Physicochemical Characterization and Antimicrobial Activity of Swietenia Macrophylla King Seed Oil

S. B. Mohammed [1], N. H. Azhari[1], Y. M. Mashitah[1], N. H. Abdurahman[2], A. S. Mazza[2]
[1]Faculty of Industrial Sciences and Technology, University Malaysia Pahang, 26300, Kuntan, Pahang, Malaysia
[2]Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang, 26300, Kuntan, Pahang, Malaysia

***Abstract*— Diseases transmitted by microbes such as bacteria and fungi, considered one of the main health problems in many countries worldwide. Plants are rich sources for wide variety of phytochemicals which are useful and used to cure many diseases. The purpose of this work was to determine: the oil content, physicochemical characterization, and antimicrobial activities of seeds oil from plant, genus Swietenia of which S. macrophylla (Family: Meliaceae) belongs to. The lipids were extracted with diethyl ether and hexane by solvent semi-continuous extraction method (Soxhlet) for 6 hours. The free fatty acids (FFA) analyzed by GC-MS. In the antimicrobial test, six microorganisms were evaluated by disk diffusion method. The microbes were: four Multiple-drug-resistant bacteria namely; Staphylococcus aureus ATCC1026, Salmonella typhimurium ATCC14038, Pseudomonas aeruginosa ATCC15442, and Escherichia coli ATCC10536; and two funguses; Candida albiacans MTCC 183 and Aspergillus niger MTCC 16404. The oil contents were, 39% and 42.7%; the major fatty acid compositions were; linoleic: (37.50 and 39.21%), oleic: (18.82 and 22.03%), stearic: (16.75 and17.65%), and palmitic: (14.62 and 15.47%) for diethyl ether and hexane respectively. Physicochemical characteristics results showed the oils contain appreciable level of unsaturation. Triacylglycerols and neutral lipids were, 85.58 and 86.70% for diethyl ether, whereas the values 86.50% and 87.99% for hexane respectively. S. macrophylla seed oils demonstrated inhibitory activity towards all tested microorganisms except E. coli. S. macrophylla seed oils demonstrated the potential antimicrobial agent for certain types of bacteria and fungi, such as S. typhimurium and A. niger. Moreover, TLC analysis showed the presence of other constituents such as sterols and this may warrant further research.**

Keywords*— Swietenia macrophylla King; Seed oil; Physicochemical characteristics; Antimicrobial activity.

I. INTRODUCTION

Nowadays, diseases transmitted by microbes such as bacteria and fungi are one of the health problems in many countries worldwide. Plants are rich source for wide variety of secondary metabolites such as terpenoids, alkaloids, flavonoids, and tannins [1], which have been reported to have antimicrobial properties. According to the World Health Organization [2] up to 80% of the population in developing countries, resort to traditional medicine, including medicinal plants to help meet their primary care needs. Thus, most people in the world using plants in their conflict with microbes.

The plant, genus Swietenia of S. macrophylla (Family: Meliaceae) locally known in Malaysia as "Sky fruit". Economically important timber tree, it is native to a number of North and South American countries including the USA, Mexico, Bolivia and central Brazil [3]. Previously, studies on the plant parts shows different phytochemical groups, such as alkaloids, terpenoids, anthraquinones, cardiac glycoside, saponins, phenols, flavonoids, volatile oils, phospholipids and long chain unsaturated acid [4]. In Malaysia the seed of the plant are used to treat diabetes, hypertension, and to relive pain [5]. Several reports shown, species of this plant have antimicrobial, antidiarrheal, antioxidant effects, antidiabetic, antinociceptive, anticancer, and hypoglycemic activities [6]. So far, very few reports published on the physicochemical properties and antimicrobial activities of the seed oil from the Malaysian S. macrophylla King. Therefore, the aim of this study was to determine the oil content, physicochemical properties, as well as antimicrobial activities of Malaysian S. macrophylla seed oil.

II. MATERIALS AND METHODS

A. Plant Material

Seeds of *S. macrophylla* King were collected on 16 December, 2010 from Kulim, Bukit Mertajan, Pulau Penang, Malaysia. The taxonomy identification of the plant was done by Botanist of the School of Environment Science and Natural Resources, National University of Malaysia, Bangi, Selengor, Malaysia.

B. Extraction of Seed Oil

The seeds (10 g) of *S. macrophylla* were divided into the seed coat and kernel parts. The seed coat part (2.5 g) was discharged. The kernel part (7.5 g) was grounded into powdered form using manual laboratory grinder and dried in air circulation oven at 50 °C for 1 h. The seed oil was extracted for 6 hours, using solvent semi-continuous extractor (Soxhlet), used hexane and diethyl ether as solvents for qualitative and quantitative comparison. Solvents were evaporated by the rotary evaporator. The oil contents were calculated based on kernel weight and expressed in (w/w %).

C. Physicochemical Characteristics

Specific gravity was determined at 30 °C with pycnometer (Cole-Parmer, Illinois, USA), whereas refractive index was determined at 27 °C using Abbey Refractometer (ATAGO T-series, Model-3T, Texas, USA) following [7] method. The oil viscosity was determined with Rheometer. [8] Testing methods were used for determination of pour, cloud, flash and fire points. Standard methods described by American Oil Chemists' Society [9] were used for determination of moisture, Ash, crude fiber, crude fat and free fatty acid (FFA) contents. Iodine value was determined using Wij's method as reported in AOCS methods [9]. Saponification value, unsaponifiable matter content, and acid values were determined by methods described by [10]. Peroxide value was determined according to the methods described by [11]. Lipids content was estimated by the method of [12] using a solvent mixture of chloroform and methanol in ratio 2:1 (v/v).

D. Fatty Acid Methyl Esters (FAMEs)

Accurately, 100 mg seed oil was dissolved in 10 mL hexane (Merck, HPLC grade) in test tube, and 1 mL of 2M methanolic KOH was added. The tube was vortex occasionally. After 15 min the fatty acid methyl ester rich upper layer was removed and washed with water and analyzed by GC-MS. GC-MS analyses were performed on an Agilent 6890 series with capillary column HP-5 (30 m × 0.25 mm ID, 0.25 µm). Hydrogen was carrier gas, flow rate: 1 mL/min, injection volume: 1 µL, injection temperature was 250 °C. Oven temperature initially maintained at 50 °C for 2 min, and then programmed at the rate of 25 °C /min up to 200 °C for 1 min, then again programmed at rate of 30 °C up to 230 °C and finally raised up to 280 °C for 18 min. The identification of the components was based on the comparison of their mass spectra with those in the system spectral library.

E. Acylglycerol and Lipid Composition

The oil was separated into mono-, di- and tri-acylglycerols by silica gel (60-120 mesh) column chromatography according to method described by [13]. For quantitative determination of acylglycerol classes, the sample (900 mg in 3 mL petroleum ether) was adsorbed on the top of the column; tri-acylglycerols were eluted with benzene, di-acylglycerols with a mixture of diethyl ether and benzene in ratio 1:9 (v/v), and mono-acylglycerols with diethyl ether. Approximately, 1.5-2 mL/min fractions were collected. Thin layer chromatography (TLC) used for monitoring the elution and/or confirmation of purities of separated fractions. hexane:diethyl ether 4:1 (v/v) as solvent system. Spots were visualized with chromic-sulphuric acid at 180 °C.

A total of 800 mg seed oil fractionated into three major lipid groups: neutral lipid, glycolipid, and phospholipid by silica gel column chromatography [13]. Neutral lipids were eluted with chloroform, glycolipids with acetone, and phospholipids with methanol. Approximately, 0.5-1.0 mL fractions were collected per min, and elution was monitored by TLC. Solvents were evaporated in vacuum rotary evaporator and percentages of these fractions were determined by gravimetric method.

F. Antimicrobial Activity

The antimicrobial efficacy of *S. macrophilla* seed oils were tested against four pathogenic bacteria: *S. aureus*, *S. typhimurium*, *P. aeruginos* and *E. coli*; and two fungus: *C. albiacans* and *A. niger* by using the disk method Kirby-Bauer method as described by [3]. Nutrient agar medium (NA) was used for determining antibacterial activity, whereas potato dextrose agar medium (PDA) was selected for antifungal activity. Stock solution of 20 mL of 1% was prepared from the 200 mg of seed oil in 20 mL of distilled water (DMSO used to dissolve the oil in water). The stock solution then serially diluted to concentrations; 10, 50, 100, and 1000 µg/mL for antimicrobial test. Streptomycin and fluconazole (10 µl/disc for each) were used as positive controls in antibacterial and antifungal tests resp., while 5% DMSO was used as negative control. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm with a transparent scale.

III. RESULTS AND DISCUSSION

A. Oil Content and Physical Characteristics

Table 1 shows, the oil content and physical characteristics of oils extracted with diethyl ether and hexane from seeds of Malaysian *S. macrophylla*. The oil content was, 39% and 42.7% for diethyl ether and hexane respectively. As shown in Table 1, no clear significant ($p>0.05$) in terms of oils properties depending on the extraction solvent have been observed in this study. [1] Reported that, oil content of seeds extracted by petroleum ether from two Indian species namely; *S. macrophylla* king and *S. mahogani* jacq., were 65.7% and 64.9% respectively. Different yields of extract might be influenced by the polarities of solvents [15]. [16] Reported different pretreatments significantly ($p<0.05$) affected yield and peroxide value of the extraction oils.

The obtained oils in this study are light yellow, bitter in taste and have smell like plant material. They are liquids at room temperature at (27±2 °C). The Flash points were 249 and 251 °C, while fire points were 260 and 263 °C, for oils of hexane and diethyl ether resp. The flash and fire points differ considerably from those previously reported for oil from *S. mahogany* grown in Bangladesh (90 and 100 °C). Fire and flash points of the fatty material are measured of its thermal stability when heated in contact with the air. Fatty acids are much less stable than acylglycerols; hence the fire and flash points of ordinary oils depend principally upon their content of free fatty acids [13]. The characterization of pour and cloud point is very important in emulsion transportation, because cloud point determines that at which temperature the emulsion start not to flow in the pipeline. While the pour point determines, at which temperature the emulsion start to flow after it freeze. High pour and cloud points reflect high oil wax content. Crude oil pour points vary between 52-60 °C [17]. From Table 1, it can be seen that the pour and cloud point for Malaysian *S. macrophylla*

seed oil were almost same as the Bangladesh seed oil, which indicates that both oils have same wax content.

B. Physicochemical Characteristics

Table 2 shows, the physicochemical characteristics of Malaysian *S. macrophylla* seeds oil compared worldwide. The specific gravity found to be 0.9432 and 0.9543 at 30 °C, refractive index were 1.459 and 1.612 at 27 °C for the oils obtained by hexane and diethyl ether respectively. Authors reported the refractive indexes of seed oils extracted from mahogany species, from different countries e.g. India, Bangladesh and Mexican were 1.4692, (1.4683-1.4751) and 1.470 respectively. The same authors, also reported that the specific gravity of seed oils extracted from two *S. mahogany* grown in Bangladesh were 0.9169 and 0.9334 [13], [18], [19], [20]. Specific gravity and refractive index are very stable parameters and should be used for checking identity of oils. The viscosity of the oil was found to be (401.77 and 412.0) at 30 °C for diethyl ether and hexane respectively. Which were lower than that of *S. mahogany* grown in Bangladesh (459.32). 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.

The moisture, Ash, and crude fiber contents of oil extracted with hexane and diethyl ether were (2.6%, 1.91%), (2.5%, 2.0%) and (72.3%, 71.1%) respectively. The obtained results in current study almost similar to that seeds oil of *S. mahogany* grown in Bangladesh [13]. The crude fat of the oil in range 71.1 to 72.3%, significantly differ from the mahogany species grown in Bangladesh. The iodine value (degree of unsaturation) of the Malaysian *S. macrophylla* seeds oil (by both solvents) was found to be 71.05, which was lower than the values 109.7 and (92.5-94.7) for Indian and Bangladesh resp. Therefore, Malaysian *S. macrophylla* seed oil in contrast to its counterparts grown in Indian and Bangladesh. As a result, the Malaysian oil has less tendency of rancidity by oxidation. The acid value and percentage of free fatty acid (as oleic) of the oil extracted by hexane and diethyl ether were (14.32 and 10.59) and (5.23 and 4.36) resp; which are almost similar to that grown in Bangladesh (0.87-11.1) and (4.36-5.23) [13], [19]. The percentage of free fatty acids indicates, unsuitability of the Malaysian lipid for edible purpose, but it can be used as an ingredient in manufacturing. The unsaponification matter (USM%) of the oil ranged between (1.5-2.1%) being much higher than the value 0.5 % and 1.1 % for those reported in Bangladesh and India resp. The saponification value of the oil in range 162.55 to 211.75, the values are below than saponification values of plant grown in India (292.4). But in consis with that of the *S. mahogany* seed oil obtained in Bangladesh (176.82-200.3), and *S. humillis zucc* seed oil grown in Mexico (159.55). The peroxide values of the oil in range 2.25-3.25, which is slightly higher than the *S. mahogany* growing in Bangladesh (0.97-2.6). The experimental results of this study revealed that, *S. macrophylla* seed oil was valuable and could be potential for the different uses, and this is warrants further research.

C. Fatty Acid Compositions

Table 3 shows the free fatty acid compositions of Malaysian *S. macrophylla* seeds oils compared with those grown in India, Mexico, Indonesia and Bangladesh. No significant difference in terms of quantity of free fatty acid compositions, with a little exception such as Mexico. Malaysian *S. macrophylla* contains high proportion of linoleic (37.5-39.21%) and oleic (18.82-22.03%) compared to world-wide; e.g. Indian *S. macrophylla* contains, linoleic and oleic in rate of 29.3% and 14.4% respectively. Linoleic acid is desirable potential industrial uses as a drying oil. The abundance of poly-unsaturated fatty acid (PUFA), such as linoleic and oleic acids in seeds oil is indicator for many health benefits. Although, it could be paid some attention for the oil has such properties. The probability of oxidation for the oils with PUFA will be high and this will produce rancid flavor and decrease quality of oil [13].

[14] Investigated on oils obtained from *S. macrophylla* king and *S. mahogani* jacq. in India, and they reported that, the fatty acid compositions were: linoleic (29.3 and 30.5%), oleic (14.4 and 27.4%), steric (both 14.4 %), linolenic (11.9 and 12.5%), palmitic (11.6 and 12.0%), arachidic (both 1.5%), palmitoleic (both 0.3%), and eicosenoic (both 0.1%) respectively. Again in research conducted by [18], showed the fatty acid composition of the seed fat from Indian *S. macrophylla* were, linoleic (33.87%), oleic (25.30%), stearic (16.42%), palmitic (12.50%), linolenic (11.32%), and arachidic (0.56%). The latter values, for linoleic and linolenic acid differ considerably from those previously reported for oil obtained from same species grown in Mexico [18]. [21] Studied the comprehensive analysis of the composition of seed cake and its fatty oil from *S. mahogany* Jacq. growing in Bangladesh and reported that the seed cake contain 19.42% fats, and the major (>1%) constituents of the methylated fatty esters were linoleic (26.00%), elaidic (24.39%), steareic (14.32%), palmitic (12.97%), 10-methyl-10-nonadecanol (5.24%), eicosanoic acid (2.48%), 3-heptyne-2,5-diol-6-methyl 5-(1-methylethyl) (2.03%), octadecanoic acid,9,10,12-trimethoxy (1.90%), 1,3-dioxalane, -ethyl-4-methyl-2-pentadecyl (1.89%), and 2-furapentanoic acid, tetrahydro-5-nonyl (1.03%). [22] Found that the seeds *S. mahogany* of *S. mahogany* Jacq., from Indonesia contained a fixed oil containing six fatty acids namely, pamitic (18.50%), linoleic (30.55%), oleic (30.66%), steareic (17.42%), arakideic (2.33%), and behenat acid (0.54%).

D. Acylglycerol and Lipid Composition

Table 4 shows the triacylglycerols and lipid compositions of Malaysian *S. macrophylla* seeds oil compared with seeds oil of *S. mahogany* grown in Bangladesh. Triacylglycerols of the total weight of Malaysian *S. macrophylla* seed oil accounted as 87.99%. The total recovery of acylglycerol was 93.15. This result indicated the seeds oil contained lower amount of non-acyglycerol than that found in Bangladesh. Malaysian oil contained higher percentage of mono-acyglycerol, which could be separated easily by column chromatography and used as emulsifier. The current

results showed that, neutral lipids were found to be most abundant of Malaysian *S. macrophylla* seed oil; its accounts up to 87.99% of the total lipids, whereas the ratio were 5.11% and 4.23% for glycoside and Phospholipids respectively.

E. Antimicrobial Activity

The antimicrobial activity of *S. macrophylla* seeds oil against four pathogenic bacteria namely; *S. aureus*, *S. typhimurium*, *P. aeruginosa* and *E. coli*; and two fungi: *C. albiacans* and *A. niger* shown in Table 5. The inhibitory activity of the oils was extremely broad against tested microorganisms. The inhibition zones of the oils (both solvent) were 5-12, 6-22, and 5-12 mm for *S. aureus*, *S. typhi* and *P. aeruginosa* respectively. Whereas *E. coli* completely resistance to the oil and not observed any inhibition zones. It can explain that, *E. coli* is most resistance, while *S. typhimurium* was most sensitive amongs the tested organisms to this oils. Table 5 also demonstrates that, all test concentrations displayed weak activities against both tested fungi compared to standard (Fluconazole). Seed oils showed maximum antifungal effect against *A. niger* with inhibition diameter 7 mm at the highest concentration. While the lowest inhibition zones (3 mm) was recorded against *C. albicans* at the lowest concentration.

In previous studies on antimicrobial effect of seed oils from *Pentaclethra macrophylla Bent*, *Chrysophyllum albidum G. Don* and *Persea gratissima Gaerth F* on some local clinical bacteria isolates. The authors reported the inhibition zone diameters (IZD) of 5.4-29.3, 5.4-28.7, and 7.6-30.0 mm for *P. macrophylla*, *P. grattisima*, and *C. albidum* resp. The same authors also reported that the *E. coli* was the most resistance to the tested oils, and inhibition zones were 10.6, 8.5, and 9.5 mm for the three organisms respectively [23]. Previously, [13] studied antifungal activity of crude extracts obtained by petroleum ether, ethyl acetate and methanol on University of Malaysia, Selangor (UM) for the Plant identification.

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six fungi, including *A. niger* and *C. albicans*. They reported most of the extracts displayed weak activity against tested fungi. Significant antifungal inhibition (14 mm) was observed with petroleum ether extract in case of *A. niger*, which is equal to that of standard anti-fungal agent fluconazole.

IV. CONCLUSIONS

The oils content of lipids extracted by soxhlet from *S. macrophylla* king seeds with two solvents namely; diethyl ether and hexane were 39% and 42.7% respectively. The physicchemical characteristics of seed oil could be helpful for identify the quality of oil, and oil products for possible industrial or commercial uses. The major fatty acid compositions are linoleic (37.50-39.21%), oleic (18.82-22.03%), stearic (16.57-17.65%), and palmitic (14.62-15.47%). The reported oil qualities are comparable to other oils and can be useful in the manufacture of paint, varnish, and ink industries. On the other hand, the underutilized *S. macrophylla* seed oil contained higher amount of lipid which makes a good source for industrial uses. *S. macrophylla* seed oil demonstrated antimicrobial activity against all tested microorganisms except *E. coli*.; and it showed the potential antimicrobial agent for certain types of bacteria and fungi, such as *S. typhimurium* and *A. niger*. Moreover, TLC analysis showed the presence of other constituents, such as sterols and this may warrant further research.

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Table -1: Oil content and physical characteristics of seeds oil

Characteristics	Values	
	hexane	Diethyl ether
Yield (%)	42.7±2.0	39±1.6
Color	Light yellow	Light yellow
Taste	Bitter taste	Bitter taste
Smell	Seed smell	Seed smell
State at room temperature	Liquid	Liquid
Pour point (°C)	-4±0.20	-5±0.30
Cloud point (°C)	8±0.10	7±0.50
Fire point (°C)	260±2.8	263±1.0
Flash point (°C)	249±3.0	251±1.1

Table -2: Physicochemical characteristics of some mahogany species seed oil

Parameter	*S. marophylla* Malaysia		*S. macrophylla* India[a]	*S. mahogany* Bangladesh [b]	*S. humilis* Mexico[c]
	hexane	Diethyl ether			
Sp.gr. at (30 °C)	0.9543±0.003	0.9432±0.005	–	0.9169-0.9334	–
R. I. at (27 °C)	1.459±0.0041	1.612±0.0048	1.4692	1.4683-1.4751	1.470
Viscosity at (30 °C)	412.9±3.1	401.77±2.3	–	459.32	–
Moisture (%)	2.6±0.11	1.91±0.10	–	1.5	–
Ash %	2.5±0.12	2.0±0.12	–	2.8	–
Fat %	72.3±	71.1±	–	19.42	–
Crude fiber %	1.67±0.31	1.42±0.10	–	1.4	–
I. V. (Wij's)	71.05±1.7	71.06±1.3	109.7	92.5-94.69	–
A. v. (mg KOH/g)	14.32±0.41	10.59±0.22	–	0.87-11.1	–
F. F. A (%) as oleic	5.23±0.32	4.36±0.27	–	3.2-5.6	0.367
S. V. (mgKOH/g)	211.75±2.7	162.55±1.8	292.4	176.82-200.3	159.55
U. S. M (%w/w)	2.1±0.27	1.5±0.32	1.1	0.52-1.9	–
P. V (%)	3.25±0.7	2.25±0.38	–	0.97-2.6	0.739

Sources: [a] [18]; [b] [19]; [C][20]; (–) not detected.

Table -3: Fatty acids composition (%) of seed oils of mahogany species from different countries

Fatty Acid %	*S. macrophylla* Malaysia		*S. macrophylla* India[a]	*S. macrophylla* Mexico[b]	*S. mahogany* Indonesia [c]	*S. mahogany* Bangladesh[d]
	hexane	Diethyl ether				
C16:0	5.5±0.14	14.4±0.11	11.6-12.50	–	18.50	12.97
C16:1	0.54±0.02	0.54±0.02	0.3	–	–	–
C18:0	17.7±0.50	16.6±0.26	14.4-16.42	–	17.42	14.32
C18:1	18.9±0.21	22.0±0.2	14.4-27.40	24.66	30.66	24.39
C18:2	39.2±0.68	37.5±0.16	29.3-33.87	49.87	30.55	26.00
C18:3	–	–	11.32-12.5	–	–	13.5

Sources: [a] [18]; [b] [19]; [C][22]; [d][21]; (–) not detected.

Table -4: Acylglycerol and lipid composition (wt %) of *S. macrophylla* and S. *mahogany* seed oils

Parameter	Composition	Malaysia		Bangladesh[a]
		hexane	Diethy ether	
Acylglycerol	Monoacylglycerol	1.57±0.50	1.51±0.22	1.4
	Diacylglycerol	3.70±0.20	3.59±0.11	3.0
	Triacylglycerol	86.50±0.43	85.58±0.63	87.0
Lipid	Neutral lipids	87.99±0.60	86.7±1.27	89.4
	Glycolipids	5.11±0.54	5.06±0.34	4.8
	Phospholipids	4.23±0.31	3.99±0.31	3.5

Sources: [a] [19]

Table -5: Antimicrobial activates of seed oils

Inhibition zone (mm) [a]							
Seed oil	Conc. µg/mL	*S. aureus*	*S. typhi*	*P. aeruginosa*	*E. coli*	*A. niger*	*C. albicans*
Hexane	10	5	6	5	–	–	–
	50	8	9	6	–	4	3
	100	9	11	6	–	5	4
	1000	11	21	11	–	7	5
Diethyl ether	10	6	6	6	–	5	3
	50	9	13	7	–	5	3
	100	10	15	9	–	7	4
	1000	12	22	12	–	8	4
Reference	10 µg/disc	21.4	22.5	32.9	27.6	23.6	26.1
Control	5%	–	–	–	–	–	–

[a]Inhibition zone diameter including the diameter of the paper disc (6 mm) determined by disc diffusion method; Control: DMSO; References: Streptomycin for *S. aureus*, *S. typhi*, *P. aeruginosa* and *E. coli*; Fluconazole for *A. niger* and *C. albicans*; – = no growth inhibition.