Full Length Research Paper

Fatty acid composition and antibacterial activity of *Swietenia Macrophylla* king seed oil

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Accepted 11 June, 2013

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 *Swietenia 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 to 42.7% and major fatty acid compositions were linoleic (37.50 to 39.21%), oleic (18.82 to 22.03%), stearic (16.75 to 17.65%), and palmitic (14.62 to 15.47%) for diethyl ether and n-hexane, respectively. The antibacterial activity among seed oil was extremely broad and inhibition zones ranged from 0 to 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.

Key words: *Swietenia macrophylla* 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 *Swietenia macrophylla* is a plant belonging to Meliaceae family, it is extended in most tropical countries especially in Brazil, Bolivia, Mexico, Guatemala, Peru and Central America (Nour et al., 2012). The fruits are commonly known as "sky fruit" because it seems to point up towards the sky (Masoud et 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; Tan et al., 2009; Wu et al., 2012). Scientifically, the crude extract from *S. macrophylla* seeds have been reported to possess

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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 antimutagenic (Guevara et al., 1996), antinoceptive (Das et al., 2009) and antitumor (Goh and Abdul Kadir, 2011).

In the present study, aimed to probe the oil content and chemical compositions of oil extracted from *S. macrophylla* seeds, as well as to screen its antibacterial activity against four multiple-drug-resistance bacteria strains namely, *Staphylococcus aureus, Staphylococcus typhimurium*, *Pseudomonas aeruginos* and *Escherichia coli*.

**MATERIALS AND METHODS**

**Plant material source**

*Swietenia macrophylla* fruits were 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, National University of Malaysia, Bangi, Selangor. The seeds were 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 grounded seeds of *S. macrophylla* (10 g) were extracted by soxhlet apparatus for 6 h by two solvents namely: n-hexane and diethyl ether for quantitative and qualitative 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 seed oil was calculated (w/w %); and stored in a dark bottles and kept at 4°C.

**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 (30 m x 0.25 mm ID, 0.25 μm). The carrier gas was hydrogen, flow rate: 1 ml/min, injection volume: 1 μl, injector 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 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* seed oil were prepared. Stock solution of 1% oil was prepared by dissolving 100 mg of seed oil in 10 ml of solution solvent (9 ml H2O + 1 ml DMSO). The stock solution was diluted to 10, 20, 50, 100, and 1000 μg/ml, labeled and stored for further antibacterial assessment. The antibacterial activity of *S. macrophylla* seed oil 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 Mueller-Hinton agar were used for liquid 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 was plugged with cotton and sterilized by autoclave at temperature 121°C for 15 min.

**Preparation of microbial**

*S. aureus, S. typhimurium, P. aeruginos* 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 h. 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* seed oil was tested against *S. aureus, S. typhimurium, P. aeruginos* 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. macrophilla* seed oil was applied onto each paper disk, followed by incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of inhibitory zone in term of millimeters and recorded. Standard antibiotic *Ampicillin* was used as positive control while DMSO was used as negative control. Testing was done in triplicate and the average values were calculated.

**RESULTS AND DISCUSSIONS**

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

The oil content of *S. macrophylla* seed, which was 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). Table 1 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 to 39.21%) and oleic (18.82 to 22.03%) compared to world-wide such as some Indian S. macrophylla plant, which contain 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 was 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 oils. Mostafa 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%), 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%).

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.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>n-hexane</th>
<th>Diethyl ether</th>
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<tbody>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>15.47</td>
<td>14.62</td>
</tr>
<tr>
<td>Palmitolic acid (C16:0)</td>
<td>0.59</td>
<td>0.54</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>17.65</td>
<td>16.57</td>
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<tr>
<td>Oleic acid (C18:1)</td>
<td>18.82</td>
<td>22.03</td>
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<tr>
<td>Linoleic acid (C18:2)</td>
<td>39.21</td>
<td>37.50</td>
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### Antimicrobial 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 to 1000 µg/ml were 5 to 11, 4 to 20 and 5 to 11 mm for three organisms namely, S. aurous, S. typhimurium and P. aeruginosa, respectively. Whereas, E. coli completely resistant 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 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) were 5.4 to 29.3, 5.4 to 28.7, and 7.6 to 30.0 mm for P. macrophylla, P. gratissima 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).

### 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 to 39.21%), oleic (18.82 to 22.03%), stearic (16.57 to 17.65%) and palmitic (14.62 to 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.

ACKNOWLEDGEMENTS

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, Universiti Kebangsaan Malaysia for plant identification.

REFERENCES


Suliman et al. 303

Table 2. Antibacterial activity of S. macrophylla seed oil. Numbers indicate the mean diameters (mm) of inhibition of triplicate experiments. –indicates no growth inhibition.

<table>
<thead>
<tr>
<th>Bacteria inhibition zones (mm)</th>
<th>Concentration (µg/ml)</th>
<th>S. aurous</th>
<th>S. typhimurium</th>
<th>P. aerugiosa</th>
<th>E. coli</th>
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<tbody>
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<td>10</td>
<td>5</td>
<td>4</td>
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<td>20</td>
<td>8</td>
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<td>Seed oil</td>
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