Fatty Acid Composition And Antibacterial Activity Of *Swieteniamacrophylla*king Seed Oil

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Abstract

Pathogenic bacteria cause many acute and serious diseases, especially Multiple-drug-resistant strains have become such a problem due to overuse of antibiotics. Various medicinal plants are used to prevent or cure infectious diseases. The aims of this study were to determine the oil content, fatty acids compositions,and antibacterial activity of *S. macrophylla*King seed oil against four Multiple-drug-resistant bacteria namely: *Staphylococcus aurous*, *Staphylococcus typhimurium*, *Pseudomonas aeruginosa,* and *Escherichia coli* by disk diffusion method. The lipids were extracted by soxhlet using diethyl ether and n-hexane; and their free fatty acids (FFA) were analyzed by GC-MS. The oil content was 39-42.7% and major fatty acid compositions were linoleic (37.50-39.21%), oleic (18.82-22.03%), stearic (16.75-17.65%), and palmitic (14.62-15.47%) for diethyl ether and n-hexane respectively. The antibacterial activity among seed oil was extremely broad and inhibition zones ranged from 0-20 mm. These results showed the potential of *S. macrophylla*seed oil as antimicrobial agent for certain types of bacteria such as *S.typhimurium.*Moreover, the TLC analysis showed the presence of others constituents such as sterols and this may warrant further research.

Keywards: *Swieteniamacrophylla*King, Lipids, Antibacterial activity, Pathogenic bacteria

INTRODUCTION

Antibiotic medications are used to kill bacteria, which can cause serious diseases and illnesses. They have played a big role to human health even many diseases can

now be controlled by antibiotics. However,the inappropriate and irrational use of antibiotics led some bacteria to become resistant to commonly antibiotics(WHO, 2011). Also the environmental problems associated by synthesis new drugs have to consider.Consequently, there is an urgent need to discover new natural and active antimicrobial from different sources for the treatment. Natural products have been important source of new drugs (Suganya et al., 2012).

The big-leaf mahogany, genus *Swietenia*of which *Swieteniamacrophylla*is a plant belonging to Meliaceae family, it is extended in most tropicalcountries especially in Brazil, Bolivia, Mexico, Guatemala, Peru, andCentral America (Nour et al., 2012). The fruits are commonly known as “sky fruit” because it seems to point up towards the sky (Masoudet al., 2012).This economically timber tree is traditionally used for the treatment of a number of diseases including: blood pressure, diabetes, and hypertension (Dewanjee et al., 2009; Tanet al., 2009;Wu et al., 2012). Scientifically, the crude extract from *S.macrophylla* seeds have been reported to possess biological activities such as: antimicrobial (Mallik and Banik, 2012), anti-malaria (Soediro et al., 1990), anti-hepatitis (Wu et al., 2012),anti-diarrheal (Maiti et al., 2007),antioxidant (Sahgal et al., 2009;Falah et al., 2008),anti-diabetic(Dewanjee et al, 2009;Kalaivanan and Pugalendi,2011),anti-inflammatory and anti-mutagenic (Guevara et al., 1996),antinociceptive (Daset al.,2009),and antitumor (Goh and AbdlulKadir,2011).In the present study, aimed to probe the oil content and chemical compositions of oil extracted from *S. mcrophylla*seeds, as well as to screen its antibacterial activity against four multiple-drug-resistance bacteria strains namely,*S.aurous,S.typhimuriumP.aeruginos*and*E.coli.*

MATERIALS AND METHOD

Plant material source

*Swieteniamacrophylla*fruitswere collected on 16 December, 2010 from the small town of Kulim, Bukit Mertajam, Pulau Pinang, Malaysia. The taxonomy identification of plant was done by botanists of the School of Environmental Sciences and Natural Resources, Natural University of Malaysia, Bangi, Selangor. The seeds removed from matured fruits and dried (33°C) at open area with active ventilation until attained

constant weight (three weeks).The seed kernels were removed from the seed and then grind to the small pieces using domestic grinder.

Extraction of seed oil

The groundedseeds of *S. macrophylla*(10g) were extracted by soxhlet apparatus for 6 hours by two solvents namely;n-hexane and diethyl etherfor qualitative and quantitative comparison.This procedure was repeated until at least 10 ml oil was recovered. The organic solvent was evaporated by the rotary evaporator and further dried under open air. Then the percentage of seedoil was calculated (w/w %); and stored in a dark bottles and kept at 4°Cuntilanalysis or evaluated for antibacterial activity as described below**.**

Fatty Acid Methyl Esters (FAMEs)

Accurately, 100 mg seed oil was dissolved in 10 ml hexane (Merck, HPLC grade) in test tube, 1 ml of 2M methanolic KOH was added, and then the tube was vortex occasionally. After 15 min, the fatty acid methyl ester – rich upper layer was removed, washed with water and analyzed by GC-MS.

GC-MS analysis

GC-MS analyses were performed on an Agilent 6890 series with capillary column HP-5 (30m × 0.25mm ID, 0.25µm). The carrier gas was Hydrogen, flow rate: 1ml\min, injection volume: 1 µl, injector temperature was250°C.Oven temperature initially maintained at 50°C for 2 minutes, and then programmed at the rate of 25°C\min up to 200°C for one minute,then again programmed at rate of 3°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's spectral library.

Test concentrations and antibacterial investigation

Five test concentrations of *S. macrophylla* seedoil were prepared. Stock solution of 1% oil was prepared by dissolving 100mg of seed oil in 10 ml of solution solvent (9 ml

H_2O + 1 ml DMSO). The stock solution was diluted to10, 20, 50, 100, and 1000 µg/ml,labeled and stored for further antibacterial assessment.The antibacterial activity of *S.macrophylla*seedoil was tested by disk diffusion method. In this method, the filter paper disk is impregnated into sample solution, and then the impregnated disk was placed on the nutrient agar media seeded with the pathogenic organism.

Preparation of Nutrient Agar Medium

Nutrient broth (CM0001, Oxiod) and Muellar-Hinton agar were used for liquid media and solid media respectively. To prepare required volume of each medium, the amount of each of the constituents was calculated from the composition chart given for 1000 ml. Liquid and solid media were weighing in two conical flasks. Distilled water was added to complete the final volume, and then the media was mixed well and boiled to make sure the media are dissolved totally. Finally, the conical flask plugged with cotton and sterilized by autoclave at temperature 121°C for 15 min.

Preparation of microbial

S. aurous, S. typhimurium, P. aeruginosa, and E. coli were obtained from biotechnology department, Faculty of Industrial Sciences and Technology, University Malaysia Pahang, (UMP). The microorganisms were cultured on Mueller-Hinton agar at 37°C for 24 hours. A colony of single bacteria was transferred into test tube contained 2 ml sterile saline, the saline tube was vortexes to make smooth suspension, The turbidity of the suspension was compared with 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy (Tan et al., 2009).

Qualitative antibacterial activity

The antibacterial efficacy of *S. macrophilla* seedoil was tested against *S. aurous*, *S.typhimurium,P. aeruginosa*, and *E. coli*), by disk diffusion method.Briefly, sterile 6 mm whatman No, filter paper disk was placed gently on MH agar freshly seeded with bacteria, with the help of a sterile forceps to ensure complete contact with the agar surface, and *S. macrophylla*seedoil was applied onto each paper disk, followed by

incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibitory zone in term of millimeters and recorded. Standard antibiotic A*mpicillin* was used as positive control while DMSO was used as negative control. Testing was done in triplicate and the average valueswere calculated.

RESULTS AND DISCUCSSION

Oil content and fatty acid compositions of *S. macrophylla*seed oil

The oil content of *S. macrophylla* seed,which extracted by diethyl ether and n-hexane were 39% and 42.7% respectively. For quantitative comparison, the oil obtained by n-hexane slightly higher than that extracted by diethyl ether. The different yields of extract might be influenced by the polarities of solvents (Ahmed et al., 2009).Table1 shows the free fatty acid compositions of *S. macrophylla* seed oils for both solvents no significant difference in terms of quality of free fatty acid compositions.

The linoleic acid, which is desirable for the potential industrial use of the oil as a drying oil is high. Malaysian *S. macrophylla* contains high proportion of linoleic (37.5-39.21%) and oleic (18.82-22.03%) compared to world-wide such as some Indian *S. macrophylla* plant, which contain of linoleic (29.3%) and oleic (14.4%). The abundance of poly-unsaturated fatty acid (PUFA) such as linoleic and oleic acids in *S. macrophylla* seed oil indicate for many health benefits. Although also it can 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.

In previous study, authors reported that the oil content of seeds which extracted by petroleum ether from two Indian species of *Meliaceae*, namely *S. macrophylla* king and *S. mahogani*jacq., were 65.7% and 64.9% respectively; whereas, 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, (Kleiman and Payne-Wahl, 1984). Again in research conducted by Chakrabarty and Chowdhuri (1957) showed that 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%). These values for linoleic and linolenic acid differ considerably from those previously reported for oil from the same species grown in Mexico (Chakrabarty and Chowdhuri, 1957).

On the other hand, Ping et al. (2012) in their investigation on the effect of pretreatments on chemical and antioxidant properties of sky fruit *(S.macrophylla*) seed oil showed that different pretreatments significantly ($p<0.05$) affected yield and peroxide value of the extraction oilsMostafa et al. (2011) 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%), pamitic (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%). Marpaung (2003) found that the seeds 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%).

Table 1: Fatty acids composition (%) of the *S. macrophylla* seed oil

Fatty acid	n-hexane	diethyl ether
Palmitic acid (C16:0)	15.47	14.62
Palmitolic acid (C16:0)	0.59	0.54
Stearic acid (C18:0)	17.65	16.57
Oleic acid (C18:1)	18.82	22.03
Linoleic acid (C18:2)	39.21	37.50

Antibacterial activity of *S. macrophylla* seed oil

The antibacterial activity of *S. macrophylla* seed oil against *S. aurous, S. typhimurium, P. aeruginosa*, and *E. coli* was evaluated by disk diffusion method and the results shown in Table 2. The obtained results showed that the antibacterial activity of the oils was extremely broad against test organisms. The inhibition zones of the oils with concentrations ranged from 10-1000 µg/ml were 5-11, 4-20 and 5-11 mm for three organisms namely *S. aurous*, *S. typhimurium*, and *P. aeruginosa* respectively. Whereas *E. coli* completely resistance to the oils and not observed any inhibition zones (Table 2). It can explain that *E. coli* is most resistance and *S. typhimurium* is most sensitive of the tested organisms to these oils.

In previous studies on antimicrobial effect of seed oils from *Pentaclethramacrophylla* Bent, *Chrysophyllumalbidum* G. Don and *Perseagratissima*Gaerth F on some local clinical bacteria isolates. The authors reported the inhibition zone diameters (IZD) were 5.4-29.3, 5.4-28.7, and 7.6-30.0 mm for *P. macrophylla, P. grattisima, and C. albidum*respectively. 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 (Ugbogu and Akukwe, 2009).

Table2: Antibacterial activity of *S. macrophylla* seed oil. Numbers indicate the mean diameters (mm) of inhibition of triplicate experiments. –indicates no growth inhibition.

	Bacteria inhibition zones (mm)				
Seed oil	**Concentration (µg/ml)**	***S.aurous***	***S.typhimurium***	***P.aeruginosa***	***E.coli***
	10	5	4	5	-
	20	8	6	5	-
	50	8	9	6	-
	100	9	10	6	-
	1000	11	20	11	-
Ampicillin	10	7	11	6	5
	50	11	17	8	6
	1000	20	36	18	30

CONCLUSION

The oil contents of lipids extracted by soxhlet from *S. macrophylla* king seeds with two solvents namely diethyl ether and n-hexane were 39% and 42.7% respectively. The major fatty acid compositions were linoleic (37.50-39.21%), oleic (18.82-22.03%), stearic (16.57-17.65%), and palmitic (14.62-15.47%). The antibacterial activity among seed oil was extremely broad against test organisms. These results showed the potential of *S. macrophylla*seed oil as antimicrobial agent for certain types of organisms such as *S.typhimurium.* The TLC analysis shows others constituents such as sterols this may warrant further research.

Acknowledgement

The authors acknowledge Universiti Malaysia Pahang for the Graduate Research Scheme GRS No. 120379, and also we thank the Botanist of the School of Environmental Science and National Resources, UniversitiKebangsaan Malaysia for plant identification.

REFERENCES

Ahmed A, Alkarkhi AFM, Hena S, Khim LH (2009). Extraction, separation and identification of chemical ingredients of *Elephantopusscaberl* using factorial design of experiment. Int. J. Chem. 01(01): 36-49.

ChakrabartyMM and Chowdhuri KD (1957).The fatty acid composition of the seed fat from *SwieteniaMacrophylla*. J. Am.Oil Chem. Soc. 34(10): 489-490.

Das A, Sunilson JAJ, Gopinath R, Radhamani S, Nilugal K (2009). Anti-nociceptive activity of fruits of *Swieteniamacrohylla* king. J. Pharm. R. 2(9):1367-1369.

DewanjeeS, Maiti A, Das AK, Mandal SC, Dey SP (2009).Swietenine: A potential oral hypoglycemic from *Swieteniamacrophylla* seed. Fitoterapia. 80: 49-251.

FalahS, Suzuki T, katayama T (2008).Chemical constituents from *Swieteniamacrophylla* bark and their antioxidant. P. J. Biol. Sci. 11(16): 2007-2012.

Goh BH, AbdlulKadir H (2011). In vitro cytotoxic potential of *Swieteniamacrophylla* King seeds against human Carcinoma cell lines. J. Med Plant Res. 5(8): 1395-1404.

Guevara AP, Apilado A, Sakurai H, Kozuka M, Takuda H (1996). Anti-inflammatory, antimutagenicity, and antitumor promoting-activities of mahogany seeds, *Swieteniamacrophylla* (Meliaceae). Phil. J. Sc. 125(4): 271–277.

Kalaivanan K, Pugalendi KV (2011). Antihyperglycemic effect of the alcoholic seed extract of *Swieteniamacrophylla*on streptozotocin-diabetic rats. Pharmacognosy Res. 3(1): 67–71.

Kleiman R, Payne-Wahl L (1984). Fatty Acid Composition of Seed Oils of the *Meliaceae*Including One Genus Rich in cis-Vaccenic Acid. JAOCS, 61(12):1836-1838.

Maiti A, Dewanjee S, Manda SC (2007). In vivo evaluation of antidiaharrhoeal activity of seed of *Swieteniamacrophylla* King (Meliaceae).J. Pharm. Res. 6(2): 711-716.

Mallik J, Banik RK (2012). In vitro studies on antimicrobial and thrombolytic activity of *Swieteniamacrophylla* King.J. Pharm. Res. Opin. 2(5): 45-48.

Marpaung H (2003). The analysis of fatty acid compositions in the seeds of *Swietenia mahogany* JAQ.JurnalSains Kimia, 7(1): 26-27.

Masoud EAM, Haji BS, Mohammed AO (2012). The influence of sucrose ester surfactants and different storage condition on the preparation of a novel *Swieteniamacrophylla* oil nano-emulsion. Int. Res. J. Pharm. 3(6): 2230-8407.

Mostafa M, Jahan AI, Riaz M, Hossain H, Nimmi I, Miah A. Chowdhury UJ, (2011). Comprehensive analysis of the composition of seed cake and its fatty oil from *S. mahogany*Jacq.Growing in Bangladesh. J. Pharm. Sci. 10(1): 49-52.

NourAH, NourAbH, Sandanasamy JA/P, Yusoff MM (2012). Antibacterial Activity of Different Extracts of *SwieteniaMacrophylla* King.Proceedings of the 13th Seminar on Medicinal and Aromatic Plants, 25-26 September 2012, Kuala lumbur, Malaysia, pp. 17-18.

Ping CL, Ibrahim HN, Yusof MH, (2012). Effect of pretreatments on chemical and antioxidant properties of sky fruit (*Swieteniamacrophylla*) seed oil. J. TeknoldanIndustriPangan. 23(02): 205-209.

Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM (2009). In vitro antioxidant and xanthine oxidase inhibitory activities of methanolic*Swieteniamahagoni* seed extracts. Molecules, 14: 4476-4485.

Soediro IPK, Wattimena JR, Rekita S (1990). Study of the active antimalarial methanolic extract of *Swieteniamacrophylla* King (Meliaceae).Acta Pharm Indonesia. 15(1): 1-13.

Suganya S, Bharathidasan R, Senthilkumar G, Panneerselvam A (2012). Antibacterial activity of essential oil extracted from *Coriandrumsativam*(L.) and GC-MS analysis. J. Chem Pharm. Res. 4(3): 1846-1850.

Tan S-K, Osman H, Wong K-C, Boey P-L (2009). Antimicrobial and antitoxic activities of *Swieteniamacrophylla* leaf extract. As. J. Food Ag-Ind. 2(02): 181-188.

Ugbogu OC, Akukwe AR (2009). The antimicrobial effect of oils from *Pentaclethramacrophylla* Bent, *Chrysophyllumalbidum* G. Don and *Perseagratissima*Gaerth F on some local clinical bacteria isolates. Afr. J. of Biotechnol, 8(2): 285-287.

WHO,2011. Antimicrobial Resistance, World Health Organization.*www.who.int/entity/mediacentre/factsheets/fs194/en***(**Accessed on April 7, 2011)

Wu S-F, Lin C-K, Chuang Y-S, Chang F-R, Tseng C-K, Wu Y-C, Lee J-C (2012). Anti-hepatitis C virus activity of 3-hydroxy caruilignan C from *Swieteniamacrophylla* stems. J. Viral Hepat. 19(5): 364-370.