*Tropical Biomedicine* 26(3): 274–279 (2009)

# Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract

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Received 24 June 2009; received in revised form 26 August 2008; accepted 31 August 2009

**Abstract.** The present study was designed to evaluate the antibacterial activities of *Swietenia mahagoni* crude methanolic (SMCM) seed extract. The antimicrobial activity of the oily extract against Gram-positive, Gram-negative, yeast and fungus strains was evaluated based on the inhibition zone using disc diffusion assay, minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values. The crude extract was subjected to various phytochemicals analysis. The demonstrated qualitative phytochemical tests exhibited the presences of common phytocompounds including alkaloids, terpenoids, antraquinones, cardiac glycosides, saponins, and volatile oils as major active constituents. The SMCM seed extract had inhibitory effects on the growth of *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Streptococcus faecalis* and *Proteus mirabillase* and illustrated MIC and MBC values ranging from 25 mg/ml to 50 mg/ml.

## INTRODUCTION

*Swietenia mahagoni* (Linn.) Jacq., (Meliaceae), is a large, deciduous, and economically important timber tree native to the west Indies. This timber tree is mainly cultivated at tropical zone, such as India, Malaysia, and Southern China (Mulholland *et al.*, 2000). *Swietenia mahagoni* is a valuable timber tree closely related to the African genus *Khaya* and one of the most popular traditional medicines in Africa. The fruit is a brown, egg- to pear-shaped capsule about 6 to 10 cm long. When the fruit is fully ripe, the woody shell splits into five sections from the base upward and falls off to release the seeds.

The decoction of the bark of these mahoganies is extensively used as febrifuge, which could be associated with its use as an antimalarial drug. *Swietenia mahagoni* seeds have been applied as a folk medicine for the treatment of hypertension, diabetes, and malaria (Nagalakshmi *et al.*, 2001). The seeds have also been reported to have therapeutic value for treatment of cancer, amoebiasis, coughs, chest pains and intestinal parasitism. The biologically active ingredients, tetranortriterpenoids and fatty acids are considered to be responsible for these therapeutic effects (Bascal *et al.*, 1997).

Infectious diseases are the world's leading cause of premature deaths, killing almost 50 000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Ahmad & Arina, 2001). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases. According to World Health Organization (Santos *et al.*, 1995) medicinal plants would be the best source to obtain a

variety of drugs. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. The systematic screening of medicinal plants with the purpose of discovering new bioactive compounds is a routine activity in many laboratories devoted to biomedical research. Therefore, *S. mahagoni* crude methanolic seed extract is used for the screening of various microorganisms to discover new antimicrobial compounds.

## MATERIALS & METHODS

**Materials**

Dragendorff reagent, sodium hydroxide (NaOH) and several other reagents for phytochemical analysis were homemade and Folin-Ciacaleu reagent was purchased from Sigma-Aldrich, United States. Methanol (Analytical graded) and sulphuric acid ($H_2SO_4$) were purchased from Merck, Germany. Mueller Hinton agar, Mueller Hinton broth, nutrient agar, nutrient broth, Saboaroud dextrose agar, and Saboaroud dextrose broth were purchased from Himedia, India.

**Plant Materials**

The *S. mahagoni* seeds were collected in the state of Penang, Malaysia. An initial quality evaluation of the plant material was carried out to validate its authenticity. The authenticity work was carried out by botanist from School of Biological Sciences, Universiti Sains Malaysia.

**Sample preparation**

The seeds were washed with running tap water to remove dirt prior to the drying process. The seeds were cut into small pieces and dried at 40°C for a week to remove the moisture content. The seeds were powdered using a blender (New Deluhe, Suruchi, India). The powdered seeds were extracted with methanol by means of maceration method. The extract was filtered through filter paper (Whtaman No. 1). The filtrate was collected and concentrated in a rotary evaporator (RII0 Buchi, Switzerland) at 40°C. The concentrated extract was dried in an oven at 40°C for three days to obtain consistent weight and freeze dried for 2 days. The sample was stored under refrigeration (-20°C) condition for further analysis.

**Phytochemical analysis**

The condensed *S. mahagoni* crude methanolic (SMCM) seed extract will be used for preliminary qualitative screening of phytochemicals such as alkaloids (Dragendorff test), flavonoids (NaOH test), glycosides (Keller-Kiliani, conc. $H_2SO_4$), lignins (Lignin test), phenols (phenol test), saponins (Foam test), sterols and terpenes (Liberman-Burchard) and tannins (Sumitra *et al.*, 2006).

**Antimicrobial assay**

SMCM seed extract will be subjected to antimicrobial assays including agar disc diffusion test, and broth dilution assay.

**Microbial strains**

Twenty pathogenic microorganisms (local isolates) culture was used for the antimicrobial assays. The bacteria strains were grown in 50 ml of nutrient broth at 37°C and maintained in nutrient agar slant at 4°C. However the fungus strains were grown and maintained in Sabouraud dextrose agar (SDA). The Mueller Hinton agar (MHA) was also used for the determination of MIC and MBC.

**Agar disc diffusion assay**

The disk diffusion (Kirby-Baurer) technique, which is of the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS), was used for antimicrobial test. An overnight suspension culture of fifteen microbial strains was spread on MHA media. Sterile discs were prepared and placed on the culture spread agar media. The discs were impregnated with the SMCM seed extract in range of various concentrations. Methanol was used as negative control and standard antibiotics including chloramphenicol and amoxicillin as positive reference to determine the sensitivity of the strains. The inoculated

plates were incubated at 37ºC for 24 h for bacterial strains and 48 h for fungal strains (Karaman *et al.*, 2003). The antimicrobial activity was evaluated by measuring diameter of the inhibition zone around the disc (Table 2).

**Minimum inhibition concentration (MIC)**

The MIC value was studied for the microorganisms, which are determined as sensitive to test samples (SMCM seed extract) in disc diffusion assay. The inocula of microorganisms were prepared from 18 h nutrient broth cultures. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 100 mg/ml (stock concentration) in methanol and serially diluted (two-fold) to a working concentration ranging from 0.78 mg/ml to 50 mg/ml using nutrient broth and later inoculated with 1 ml suspension of the test organisms. The positive control was nutrient broth with standard reference antibiotics and inoculums and negative control was the nutrient broth and inoculums. After 18 hours of incubation at 37ºC, the test tubes were observed for turbidity. The least concentration where no turbidity was observed and noted as the MIC value. The test tubes were vortex gently to mix the content and incubated at 37ºC for 24 h (Kuete *et al.*, 2008). MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration).

**Minimum bacterial concentration (MBC)**

The MBC is defined as the lowest concentration where no bacterial growth is observed (bactericidal concentration). This was determined from the broth dilution resulting from the MIC tubes by sub-culturing to antimicrobial free agar as described in Reuben *et al.* (2008). In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37ºC for 24 h for bacteria and 48 h for fungal strains. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

## RESULTS & DISCUSSION

SMCM seeds extract was subjected to various studies as mentioned above. Preliminary qualitative phytochemical screening reveals the presence of alkaloids, terpenoids, antraquinones, cardiac glycosides, saponins, and volatile oils in SMCM seeds extract while test for tannins, flavonoids and steroids demonstrated negative responses (Table 1). Initial work on the SMCM seed extract for antimicrobial activity was carried out in our laboratory. The antimicrobial activities of this extract against Gram positive and Gram-negative bacteria, yeast and fungus were examined in terms of disk diffusion, MIC and MBC assays (Table 2 and Table 3).

The diffusion test showed that the SMCM seed extract was active against 5 Gram positive, 9 Gram-negative bacteria, and *Candida albicans* (fungus). However no significant activity showed against *Saccharomyces cerevisiae* (yeast), *Rhizopus* sp., *Penicillium* sp., and *Aspergillus fumigatus*. We found this extract more active compared to amoxicillin antibiotic (positive control) when tested against *Bacillus thuringiensis* and *Pseudomonas aeroginosa* at 1 mg/ml. Those Gram positive and Gram-negative bacteria and *C. albicans* were further evaluated for MIC and MBC determination. The MIC was defined as the lowest value concentration of the extract, which is inhibiting the growth of microorganisms. The MBC is the significance concentration of the extract which is able to kill the microorganisms. The MIC and MBC ranging from 12.5 to 50 mg/ml and 25 to 50 mg/ml for the SMCM seed extract respectively. The standard antibiotic chloramphenicol and amoxicillin (bacteria) and miconazole nitrite (fungal) inhibited the growth of the test bacterial and fungal strains at the smallest concentration. The lowest MIC and MBC recorded were 12.5 mg/ml for *Streptococcus sp.*, *Staphylococcus aureus*,

Table 1. The Phytochemical substances in crude methanolic *Swietenia mahagoni* crude methanolic seeds extract (SMCM seed extract)

| Phytochemicals | Observation | Outcomes |
|---|---|---|
| Alkaloids | Extract change into yellow-orange colour | + |
| Terpenoids | Extract colour changed to pink | + |
| Antraquinones | Formation of tomato red colour | + |
| Cardiac glycosides | Extract change into Green-blue colour | + |
| Saponins | Formation of frothing | + |
| Volatile oils | White precipitates | + |
| Tannins | No blue colour indication | – |
| Flavonoids | No pink colour indication | – |
| Steroids | No green colour visualized | – |

Notes: + *Swietenia mahagoni* has the phytochemical substances, – *Swietenia mahagoni* does not has the phytochemical compounds.

Table 2. Antibacterial activity including disk diffusion, MIC and MBC assays of *Swietenia mahagoni* crude methanolic seed extracts against various types of bacterial and fungal strains

| Bacteria strains | Zone of inhibition (mm) of various concentration of extracts, methanol and antibiotics[a] | | | | | | MIC (mg/ml)[a] | MBC (mg/ml)[a] |
|---|---|---|---|---|---|---|---|---|
| | 100 $mg.ml^{-1}$ | 10 $mg.ml^{-1}$ | 1 $mg.ml^{-1}$ | Chloram-phenicol | Amoxicillin | Methanol | | |
| **Gram positive strains** | | | | | | | | |
| *Bacillus subtilis* | 11.2 ± 2.4 | 9.6 ± 1.3 | 9.0 ± 0.7 | 24.0 ± 1.4 | 25.0 ± 2.8 | – | NA | NA |
| *Bacillus cereus* | 11.8 ± 0.8 | 11.0 ± 0.7 | 9.6 ± 0.9 | 19.0 ± 2.8 | 14.5 ± 3.5 | – | NA | NA |
| *Bacillus thuringiensis* | 15.7 ± 4.0 | 13.0 ± 0.6 | 10.0 ± 0.0 | 19.0 ± 1.0 | 9.5 ± 0.7 | – | NA | NA |
| *Enterococcus faecalis* | 11.6 ± 1.7 | 10.6 ± 1.3 | 9.5 ± 0.6 | 20.0 ± 2.0 | 31.0 ± 5.3 | – | 12.5 | 25 |
| *Staphylococcus aureus* | 12.0 ± 1.2 | 10.8 ± 1.1 | 9.8 ± 1.7 | 24.0 ± 0.0 | 30.5 ± 0.7 | – | 12.5 | 25 |
| **Gram negative strains** | | | | | | | | |
| *Escherichia coli* | 12.0 ± 0.0 | 11.7 ± 0.6 | 11.7 ± 0.6 | 16.0 ± 1.0 | 30.7 ± 1.2 | – | NA | NA |
| *Salmonella typhi* | 10.6 ± 0.9 | 9.6 ± 0.9 | 10.0 ± 1.0 | 20.7 ± 3.0 | 11.0 ± 2.0 | – | NA | NA |
| *Shigella sonnei* | 15.5 ± 2.1 | 11.8 ± 1.5 | 11.0 ± 3.0 | 20.7 ± 0.6 | 14.0 ± 4.0 | – | NA | NA |
| *Klebsiella pneumoniae.* | 10.2 ± 0.8 | 9.4 ± 0.5 | 8.3 ± 1.0 | 19.7 ± 0.6 | 18.3 ± 0.8 | – | NA | NA |
| *Pseudomonas aeroginosa* | 19.0 ± 1.0 | 17.0 ± 0.2 | 15.3 ± 3.6 | 15.0 ± 0.0 | 10.0 ± 0.0 | – | 12.5 | 25 |
| *Proteus mirabillase* | 15.3 ± 2.1 | 11.8 ± 1.5 | 11.0 ± 3.0 | 20.7 ± 0.6 | 14.0 ± 4.0 | – | 25 | 50 |
| *Enterobacter aeroginosa* | 12.0 ± 1.2 | 10.5 ± 0.5 | 9.3 ± 0.4 | 23.0 ± 0.0 | 16.0 ± 0.0 | – | NA | NA |
| *Azatobacter brandi* | 14.0 ± 4.2 | 14.5 ± 4.9 | 9.5 ± 0.7 | 24.5 ± 2.3 | 29.5 ± 1.1 | – | NA | NA |
| *Azospirillum lipoferum* | 12.2 ± 1.1 | 10.8 ± 1.6 | 10.0 ± 1.2 | 21.0 ± 1.7 | 15.3 ± 0.6 | – | NA | NA |
| *Herbaspirillum* sp. | 14.0 ± 1.4 | 12.0 ± 1.6 | 10.3 ± 1.3 | 16.0 ± 2.6 | 39.0 ± 1.7 | – | NA | NA |
| **Fungal strains** | | | | **Myconazole 1 $mg.ml^{-1}$** | | **Methanol** | | |
| *Candida albicans* | 19.0 ± 1.7 | 16.0 ± 1.6 | 16.0 ± 1.1 | 16.7 ± 3.5 | | – | 12.5 | 25 |
| *Saccharomyces cerevisiae* | NA | NA | NA | 17.0 ± 1.5 | | – | NA | NA |
| *Rhizopus* sp. | 12.8 ± 0.5 | 11.0 ± 0.5 | 9.1 ± 1.2 | 18.0 ± 1.7 | | NA | NA | NA |
| *Penicillium* sp. | NA | NA | NA | 18.0 ± 0.3 | | NA | NA | NA |
| *Aspergillus fumigatus* | NA | NA | NA | 19.0 ± 1.1 | | NA | NA | NA |

[a] The zone of inhibition is mean of three replicates for each microbial strain. Methanol does not give any affect against the microorganisms.
NA- No activity against SMCM seed extract.

*P. aeroginosa*, and *C. albicans* respectively. Similar study was also reported by Goun *et al.* (2003). They used *S. mahagoni* seed extract extracted with methylene chloride and methanol before testing their antimicrobial activity against 10 microbial species, including four bacterial pathogens, *Escherichia coli*, *S. aureus*, *Xanthomonas*

*campestris*, *Bacillus subtilis*; one yeasts, *C. albicans*; and five molds, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *A. fumigatus* and *Pythium parasitica* using the disk diffusion assay technique. The methylene chloride extract was found to be most effective and inhibited 7 microorganisms tested. In contrast, the methanol extract was found not active against all of the tested microorganisms except for *R. solani*.

Besides the seed, other parts of this plant such as leaf and bark have also been tested for antimicrobial activities by using chloroform and ethyl acetate by Haque *et al.* (2009). The extracts were tested for antibacterial activities against 12 (4 Gram positive namely *Bacillus megaterium*, *B. subtilis*, *S. aureus* and *Staphylococcus lutea* and 8 Gram negative namely *Salmonella paratyphi*, *Salmonella typhi*, *Vibrio parahemolyticus*, *Vibrio mimicus*, *E. coli*, *Shigella dysenteriae*, *P. aeruginosa* and *S. boydii*) human pathogenic bacteria. The chloroform and ethyl acetate extracts were used in concentration of 500 ìg disc$^{-1}$. The chloroform extracts of leaf and bark showed activity against most of the test bacteria. But the chloroform extract of seed showed activity against only *B. megaterium*, *S. paratyphi*, *S. dysenteriae*, *P. aeruginosa* and *S. boydii*. The highest activity of chloroform extract was recorded 16 mm disc$^{-1}$ in case of seed against *S. paratyphi*. A good activity was recorded by the ethyl acetate extract of bark against all the test organisms in comparison to others. Among the ethyl acetate extracts of *S. mahagoni*, bark showed highest activity and was found 18 mm disc$^{-1}$ against *B. megaterium*, *S. paratyphi* and *P. aeruginosa*

This findings show that the extract might be potential anticandidal and antibacterial agents even against some resistant strain such as *S. aureus*. However, the SMCM seed extract was more active against *C. albicans* strain compared to the other screened bacterial and fungal strains. The inhibition zone for this fungal yeast is 16.0±1.1 at 1 mg.ml$^{-1}$. The MIC and MBC values are 12.5 mg/ml and 25 mg/ml respectively for *C. albicans*. The presence of bioactive compounds including the phytochemical substances in the extract has been associated to antibacterial activities and thus has curative properties against pathogens. Several limonoids were previously isolated from *S. mahagoni* (Govindachari *et al.*, 1999). Isolation of triterpenes also was reported on *S. mahagoni* (Ekimoto *et al.*, 1991). The antibacterial activity of the isolated limonoids 1 and 2 has been reported by Shahidur Rahman *et al.* (2009) against pathogenic bacteria. They used the isolated limonoids 1 and 2 to test their antimicrobial activity against eight multiple-drug-resistant bacterial strains (clinical isolates) including four Gram positive (Group A b haemolytic *S. aureus*, *S. aureus*, *S. pneumoniae* and *Haemophillus influenzae*) and four Gram-negative (*E. coli*, *Klebsiella pneumoniae*, *S. typhi*, and *S. paratyphi*) strains by the conventional disc diffusion method. While both compounds were active against all test organisms, compound 2 showed overall more potent activity than compound 1. While the most potent activity of limonoid 1 was observed against *H. influenzae*, *S. typhi*, and *S. paratyphi*, limonoid 2 was most active against *Streptococcus pneumoniae*, *S. typhi*, and *S. paratyphi*. The lowest activity was observed against *K. pneumoniae* for both compounds. Moreover, the antimicrobial activity of triterpenes isolated from *Drypetes inaequalis* was reported by Awanchiri *et al.* (2009) against against pathogenic bacteria including one Gram positive (*S. aureus*) and four Gram negative (*E. coli*, *S. typhi*, *S. dysenteriae*, *K. pneumoniae* and *P. aeruginosa*) strains. Hence, it is possible that these two compounds were mainly responsible for the observed antimicrobial effects in this study.

Therefore, SMCM seed extract could serve as a remedy to such resistance and infectious microbial strains. Plant-based antimicrobials have vast medicinal prospective as they can serve the purpose with minor side effects that are habitually associated with synthetic antibiotics. This study also provides some validity for the use of the higher plant parts in traditional medicine and as a source of chemotherapeutic agents.

In conclusion, SMCM seed extracts possess a broad spectrum of activity against a panel of bacteria and fungus respectively. These promising extracts open the possibility of finding new clinically effective antibacterial compounds. Further purification of the active compounds and *in vivo* evaluation of antimicrobial activity along with toxicity studies of the extracts from SMCM are therefore suggested for further studies.

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