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Physico-Chemical Characterization, Antimicrobial Activity and Toxicity Analysis of *Swietenia mahagoni* Seed Oil

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ABSTRACT

Oil extracted from *Swietenia mahagoni* seed was studied with a view to finding out its suitability for ethnomedical uses with special focus on antimicrobial and toxic behavior. Some of its physical and chemical properties were examined and compared with those of standard oils: olive, sunflower, cotton seed, Linseed, soybean, coconut, palm and castor. The refined oil was found to show good to moderate activity against disease causing bacteria *viz. Shigella dysenterial, Salmonella typhi, Staphylococcuss aureus* and fungal pathogens viz. *Macrophomina phascolma, Alternaria alternata, Curvularia lunat*a. Different extracts from *Swietenia mahagoni* seed showed minimal toxic effect while applied on predatory fish's viz. *Heteropneustes fossilis* and *Anabas testudineu*s.

Key Words: *Swietenia mahagoni;* Seed oil; Seed extract; Physico-chemical characteristics; Antimicrobial activity; Toxicity

INTRODUCTION

Swietenia mahagoni is an evergreen to semi-evergreen hardwood timber species of the family *Meliaceace* having seeds chestnut brown in colour, 4-5 cm long, compressed, crested and extended into a wing at the attachment end (Troup, 1921; Lyhr, 1992; Schmidt & Jøker, 2000). This plant species has ethnomedical uses (Goun *et al*., 2003) and *in vitro* antibacterial and antifungal property of its extract has been reported (Goun *et al.*, 2003). The seed extracts of *S. mahagoni* has also been found to inhibit platelet-activating factor (PAF)-induced platelet aggregation (Ekimoto *et al.*, 1991). In Bangladesh, *S. mahagoni* is widely planted in homesteads, roadsides and marginal lands (Hossain & Rakkibu, 1993; Misbahuzzaman & Ahmed, 1993; Millat-e-Mustafa *et al.*, 1994; Uddin *et al.*, 1998). Study on physico-chemical properties, antimicrobial activity and toxicity of the seed oil extracts of some widely distributed plant species of Bangladesh viz. *Azadirachta indica* (Majumder *et al.*, 1997), *Abelmoschus esculentus* (Majumder & Hoque, 1999), *Melia azedarach* (Hashem *et al.*, 2002) have been conducted in our Laboratory in the recent past and as a continuation of those works, we are reporting the same for *S. mahagoni*. The objective of the study is to find out the possible reasons behind its ethnomedical importance with special focus on antimicrobial and toxic behavior.

MATERIALS AND METHODS

Oil was extracted from cleaned, dried, dehulled and crushed seeds of *S. mahagoni* by solvent extraction method using petroleum ether as the solvent. The extracted oil was then purified (Majumder *et al.*, 1997) and dried completely by blowing a slow stream of nitrogen gas. The yield was 47.5%.

Refractive index, specific gravity (Griffin, 1927), co-efficient of viscosity and energy of activation for viscous flow (Kitchener, 1965) of the oil were determined by standard procedure.

Acid value, iodine value, elaidin test, reichert-meissl value, saponification value (Sharma, 1995) and saponification equivalent, the quantity of unsaponifiable matter (Williams, 1966), acetyl value (Griffin, 1972), thiocyanogen value, henher value, peroxide value (Morris, 1965) and polenske value (Ranganna, 1991) of the oil were estimated using the standard methods.

Thin Layer Chromatographic (TLC) investigation of the fatty acids present in the oil of was done in various solvent systems by converting the acids into their corresponding methyl esters (Mangold & Kammereck, 1961; Loury, 1966, 1967). After development of the chromatogram, the fatty acid composition was identified by comparing with the R_f values of methyl esters of standard fatty acids.

The fatty acid composition of the seed oil was also investigated by GLC (Gas Liquid Chromatography) after conversion of the acids into the corresponding methyl esters (Mangold & Kammereck, 1961; Loury, 1966, 1967). To study the sample by GLC, a portion of the converted sample was injected into one end of the column of the GLC equipment (PYE-UNICAM PU 4500, Phillips) having a flame ionization detector and a chart recorder. The column (internal diameter 2 mM, length 1.5 meter) was filled with 10% diethyl glycol succinate (DEGS) on 100-200 (British Std. Sieve) mesh. The injector temperature was 230^{o}C and the detector temperature was 250^{o}C. The temperature of the column was programmed initially at 100^{o}C for 1 minute, then allowed to rise to 225^{o}C at a rate of 4^{o}C/min. Nitrogen gas was used as the carrier gas at a flow rate of 11.3 mL/min. Standard methyl esters of caprylic, nonanoic,

capric, undecanoic, lauric, myristic, palmitic, stearic, oleic, arachidic and behenic acids (Sigma Chemical Company, USA) were used for identification of the peaks. Peak position and relative retention time of those standard methyl esters in GLC-Chromatograph are shown in Fig. 1 and denoted as [1]–[11], respectively. The fatty acids present in the lipid under investigation were thus identified by comparison of relative retention time and peak position. The percentage of the acids was computer estimated from the GLC peaks.

The seed oil was screened for its antimicrobial activity against selected disease-causing bacteria and fungal pathogens. Disc diffusion method (Bauer *et al.*, 1966; Ahmed *et al.*, 1998) and poisoned food technique (Grover & Moore, 1962; Ahmed *et al.*, 1998; Basak & Paul, 1999) were followed for screening the oils samples against the bacteria and fungi, respectively.

The toxicity of different extracts from *S. mahagoni* seed was bio-assayed upon two predatory fish viz. *Heteropneustes fossilis* and *Anabas testudineus* at various concentrations under laboratory conditions. The actual concentrations of doses which have been used in the bioassays were calculated in terms of ppm. The bioassays were run in a series of glass aquarium, netted on top, each containing five liters of tap water and the calculated amount of toxicant. In each experiment, a control was maintained. Five concentrations of each extract were used in the final experiments. In each test, a set of five test fish were released at random in each concentration and each dose of the toxicant was replicated two times. The test exposure period was 24 h. All the bioassays were conducted under laboratory conditions. The average water temperature was 26±0.5°C and average system pH during experiments was 6.2±0.05. Behavior of the fish was recorded in terms of their movements and abnormalities. The rate of fish mortality was counted only with those fishes which were killed within 24 h after treatment.

RESULTS AND DISCUSSION

Solvent extracted seed oil of *S. mahagoni* has been investigated for some physical and chemical characteristics as well as for antimicrobial capability and toxicity to determine its nature and to find out its suitability for a given purpose. Citation (Table I) and discussion on the explored data are as follows.

Physical characteristics. The refractive index of *S. mahagoni* oil was found to be 1.4751 at 30°C, which is almost the same as that of soybean oil (1.4723–1.4756). The result indicates that the oil consists of glycerides of long chain saturated fatty acids.

Fig. 1. GLC-Chromatograph of the standard fatty acid methyl ester mixture and of *S. mahagoni* seed oil

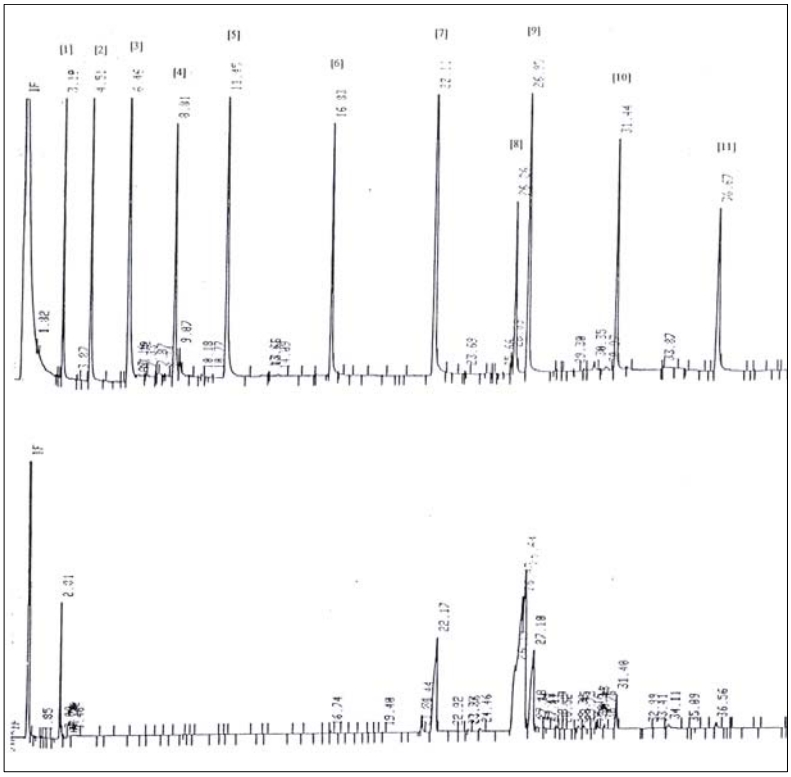

Table I. Physical and chemical constants of the seed oil of *S. mahagoni* and some important commercial oils (Williams, 1966; Lange, 1987; Das, 1989)

Name of the sample	Sp. gr. at 15.5^{0}C	R. I. at 15.5^{0}C	Viscosity mp at 27^{0}C	S. V.	S. E. V.	A. V.	F. F. A % as oleic	I. V.	T. V.	Acetyl Value	U. S. M. %	R. M. V.	P. V.	H. V.
Olive oil	0.915-0.919	1.4657-1.4667	466.81	190-195	287-295	0.2-6	0.25-0.60	80-88	75-83	10.04	0.5-1.2	0.6-1.5	0.5	0.6
Sunflower oil	0.924-0.926	1.4659-1.4721	331.12	190-194	287-295	0.6-2.4	0.15-045	125-140	78.4-81.3	--	0.3-0.9	0.5	--	--
Cotton seed oil	0.921-0.922	1.4743-1.48	358.43	192-198	283-292	1.0-5.0	0.4-0.9	103-111	61-69	7.5-12.5	0.8-18	0.95	--	94.2
Linseed oil	0.931-0.938	1.479-1.480	296.08	189-195	287-296	4.0	0.5-0.75	175-200	--	4-12	1.0-1.5	--	--	94.8
Soybean oil	0.920-0.922	1.4723-1.4756	284.98	190-195	287-295	0.3-3	0.35-0.85	129-137	77-85	--	0.7-1.6	0.5-2.5	0.2-1	--
Coconut oil	0.926	1.4530	297.90	255-260	210-250	1-10	--	8.2-9.6	6.1-7.0	--	0.15-0.7	7.0-8.0	15-17	82
Palm oil	0.837	1.4510	309.24	204-207	220-250	--	--	53.3-57	--	--	0.2-0.5	5-7	10-12	--
Tung oil	0.939-0.945	1.515-1.52	--	190-197	--	--	--	170-187	--	--	0.4-1	--	--	--
Castor oil	0.9561 at 27^{0}C	1.4761	293.42	178.08	--	0.6	--	84-90.1	--	--	0.02-0.03	1.2	--	--
Seed oil of S. mahagoni	0.9334 at 30^{0}C	1.4751 at 30^{0}C	459.32 at 30^{0}C	176.82	317.27	0.87	3.2	94.69	20.44	0.69	0.52	0.36	0.97	81.9

The specific gravity of the oil was found to be 0.9334 at 30°C. The value is close to that of Neem seed oil (0.9109) and Bohera seed oil (0.9111).

The viscosity of the oil was 459.32, 366.94, 301.22, 252.83, 209.50 and 185.26 millipoise at 30, 35, 40, 45, 50 and 55°C, respectively. The energy of activation for viscous flow was 3.047 kcal per mole. In general, the viscosity of the lipids (fats/oils) increases with the increase of intermolecular hydrogen bonding. The low viscosity suggests that there are a few hydroxyl groups in the molecule, which is supported by the low acetyl value (0.69) and low refractive index of the oil, as well.

Chemical characteristics. The iodine value (degree of unsaturation) of the *S. mahagoni* seed oil was found to be 94.69, which is almost similar to that of castor oil (84.0–90.08). The value supports that the oil is unsaturated but not highly. The saponification value of the oil was found as 176.82, which is almost the same as that of castor oil (175.0–183.0). This comparatively lower saponification value indicates the presence of higher fatty acids in higher proportions. The saponification equivalent of the oil has also been calculated and found to be 317.27.

The Henher value which is a measure of water insoluble fatty acids in oil or fat was found to be 81.9 indicating the presence of higher percentage of water insoluble fatty acids with high molecular weight in the oil.

The Reichert-Meissl value (R.M.V.) of the oil was found to be 0.36, which is the measure of volatile water soluble fatty acids present in the oil or fat. Relatively lower R.M.V. of the oil is an indication of low content steam volatile fatty acids. The Polenske value of the oil was found to be 0.97, which indicates low content of the volatile alcohol soluble but water insoluble fatty acids in the oil. The low R.M.V. and Polenske value indicate presence of comparatively higher fatty acids in the oil as indicated by the low saponification value (176.82) of the oil.

The percentage of unsaponifiable matter (U.S.M.%) of the oil was found to be 0.52% (w/w) which indicates that the lipid contains a small amount of unsaponifiable sterols, tocopherols, vitamins A and D and hydrocarbons (Chatten, 1966; Helal Uddin *et al*., 2004).

The peroxide value is the indication of unsaturation of oil and was found to be 39.65 millimoles per 1000 g for the *S. mahagoni* seed oil. The low peroxide value supports the observation indicated by low iodine value of the oil. The fact is also supported by the Thiocyanogen value of the oil which was found to be 20.44.

The acid value and percentage of free fatty acid (as oleic) of the oil were found to be 0.87 and 3.2%. Such percentage of free fatty acids indicates unsuitability of the lipid for edible purpose but it can be used as an ingredient in soap manufacture. The acetyl value of the oil was found to be 0.69 indicating low content of free hydroxyl groups in the oil. The fact is also supported by the low viscosity and low refractive index of the oil.

Table II. Fatty acid composition of *S. mahagoni* seed oil

Name of the fatty acid	Inference	Retention time (RT)	Relative percentage
Myristic acid	$C_{14:0}$	16.74	0.56
Palmitic acid	$C_{16:0}$	22.17	52.01
Stearic acid	$C_{18:0}$	26.26	36.01
Oleic acid	$C_{18:1}$	27.18	0.88
Arachidic acid	$C_{20:0}$	31.40	9.12

Table III. Antibacterial activity of *S. mahagoni* seed oil

Name of the bacteria	Diameter of inhibition zone (mm)	
	10 µl oil	20 µL oil
Shigella dysenterial	12	13
Salmonella typhi	13	15
Staphylococcuss aureus	3	2

Table IV. Antifungal activity of *S. mahagoni* seed oil

Name of the fungus	Radial growth inhibition (mm)	
	10 µL oil	20 µL oil
Macrophomina phascolma	3.27	3.93
Alternaria alternata	2.80	3.01
Curvularia lunata	2.37	2.46

Table V. Percentage mortality of *Heteropneustes fossilis* at different concentrations of various solvent extracts of *S. mahagoni* seed for 24 h exposure

Name of the solvent extract	Dose in ppm	Rep-1		Rep-2		No. of fish killed	Mortality %
		No. of fish taken	No. of fish killed	No. of fish taken	No. of fish killed		
	50	5	0	5	0	0	0
	100	5	0	5	2	2	20
Distilled water	250	5	1	5	1	2	20
	500	5	1	5	2	3	30
	750	5	2	5	2	4	40
	10	5	1	5	1	2	20
	50	5	2	5	1	3	30
50% ethyl alcohol	100	5	2	5	2	4	40
	250	5	2	5	3	5	50
	500	5	4	5	4	8	80

Table VI. Percentage mortality of *Anabas testudineus* at different concentrations of various solvent extracts of *S. mahagoni* seed for 24 h exposure

Name of the solvent extract	Dose in ppm	Rep-1		Rep-2		No. of fish killed	Mortality %
		No. of fish taken	No. of fish killed	No. of fish taken	No. of fish killed		
	50	5	0	5	0	0	0
	100	5	1	5	0	1	10
Distilled water	250	5	1	5	1	2	20
	500	5	1	5	2	3	30
	750	5	2	5	1	3	30
	10	5	1	5	2	3	30
	50	5	1	5	3	4	40
50% ethyl alcohol	100	5	3	5	3	6	60
	250	5	4	5	3	7	70
	500	5	4	5	5	9	90

The drying property of the oil was examined. After the experiment, it was found that the seed oil thickened into solid after 24 hours. Again, since the oil has the iodine value of about 94.69, hence the oil is of nondrying type (Das, 1982). The study of the effect of storage for about a year on the oil showed a significant variation in acid value, peroxide value, R.M.V., Thiocyanogen value and iodine value. Acid value and peroxide value increase with increasing time of storage and R.M.V., Thiocyanogen value and iodine value decrease with increasing time of storage i.e., quality of the oil deteriorates with increasing time of storage and the deterioration level is up to 8-15%.

Characteristics of some important commercial oils and *S. mahagoni* seed oil are given in Table I.

Chromatographic Examinations

TLC analysis. The fatty acid methyl esters mixture obtained from the oil was subjected to TLC examination and the fatty acid composition was identified by comparing the R_f values of methyl esters of standard fatty acids in different solvent systems. From the results, it may be suggested that the oil contains myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$).

GLC analysis. GLC analysis of *S. mahagoni* seed oil shows that about 98% of the weight of the fatty acids consists of saturated acids which include following even numbered normal fatty acids: palmitic acid, stearic acid, arachidic acid and myristic acid. A very small amount of unsaturated fatty acid i.e. oleic acid was also found in the oil. GLC chromatograph of the *S. mahagoni* seed oil is shown in Fig.1 and detailed fatty acid composition in Table II.

Antimicrobial activity. Antibacterial and antifungal activities of *S. mahagoni* seed oil were investigated by standard methods against selected fungus and bacteria. The selection was made on the basis of the availability of the bacteria and fungus species in the Laboratory.

In Table III, antibacterial activity of *S. mahagoni* seed oil against three disease causing bacteria viz. *Shigella dysenterial, Salmonella typhi, Staphylococcuss aureus* is listed. From the table, it is clear that the oil showed maximum zone of inhibition against *Salmonella typhi* and the minimum against *Staphylococcuss aureus*.

Antifungal activity of *S. mahagoni* seed oil against three fungal pathogens viz. *Macrophomina phascolma, Alternaria alternata, Curvularia lunata* have been listed in Table IV. It is evident from the result that the oil showed the maximum degree of radial growth inhibition against *Macrophomina phascolma* and the minimum against *Curvularia lunata*.

Toxicity. The toxicity of different extracts from *S. mahagoni* seed were studied by applying them on two predatory fish species viz. *Heteropneustes fossilis* and *Anabas testudineus*. Upon exposure to the extract *H. fossilis* showed vigorous movement and were repeatedly rising towards the surface probably for taking air. With erratic movement and having no control on the balance, the exposed species became paralyzed and straightened. Then they slowly settled to the bottom of the aquarium-water and ultimately died after different intervals. The effect of distilled water extract on *H. fossilis* showed that the lowest mortality (0%) occurred with 50 ppm dose and the highest (40%) with 750 ppm dose. However, 50% ethyl alcohol extract gave the lowest mortality (20%) with 10 ppm dose and the highest (80%) with 500 ppm dose (Table V). In case of *A. testudineus*, the affected fishes jumped upwards then they started moving up and down rapidly. Subsequently, their movement became slow and stopped; gradually they

allayed to the bottom of the aquarium-water and ultimately died after different intervals. With *A. testudineus* the distilled water extract showed mortality of 0% with 50 ppm and 30% with 500 and 750 ppm doses (Table VI). Whereas, with 50% ethyl alcohol extracts the lowest and highest mortalities were 30 and 90% with 10 and 500 ppm doses, respectively. The relative toxicity of the extracts on both *H. fossilis* and *A. testudineus* can be shown as follows: Toxicity of 50% ethyl alcohol extract > Toxicity of distilled water extract. The findings are in close conformity with that of Latifa *et al.* (1992), Latifa and Begum (1993) and Nasiruddin *et al.* (1998). From the results, we can also conclude that the toxic ingredients present in the seed of *S. mahagoni* were highly soluble in ethyl alcohol but least in distilled water. Similar solubility behavior was also observed for other plant species with the same solvents (Ameen *et al.*, 1983). However, though the observed toxic effect is minimal, further study is yet required to ascertain the drugs responsible for the toxicity and to gather information regarding the spectrum of their toxic effects.

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