Antibacterial Activity of Different Extracts of *Swietenia Macrophylla* King

Azhari H. Nour[1*], Abdurahman H. Nour[2], Jessinta A/P Sandanasamy[1] and Mashitah M. Yusoff[1]

[1]Faculty of Industrial Sciences and Technology, University Malaysia Pahang, Malaysia

[2]Faculty of Chemical and Natural Resources Engineering, University Malaysia Pahang, Malaysia

*azharyhamid@yahoo.com

Abstract: The study was carried out to screen antimicrobial activity for a new natural, nontoxic and effective antibiotic from plant extract. In this study, *Swietenia Macrophylla* King was selected and antibacterial activity of extracts from different parts (leaf, fruit cover, seed cover) with various solvents or different fractions of the extracts was studied in laboratory test. The antibacterial activity of these extracts was assessed against two multiple-drug-resistance bacteria strains namely, *Escherichia coli* and *Staphylococcus aureus* by the well diffusion method. For the antibacterial bioassay, four concentrations (10, 50, 100 and 150 µg mL^{-1}) of each extract solutions were prepared. The antibacterial activity among extracts was extremely broad against both test organisms. Minimum inhibition concentration was done serial dilution method. The MIC values of the extracts ranged from 8.1 to 21.2 µg mL^{-1}. Among fractionated extracts, fraction 4 (F4) of fruit cover more potent than other fractions, whereas among solvent crude extracts; the ethanol extracts from fruit cover (EFC) displayed overall more potent activity than other parts against both tested bacteria. The experimental results obtained from this study suggest that *Swietenia Macrophylla King* is promising as natural antibacterial. Some bioactive groups were screened by TLC; and the results obtained were positive and this may warrant further research to determine the bioactive compound(s).

Keywards: *Swietenia Macrophylla* King; Antibacterial; Multiple-drug-resistance; *E. coli*; *S. aureus*

INTRODUCTION

Natural products play an important role in discovery and development of new products (Cantrell et al., 2012). In the 20 century, there are sharply improves of the science and advance in technology in the medical field to improve the health quality of human. However, there are still have a lot of human affected by some of dangerous bacterial, fungus, and virus. Many vaccines and antibacterial agents in the market nowadays are less efficiently. It is due to the global increase in resistance of bacteria to drugs. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). Thus, the need for the development of newer drugs to treat infections caused by these MDR bacterial species has never been more paramount (Nascimento et al*.,* 2000)*.*

Synthesis a new drug is too expensive, waste products in process, the drug is not stable and may be create toxic as by products. Therefore, there is more favorable used national product to form drug. By this way, there are more safety, friendly environment, and cheaper in price.

Swietenia macrophylla has a wide natural distribution, extending from Mexico to Bolivia and central Brazil, the plant commonly known in Malaysia as “sky fruit” or Cheria mahogany (Krisnawati et al., 2011). Its extracts especially the plant seed have medical efficacy. It is proven traditionally and scientific used to cure malaria, anemia, Diarrhea, fever, dysentery, hypertension, cancer, coughs, chest pains, intestinal parasitism, and anti-ulcer activity (Al-Radahe et al., 2012; Chen et al., 2010; Goh and Abdul Kadir, 2011; Nagalakshmi et al., 2001; Maiti et al., 2007). It is also proves in treatment of diabetic (Dewanjee and Maiti, 2011; Li et al., 2005), antibacterial (Haque et al., 2009; Majid et al., 2004) as well as antioxidant activities (Sahgal et al., 2009). The aim of this study, to screen antibacterial activity of crude ethanol and acetone extracts from different parts

(leaf, fruit cover, seed cover) of *Swietenia Macrophylla* King and its fractions against two multiple-drug-resistance bacteria strains namely, *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Plant material source

Swietenia Macrophylla King Plant was collected on 16 December, 2010 from the small town of Kulim, Bukit Mertajam, Pulau Pinang, Malaysia. The taxonomy identification of plant was done by a Botanist of the School of Environmental Sciences and Natural Resources, Natural University of Malaysia, Bangi, Selangor. The parts were separated into leaves, fruit, seed and fruit cover; and shade-dried for 20 days. The dried samples were powdered using electrical blender. The seed kernels were removed from the seed and then grind to the small pieces.

Crude ethanol and acetone extracts

100 g of shade-dried and powdered of each plant parts (leaves, fruit and fruit cover) were macerated in ethanol and acetone. The macerated extracts were filtered after 5 days and concentrated by reducing the solvents through rotary evaporator and further drying on petri dishes under open air.

Fractionation of Plant extracts

Each grinded Plant part (100 g) were defatted with 500 mL of hexane. The defatted material was shaken for 30 min and the solution was filtered out using sieve. Next, hexane added to the filtered residue and was shaken again, repeating for 2 cycles. The filtered residue of stems obtained was soaked with 4:1 ratio of methanol to water solution. This mixture of solution was left to settle for a day. The solution cake formed was sonicated in the ultrasonic bath for about 30 min and filtered out. The filtered residue was added with methanol and water solution (4:1), shaken for 30 min and filtered. This step was repeated for 3 cycles. The filtrates were collected and evaporated by the rotary evaporator until one third (1/3) of the extract is left in the evaporator flask

(fraction 1). The concentrated extract was acidified with sulfuric acid 50 % at pH 2. Chloroform was added to the acidified extract to the ratio of 2:1 and was shaken for 30 min. This step was repeated for three cycles. The aqueous and organic layer of the Plant extract was separated using the separation funnel. The organic layer was collected and evaporated using rotary evaporator until one third (1/3) of extract was left. The concentrated organic extract was obtained as fraction 2. Next, the aqueous layer was basified to pH 10 with 25 % ammonia solution under the fume hood and was partitioned with solvent chloroform: methanol (3:1). The aqueous extract was partitioned in ratio of 1:2 with the solvent using a separation funnel. The organic layer was separated from the separation funnel and rotary evaporated to 1/3 of its original volume to obtain fraction 3. Finally, the aqueous layer was removed from the separation funnel as fraction 4. The solvent fractionation method used to extract Plant extracts are based on the method by Harbone, (1998). All fractions collected were dried individually on a petri dish.

Preparation of test concentration

The crude solvent extracts and fractions of each part of *Swietenia Macrophylla* King were dissolved in water using dimethyl sulfoxide (DMSO) in order to prepare the dilute solutions. Stock solutions of 20 mL of 1% were prepared from the 200 mg of extracts in 20 mL of water solvent (19 ml H_2O + 1 ml DMSO). The stock solution then serially diluted with water to prepare further concentration. The test concentrations were prepared as 10, 50, 100 and 150 µg mL^{-1}. Finally, the test concentrations stored in labeled specimen bottles for further antibacterial activities bioassay.

Preparation of Liquid and Solid Media

For liquid media, nutrient broth (CM 0001, Oxoid) was prepared by adding 13 g of nutrient broth powder to 1 liter of distilled water. The media was mixed well and sterilized by autoclaving at 121 °C for 15 minutes. Mueller-Hinton agar (CM 0337, Oxoid) was used for solid media where 38 g of media powder was dissolved in 1 litre of distilled water. The media were boiled to make sure the medium are dissolved totally. The solution was sterilized by autoclaving it at 121 °C for 15 minutes.

Preparation of Test Microorganisms

Gram-positive bacteria *S.aureus,* and Gram-negative strain *E.coli,* were cultured overnight at 37 °C on Mueller-Hinton agar. The bacterial isolates from the agar plate cultured before were first grown in a nutrient broth for 24 hours before use and was standardized to 0.5 McFarland standards. An alternate method of determining if the broth culture has achieved the turbidity of the 0.5 McFarland standards was done using a spectrophotometer. The tube was placed in the spectrophotometer and the absorbance at 600 nm was read; where the absorbance of the 0.5 McFarland standard was approximately 0.132 or 1.5 x 10^8 CFU/mL or a percentage transmission equal to 74.3 (Schetz and Hoger, 2009).

Qualitative Antibacterial Activity

The antibacterial efficacy of different *S. Macrophilla* extracts was tested against *E. coli* and *S. aureus* by agar well diffusion method (Shailendra Kumar et al., 2010). Briefly, 24-hours old broth cultures of test bacteria were swabbed on sterile Mueller-Hinton agar plates using sterilize cotton swab. Then, 6 mm wells were punched onto the agar using sterilized cork borer. Different extracts with different concentrations were tested against these bacteria by carefully added to the respectively labeled well at fixed volume of 50 µL per well. The plates were incubated at 37 °C for 24 hours in upright position, and the diameter of a zone of inhibition was measured in millimeters and recorded. Streptomycin was used as a positive control while DMSO used as a negative control. Triplicates were maintained in each extract at different concentration and the average values were calculated (Arshad et al., 2010).

Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration of Plant extract at which the microorganism does not demonstrate visible growth. Stock solutions of the different plant extracts prepared. Later, serial of two fold dilutions was performed. Bacterial inocula (200 µL) were dispensed into each well (adjusted to approximate 0.5 McFarland)

using transparent Nunc. 96 well plates. The first three wells contained 200 µL bacterial inoculates were used as positive control and another three wells contained 200µL broth as blank. The plates were covered with sterilized plate sealers. The microtiter plate was incubated at 37 °C for 24 hours with 20 rpm shaking. Microbial growth in each medium was determined using two methods; (i) visible growth using naked eyes where there is no turbidity visible occurred and (ii) using Tecan Biorad Infinite Series 200 Pro with Magellan software by taking the reading of each well at absorbance of 600 nm.

Minimum Bactericidal Concentration (MBC)

The determination of MBC was determined by plating out the broth culture from each microliter well (used in MIC) into an agar plate and incubates overnight at 37 °C. The MBC is the concentration at which no growth occurs.

Statistical analysis

All the experiments were conducted in triplicate, and statistical analyses of the data were performed by one-way analysis of variance (ANOVA) using IBM SPSS Statictics 19 software (Sun Microsystems, Inc., USA). A probability value at $p \leq 0.05$ was considered significantly difference. Data is presented as mean values calculated from triplicate determinations.

RESULTS AND DISCUSSION

Table 1 shows the inhibition activity of crude solvent extracts and fractions using disc diffusion assay, and Table 2 shows the minimum inhibitory concentration (MIC) of *Swietenia macrophylla* King in serial dilution method. This study has evaluated the antibacterial activity of ethanol and acetone extracts and fractions from different parts of *S. macrophylla* against *S.aureus* and *E.coli* bacteria. Both solvents were proved to be good solvents in extracting inhibitory substances from some tested plant parts. The antibacterial effect of a crude ethanol and acetone extracts of *S. macrophylla* parts has

demonstrated varying levels of activity when screened against *S.aureus*, but some were found to have less effect on *E.coli.* Assays showed that among fractionated extracts, fraction 4 of fruit cover (FCF4) is more active than other fractions; the inhibition zones against both bacteria (≥ 16 mm). Fraction 4 of leaf (LF4) and fraction 2 of fruit cover (FCF2), both showed good inhibition (≥ 16 mm) against *S.aureus,* but less against *E.Coli* (12-15 mm). Fractions 3 from two parts (FCF3, and SCF3) do not showed growth inhibitory activity for both bacteria. Whereas, among solvent crude extracts; the ethanol extracts from fruit cover (EFC) was displayed overall more potent activity than other extracts against both tested bacteria. The inhibition zones were (≥ 16 mm) and (12-15 mm) for *S.aureus* and *E.Coli* respectively. However, extracts of ethanol seed cover (ESC) and ethanol leaf (EL), both showed inhibition zones of (12-15 mm) against both tested bacteria. Acetone seed cover (ASC) showed better growth-inhibitory activity on *S.aureus* (12-15 mm), but less against *E.Coli* (8-11 mm). Acetone leaf extracts do not showed growth-inhibitory activity on *E.coli* (<8 mm).

In contrast, Eloff (1998) and Cowan (1999) found that methanol was more efficient than acetone in extracting phytochemicals from plant materials. In present study, ethanol extracts showed inhibition zones better against both tested bacteria than acetone extracts. Meaning, ethanol extracted more inhibitory principles from those plant parts than acetone. In previous studies of *Swietenia Macrophylla* King Extract of different solvent shows different activities. Tan et al., (2009) reported the antimicrobial activities of methanol, dicholoromethane, and n-hexane extracts of *Swietenia Macrophylla King* leaves are showed inhibition activities. The cytotoxicity effect of a crude ethanol extract of *S. macrophylla* seeds has demonstrated varying levels of cytotoxicity when screened against KB, Ca SKi, HCT 116, Hep G2 and MCF-7 cancer cell lines (Goh and Abdul Kadir, 2011).

Some phytochemical classes were screened by TLC; and the results obtained were positive. Therefore, the experimental results obtained from this study suggest that

Swietenia Macrophylla King is promising as natural antibacterial; and this may warrant further research to determine the bioactive compound(s).

Table 1: Inhibition activity of plat extracts and fractions using disc diffusion assay (800 µg/ disc)

Inhibition zone (mm)		
Extracts and fractions	*E.Coli*	*S.aureus*
ESC	+	+
ASC	–	+
EL	+	+
AL	– –	+
EFC	+	++
AFC	–	+
LF1	+	+
LF2	–	+
LF3	– –	–
LF4	+	++
FCF1	+	+
FCF2	+	++
FCF3	– –	– –
FCF4	++	++
SCF1	+	+
SCF2	+	+
SCF3	– –	– –
SCF4	–	+
Sm	++	++
Control	– –	– –

ESC: Ethanol Seed Cover; ASC: Acetone Seed Cover; EL: Ethanol Leaf; AL: Acetone Leaf; EFC: Ethanol Fruit Cover; AFC: Acetone Fruit Cover; LF1-LF4: Leaf fractions 1-4; FCF1-FC4: Fruit Cover Fractions 1-4; SCF1-SCF4: Seed Cover Fractions 1-4; Sm: Streptomycin, Control= 1% DMSO.;

– –: no inhibition (>8 mm, diameter of disc); –: inhibition zone (8-11 mm) (); +: inhibition zone (12–15 mm); ++: inhibition zone (≥16 mm).

Table 2: Minimum inhibitory concentration (MIC) of some extracts of S.M in serial dilution method

Minimum Inhibitory Concentration MIC (g mL^{-1})		
	Bacterial specie	
Extracts and fractions	*E.Coli*	*S.aureus*
ESC	11.5±0.5	13.1±0.3
ASC	8.4±0.1	11.7±0.4
EL	11.3±0.1	12.2±0.2
AL	8.1±0.4	10.5±0.1
EFC	11.2±0.2	15.0±0.3
AFC	8.6±0.2	12.3±0.1
LF1	12.3±0.4	14.1±0.1
LF2	9.1±0.4	13.2±0.1
LF3	8.1±0.1	8.3±0.5
LF4	15.2±0.3	18.8±0.2
FCF1	11.5±0.4	14.5±0.1
FCF2	14.1±0.4	16.5±0.1
FCF3	8.4±0.2	9.3±0.3
FCF4	19.0±0.1	21.2±0.3
SCF1	12.1±0.4	13.5±0.1
SCF2	11.1±0.4	14.5±0.1
SCF3	8.6±0.3	9.1±0.5
SCF4	10.2±0.3	12.8±0.2
Sm	21.3±0.1	22.1±0.3

ESC: Ethanol Seed Cover; ASC: Acetone Seed Cover; EL: Ethanol Leaf; AL: Acetone Leaf; EFC: Ethanol Fruit Cover; AFC: Acetone Fruit Cover; LF1-LF4: Leaf fractions 1-4; FCF1-FC4: Fruit Cover Fractions 1-4; SCF1-SCF4: Seed Cover Fractions 1-4; Sm: Streptomycin

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