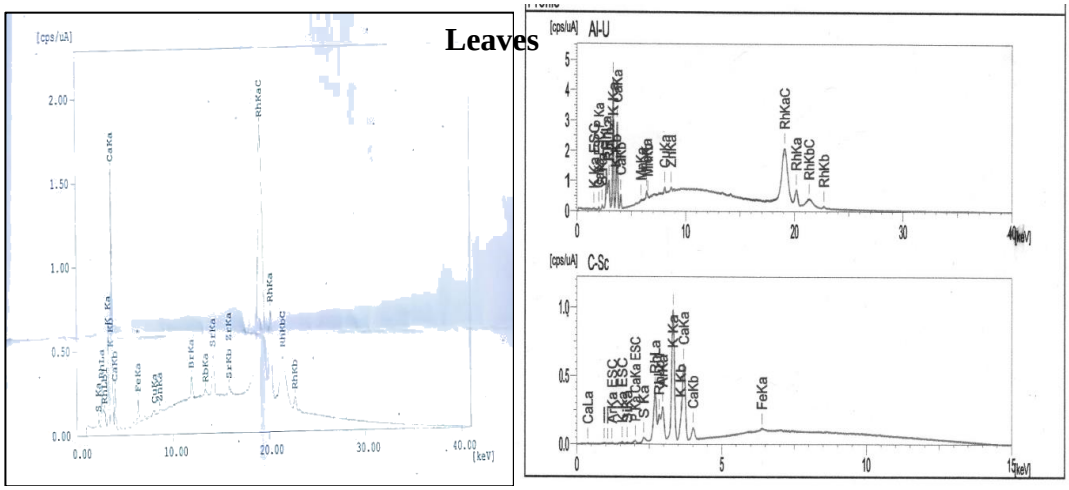


Figure 2. ED-XRF spectra of leaf and seed of *Swietenia mahagoni* L. Jacq. (Mahogany)

Table 2. Relative Abundance of Some Elements in Leaves and Seeds of *S. mahagoni* (Mahogani) by EDXRF Method

Mineral Elements	Relative Abundance (%)	
	Leaves	Seeds
K	21.137	46.87
Ca	70.943	45.343
S	3.958	2.185
Si		2.027
P		1.742
Fe	2.035	0.899
Mn		0.344
Cu	0.237	0.320
Zn	0.153	0.269
Sr	0.800	
Rb	0.158	
Zr	0.013	

Extraction of Leaf Essential Oil and Analysis

Only very small amount of essential oil was obtained from the leaf by steam distillation. GC-MS analysis (Figure 3) of the essential oil suggests 4-hydroxy-4-methyl hexa-5-enoic acid (RT 14.42 min), 2,6,10-trimethyltetradecane (RT 16.19 min), 3,5-dimethoxycinnamic acid (RT 17.69 min) and methyl-4-(1,2 –dioxolan-2-yl) benzoate (RT 17.69 min).

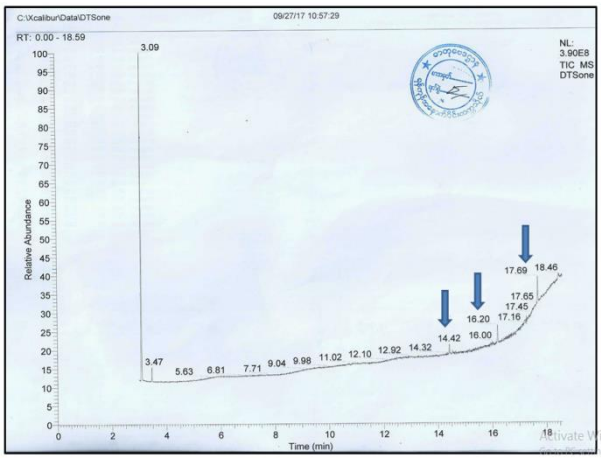


Figure 3.Gas Chromatogram of the essential oil extracted from leaf

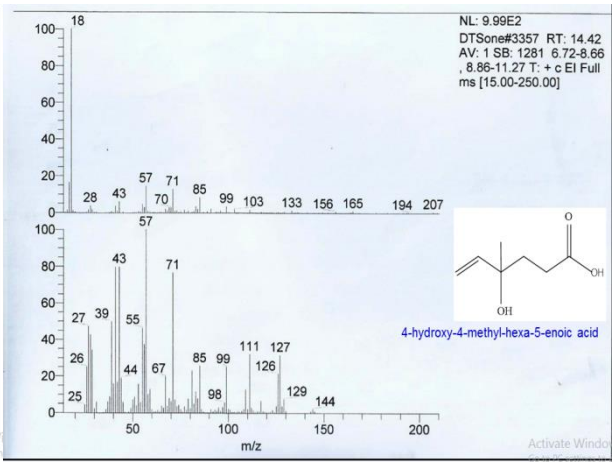


Figure 4. Mass spectra of eluted compound at RT 14.42 min and database compound

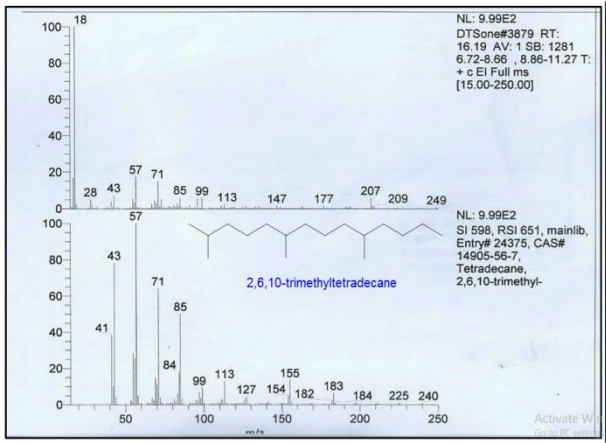


Figure 5. Mass spectra of eluted compound at RT 16.19 min and database compound

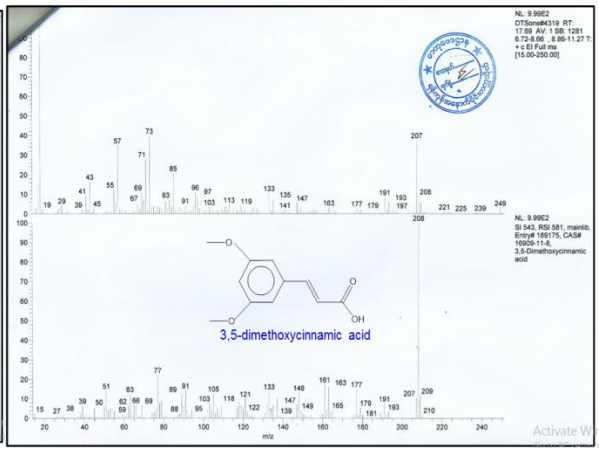


Figure 6. Mass spectra of eluted compound at RT 17.69 min and database compound

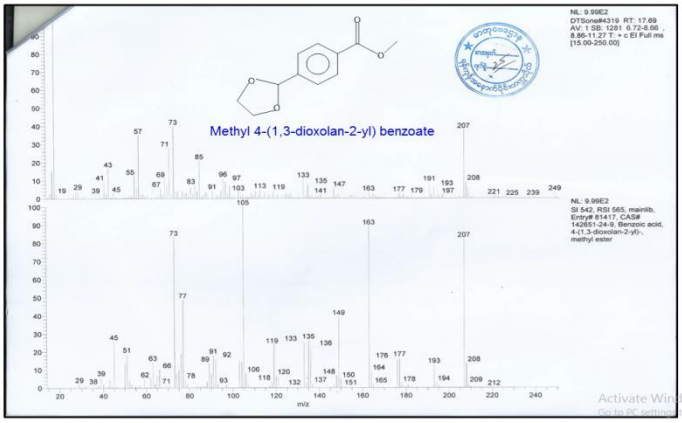


Figure 7. Mass spectra of eluted compound at RT 17.69 min and database compound

Extraction of Seed Oil and Analysis

After the transesterification reaction of the extracted seed oil, the product mixture was observed as two layers, FAME in the upper and glycerol in the lower layer. The purified FAME mixture from chromatography (Figure 8) was analysed by FT IR (Figure 9 and Table 3).

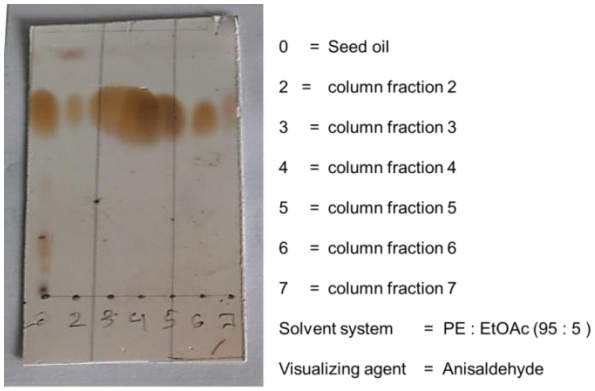


Figure 8 . TLC monitoring of fractionated methylated seed oil

Table 3. Assignment of IR Bands of the Mixture of Methyl Esters of Fatty Acids Prepared from the Seed Oil

Wavenumber (cm^{-1})	Vibrational mode	Functional group
3009	=C-H stretching	Olefin
2951	CH_3 asymmetric stretching	CH_3
2922	CH_2 asymmetric stretching	CH_2
2853	CH_2 symmetric stretching	CH_2
1745	C=O stretching	ester
1459	CH_2 bending & CH_3 asymmetric bending	CH_3 & CH_2
1377	CH_3 symmetric bending	CH_3
1245, 1198, 1167	C-O stretching	Methyl ester of long chain fatty acid
909	= C-H out-of-plane bending	Trans C=C double bond
722	CH_2 rocking,	chain $\geq 4CH_2$

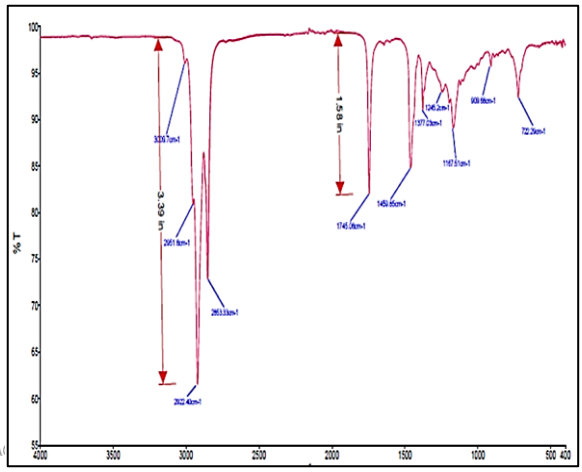


Figure 9. FTIR spectrum of the mixture of methyl esters of fatty acids (FAME) prepared from the extracted seed oil

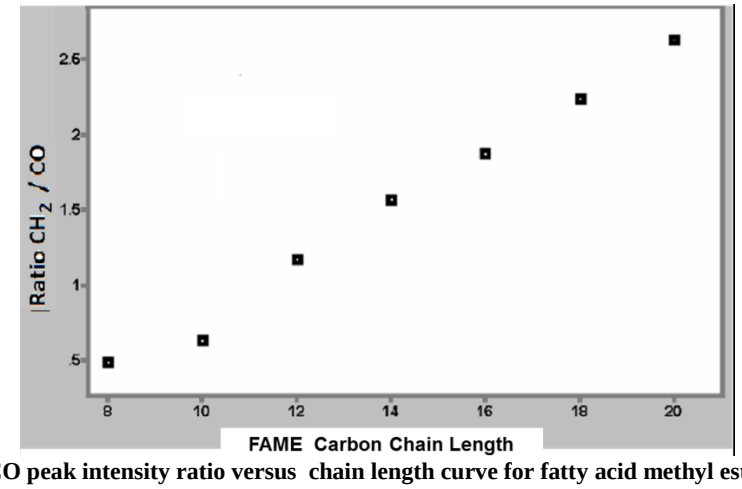


Figure 10. CH_2/CO peak intensity ratio versus chain length curve for fatty acid methyl esters*

The average chain length of fatty acids in the oil can be estimated from the value (2.2) of the ratio of peak intensityfor CH_2 and CO stretching (Figure 9) and the CH_2/CO intensity ratio versus chain length curve (Figure 10).

$$\frac{Intensity\ of\ CH_2\ \ peak\ at\ 2922\ cm^{-1}}{Intensity\ of\ CO\ \ peak\ at\ 1745\ cm^{-1}} = \frac{3.39\ in}{1.58\ in} = 2.2$$

This indicates that the average chain length, *i.e.* the number of carbons in the chain in the constituent fatty acids is round about 18.

GC-MS analysis Figure 11. of the FAME mixture suggests 2-hydroxy-4-methyl pentanoate (RT 14.37 min), (11E, 14E)-11, 14-eicosadienoic acid (RT 15.80 min) and di and mono-methyl esters of sebacic acid (RT 15.85 min).

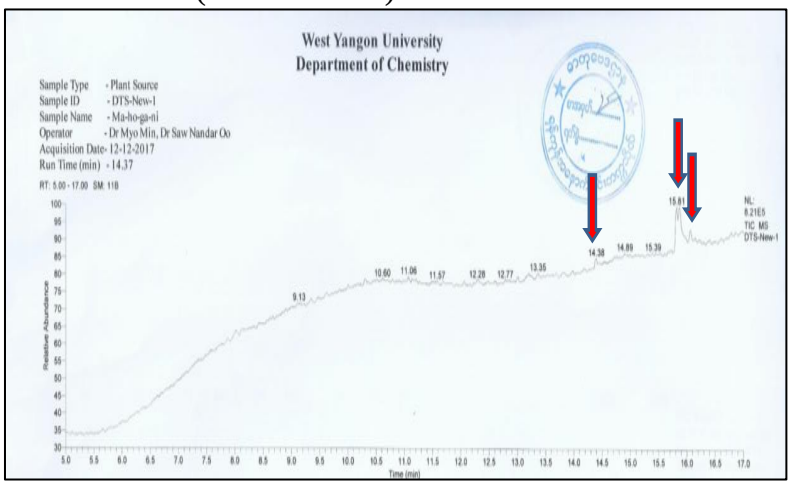


Figure 11. Gas Chromatogram of the mixture of methyl esters prepared from the seed oil

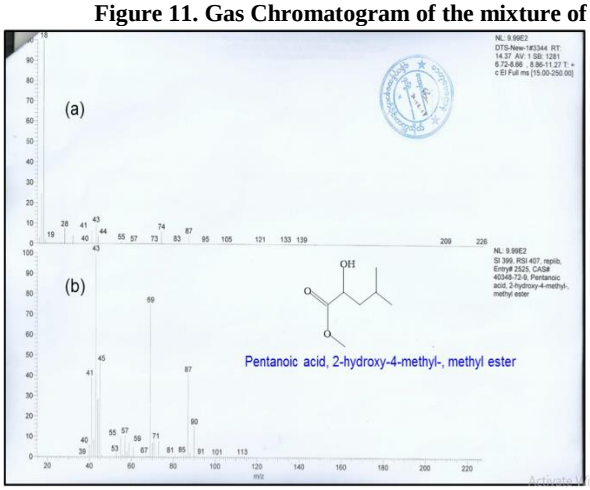


Figure 12. EI mass spectra (a) of eluted compound at RT 14.37 min and (b) methyl2-hydrox-4-methylpentanoate (database)

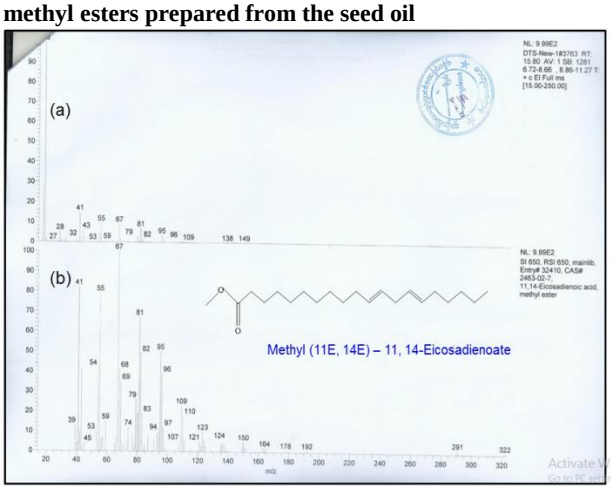
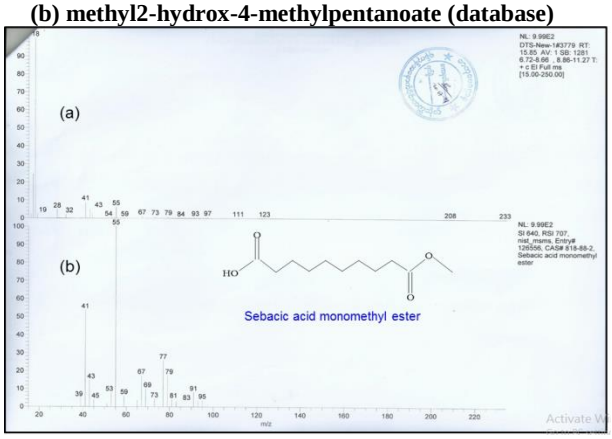
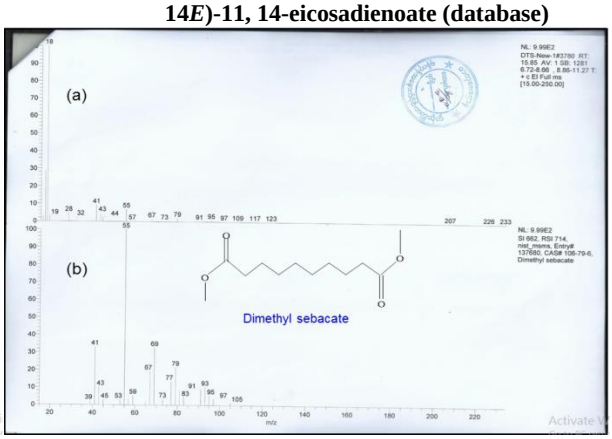


Figure 13. EI mass spectra of (a) eluted compound at RT 15.80 min and (b) methyl (11*E*, 14*E*)-11, 14-eicosadienoate (database)





Conclusion

From the results of preliminary phytochemical tests of leaf and seed samples of *S.mahagoni*, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds and saponins are present but reducing sugars, starch and tannins are absent.

Nutritional analyses showed that mahogany leaf and seed contained 18.10 % and 7.25 % of moisture, 6.54 % and 2.50 % of ash, 8.42 % and 17.49 % protein, 41.14 % and 22.23 % of fiber, 2.16 % and 28.77 % of fat, 23.54 % and 21.75 % of carbohydrate, respectively on the basis of dry samples. The energy value of the leaf and seed were observed to be 147.28 and 403.89 (kcal/100g) respectively.

Ca (70.943 %) was the most abundant element in the leaves sample followed by K, Si, Fe, S, Sr and Cu in relatively small quantities. But K (46.87 %) was the most abundant element in the seed sample. Ca (45.343 %) was nearly abundant as K, followed by S, Si, Fe, Cu and Zn in relatively small quantities in the seed sample.

From the GC-MS analysis, 4-hydroxy-4-methylhexa-5-enoic acid (RT 14.42 min), 2,6,10-trimethyltetradecane (RT 16.19 min), 3,5-di methoxycinnamic acid (TR 17.69 min) and methyl 4-(1,5-dioxolan-2-nyl) benzoate (RT 17.69 min) were suggested in the leaf essential oil and methyl esters of 2-hydroxy-4-methylpentanoic acid (RT 14.37 min) (11E, 14E)-11,14-eicosadienoic acid (RT 15.80 min) and di and mono-methyl esters of sebacic acid (RT 15.85 min) were suggested in the FAMEs of seed oil.

It is hoped that the knowledge of the chemical compositions of the seed oil and the leaf essential oil of mahogany will make useful contribution to further understanding of the medicinal values of the plant.

References

Application Note 005. (2018). "IR Analysis of Fatty Acid Methyl Esters" http://www.spectra-analysis.com/documents/AppNote005FAME.pdf

Addisu, S., D. Mohamed and S. Waktole. (2014). "Efficacy of Botanical Extracts against Termites, *Macrotermes spp.*, (Isoptera : Termitidae) under Laboratory Conditions". *International Journal of Agricultural Research,* **9** (2), 6 - 73

Chieng, T. C., Z. B. Assim and B. A. Fasihuddin. (2008). "Toxicity and Antitermite Activities of the Essential Oils From *Piper sarmentosum*". *The Malaysian Journal of Analytical Sciences*, **12** (1), 234 – 238

Hundley, H.G., and Chit Ko Ko (1986). "List of Trees, Shrubs, Herbs and Principle Climbers etc." Myanmar :4th Edn., Union of Burma, Government Printing and Stationary, Rangoon

Eco India. (2016). *Indian Mahogany* http://www.ecoindia.com/flora/trees/indian-mahogany-tree.html

Falah, S., T. Suzuki and T. Katayama. (2008). "Chemical Constituents from *Swietenia macrophylla* Bark and their Antioxidant Activity". *Pak. J. Biol. Sci.,* **11** (16), 2007 - 2012

Harsha, K., K. Amit, P. Preeti and K. Harendra. (2014). "Anti-Termite Activity of Heartwood of *Dalbergia sisso* Roxb. Ex. DC.". *World Journal of Pharmacy and Pharmaceutical Sciences,* **3** (6), 673 – 679

Jenny, M. W., H. Michael, R. Tracey and M. A. Heather. (2002). "Bioactivity of *Backhousia citriodora* : Antibacterial and Antifungal Activity". *Journal of Agricultural and Food Chemistry*, **51** (1), 76 - 81

Suliman, M. B., Nour, A. H. Yusoff, M. M. (2013). "Fatty Acid Composition and Antibacterial Activity of *Swietenia macrophylla* King Seed Oil". *African Journal of Plant Science,* **7** (7), 300-303anandan

Subhadip, H., M. Archana and P. Pinkee. (2012). "*In Vitro* Anthelmintic Activity of *Swietenia mahagoni* Seed Extracts Against Pheritima Posthuma" *International Journal of Pharmaceutical Sciences Review and Research,* **12** (1), 111-113

Sundar, D. S., S. Anandan and S. K. R. Namasivayam. (2013). "Antifungal Activity of *Swietenia mahogany* on *Candida albicans* and *Cryptococcus neoformans*". *Journal of Microbiology and Antimicrobials*, **5** (6), 55-59

Wikipedia. (2016). *Swietenia mahagoni* https://en.wikipedia.org/wiki/Swietenia_mahagoni

Yuan, Z. and X. P. Hu. (2012). "Repellent, Antifeedant, and Toxic Activities of *Lantana camara* Leaf Extract Against *Reticulitermes flavipes* (Isoptera : Rhinotermitidae)". *J. Econ. Entomol.*, **105** (6), 2115-2121