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In vitro Antibacterial and Cytotoxic Activities of Different Parts of Plant *Swietenia mahagony*

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Abstract: Crude extracts from different parts (leaf, bark and seed) of *Swietenia mahagony* (Family: Meliaceae) were screened for their antibacterial activity against 4 Gram positive and 8 Gram negative bacteria. Disc diffusion technique was used for *in vitro* screening. Among the crude extracts, chloroform and ethyl acetate extracts of leaf and bark showed good activity against all the tested organisms. The chloroform and ethyl acetate extracts of seed exhibited little or positive effect against most of the tested bacteria. The activities were compared to a standard antibiotic-kanamycin. Cytotoxic activity of crude extracts were determined using brine shrimp lethality bioassay and standard vincristine sulphate was used as positive control. The chloroform extract of seed and ethyl acetate extract of bark showed good cytotoxic activities and the LC_{50} values were found 13.75 and 11.64 μg mL^{-1}, respectively.

Key words: *Swietenia mahagony*, Meliaceae, antibacterial, cytotoxic activity

INTRODUCTION

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments (Dulger *et al*., 2004). In recent years, attempts have been taken to investigate the indigenous drugs against infectious diseases in order to help developing safer antimicrobial drugs (Rahman *et al*., 2001). In the continuation of this strategy of new drug discovery we have studied the different parts of the plant *Swietenia mahagony* for their antibacterial and cytotoxic activities.

Swietenia mahagony, commonly known as the West Indian Mahogany, is a species of Swietenia which belongs to the family Meliaceae. The plant is native to Southern Florida and the islands of Cuba, Jamaica and Hispaniola. The plant under investigation has many traditional uses. The seeds have been used for leishmaniasis and abortion medicine by an Amazonian Bolivian ethnic group (Bourdy *et al*., 2000) and for the treatment of hypertension, diabetes and malaria as a folk medicine in Indonesia (Kadota *et at*., 1990a). The bark has been used as an astringent for wounds and occasionally for tanning because of the rich red color (Falah *et al*., 2008).

Three novel ring-D opened limonoids corresponding to the phragmalin 8, 9, 14-orthoacetate with the addition of methyl 2, 30-orthoacetate or a propionate, swietenialides A, B, C and two ring-D opened phragmalin-type 1, 8, 9-orthoacetates, swietenialides D and E, were isolated together with one known mexicanolide, 2-hydroxyswietenin, from the ether extract of the stem bark of *S. mahagony* (Saad *et al*., 2003). From the seeds of *S. mahagony,* 18 tetranortriterpenoids, (five swietenins (B-F), three acylswietenolides, seven swietemahonins (A-G), swietemahonolide, mahonin and secomahoganin) related to swietenine and swietenolide were isolated and identified (Kadota *et al*., 1990a-c). *S. mahagony* extract shows agonistic activity to PPAR γ and gives ameliorative effects on diabetic db/db mice (Shen *et al*., 2005). Hence, this *in vitro* study was aimed at screening *S. mahagony* plant for their antibacterial and cytotoxic activities, evaluating their potential uses and determining whether their uses in traditional medicine are justified.

MATERIALS AND METHODS

Plant material: The leaf, bark and seed of *Swietenia mahagony* were collected in January 2008 from the adjoining areas of Southeast University, Bangladesh and identified by Dr. M.A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh and Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen of this collection has been deposited under the accession No. DACB 32527.

Plant material extraction and fractionation: The fresh leaf, bark and seed were washed, sun dried and ground. The ground leaf (100 g), bark (80 g) and seed

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(40 g) were extracted with ethanol at room temperature for 7 days. The extract was then concentrated by using a rotary evaporator. A portion of the concentrated ethanol extracts of leaf, bark and seed were fractionated by the modified Kupchan partitioning method (Van Wagenen *et al*., 1993) into n-hexane, chloroform and ethyl acetate.

Antibacterial test: The antibacterial assay was performed by disc diffusion technique (Rios *et al*., 1988). The samples solutions of the materials to be tested were prepared by dissolving a definite amount of material in appropriate solvent to attain a concentration of 50 mg mL^{-1}. Ten microliter of such solution was applied on sterile disc (5 mm diameter filter paper) and allowed the solvent to dry off in an aseptic hood. Thus, such discs contain 500 µg of crude extracts. To compare the activity with standard antibiotics, Kanamycin (30 µg $disc^{-1}$) was used. Discs containing 500 µL chloroform and ethyl acetate were used as a negative control.

All extracts of collected plants were tested against four Gram positive (*Bacillus megaterium*, *B. subtilis*, *Staphylococcus aureus* and *Sarcina lutea*) and eight Gram negative (*Salmonella paratyphi*, *S. typhi*, *Vibrio parahemolyticus*, *V. mimicus*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *S. boydii*) bacteria. Briefly, in this study the test discs and standard disc were placed in a Petri dish seeded with particular bacteria and then left in a refrigerator at 4°C for 12-18 h in order to diffuse the material from the discs to the surrounding media in the Petri dishes. The Petri dishes were then incubated at 37°C for overnight to allow the bacterial growth. The antibacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm.

Cytotoxicity screening: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Zhao *et al*., 1992). Here, simple zoological organism, brine shrimp (*Artemia salina*) was used as a convenient monitor for the screening.

The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% w/v NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method (Meyer *et al*., 1982). The test samples (extracts) were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus sea water (3.8% w/v NaCl in water) to attain concentrations -5, 10, 20, 40 and 80 µg mL^{-1}. A vial containing 50 µL DMSO diluted to 5 mL was used as a control. Standard Vincristine sulphate was used as positive control (Hossain *et al*., 2004; Khan *et al*., 2008; Nikkon *et al*., 2003). Then matured shrimps were applied to each of all experimental vials and control vial. The number of the nauplii that died after 24 h was counted. The findings were transformed to probit analysis for the determination of LC_{50} values of the compound.

RESULTS

The result of antibacterial screening: The chloroform and ethyl acetate extracts of *S. mahagony* were tested for antibacterial activities against 12 (4 Gram positive and 8 Gram negative) human pathogenic bacteria. The chloroform and ethyl acetate extracts were used in concentration of 500 µg $disc^{-1}$. 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 *Bacillus megaterium*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Pseudomonas 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. mahagony*, bark showed highest activity and was found 18 mm $disc^{-1}$ against *B. megaterium*, *S. paratyphi* and *P. aeruginosa* (Table 1, 2).

The result of brine shrimp lethality bioassay: The cytotoxic activities of all the extracts (chloroform and ethyl acetate) of *S. mahagony* were studied by brine shrimp lethality bioassay. The LC_{50} values of chloroform extracts of leaf, bark and seed were 30.34, 30.49 and 13.75 µg mL^{-1}, respectively (Fig. 1). The LC_{50} values of ethyl acetate extracts of leaf, bark and seed were 18.78,

Table 1: *In vitro* antibacterial activity of chloroform extracts of *Swietenia mahagony* with standard Kanamycin

	Diameter of zone of inhibition (mm)			
Name of bacteria	Leaf 500	Bark 500	Seed 500	Kanamycin 30
	(µg $disc^{-1}$)			
Gram positive bacteria				
Bacillus megaterium	12.5	12.0	13	25
Bacillus subtilis	12.0	14.0	+ve	25
Sarcina lutea	11.0	12.0	+ve	26
Staphylococcus aureus	12.0	13.0	+ve	28
Gram negative bacteria				
Escherichia coli	11.0	13.0	+ve	24
Salmonella typhi	12.5	12.0	+ve	24
Salmonella paratyphi	12.0	12.0	16	23
Vibrio mimicus	10.0	11.0	+ve	23
Vibrio parahemolyticus	11.0	12.0	+ve	26
Shigella boydii	10.0	11.5	12	23
Shigella dysenteriae	10.0	14.0	13	27
Pseudomonas aeruginosa	12.0	15.0	13	27

+ve: The extract has positive effect against the test organism that is it produces slightly clear zone around the disc

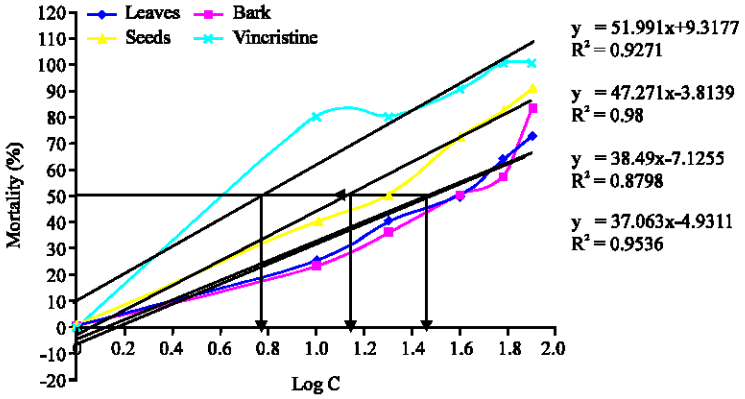

Fig. 1: Determination of LC_{50} values for standard and chloroform extracts of leaf, bark and seed of *Swietenia mahagony* from linear correlation between logarithms of concentration versus percentage of mortality

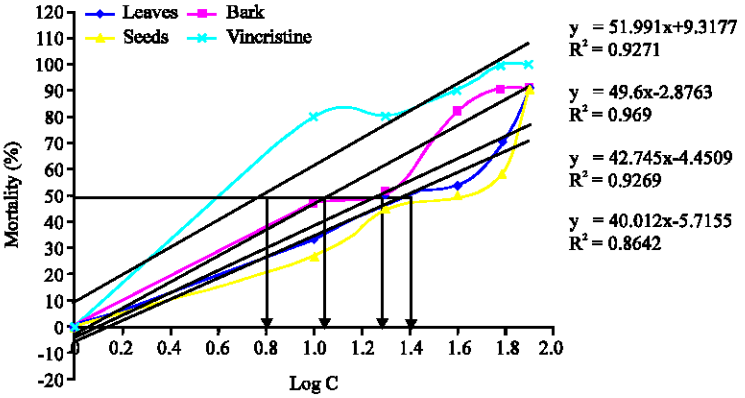

Fig. 2: Determination of LC_{50} values for standard and ethyl acetate extracts of leaf, bark and seed of *Swietenia mahagony* from linear correlation between logarithms of concentration versus percentage of mortality

Table 2: *In vitro* antibacterial activity of ethyl acetate extracts of *Swietenia mahagony* with standard Kanamycin

Name of bacteria	Diameter of zone of inhibition (mm) Leaf 500	Bark 500	Seed 500	Kanamycin 30
	(μg disc^{-1})			
Gram positive bacteria				
Bacillus megaterium	11	18	10	25
Bacillus subtilis	10	11	+ve	25
Sarcina lutea	11	11	+ve	26
Staphylococcus aureus	12	13	+ve	28
Gram negative bacteria				
Escherichia coli	9	12	+ve	24
Salmonella typhi	7	11	+ve	24
Salmonella paratyphi	11	18	12	23
Vibrio mimicus	8	11	+ve	23
Vibrio parahemolyticus	9	12	+ve	26
Shigella boydii	11	17	11	23
Shigella dysenteriae	11	16	11	27
Pseudomonas aeruginosa	10	18	12	27

+ve: The extract has positive effect against the test organism that is it produces slightly clear zone around the disc

11.64 and 24.68 μg mL^{-1}, respectively (Fig. 2). The LC_{50} value of standard Vincristine was found 6.06 μg mL^{-1} (Fig. 1, 2). No mortality was found in the control group. An approximate linear correlation was observed between logarithm of concentration and percentage of mortality. For determination of LC_{50} Microsoft Excel 2002 is used.

DISCUSSION

The present study revealed that the plant *Swietenia mahagony* has got antibacterial and cytotoxic effects and may have bioactive principles. The crude chloroform and ethyl acetate extracts of leaf and bark showed antibacterial activity against both Gram positive and Gram negative bacteria. The ethyl acetate extract of bark showed the highest antibacterial activity against *Bacillus megaterium*, *Salmonella paratyphi* and *Pseudomonas aeruginosa* and was found 18 mm disc^{-1}. The results of antibacterial activity suggest that the bark is more active than leaf and seed.

The crude chloroform and ethyl acetate extracts of *S. mahagony* showed moderate cytotoxic activities. The

ethyl acetate extract of bark showed more cytotoxic effect than leaf and seed (both chloroform and ethyl acetate extracts). This study is a general agreement with the results of earlier investigations (Falah *et al*., 2008; Bourdy *et al*., 2000). Some chemical compounds have been isolated from seed (Saad *et al*., 2003) and bark (Kadota *et al*., 1990a-c) of *S. mahagony*, in spite, this results should be encouraging other researchers to more study including phytochemical (specially leaf) and biological investigation. The earlier reports of antimicrobial activities (Chandra *et al*., 2008) support the findings of present studies. Moderate cytotoxic effects of crude extracts indicate that it can be selected for further cell line assay, because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts.

It may be concluded from this study that the extracts of *S. mahagony* are active against the tested microorganisms and also have cytotoxic effects. In addition, the results confirm the use of the plant in traditional medicine. Now present study will be directed to explore the lead compound responsible for aforementioned activity from this plant.

REFERENCES

Bourdy, G., S.J. De Walt, L.R. Chávez De Michel, A. Roca and E. Deharo *et al*., 2000. Medicinal plants uses of the Tacana, an Amazonian Bolivian ethnic group. J. Ethnopharmacol., 70: 87-109.

Chandra, M., K. Harish, Tripathi, J. Srivastava and N. Rai, 2008. Antimicrobial activity of *Swietenia mahogany*, *Callistemon lanceolatus* and *Cymbopogon caesius*. Icfai Univ. J. Life Sci., 2: 36-41.

Dulger, B. and A. Gonuz, 2004. Antimicrobial activity of some Turkish medicinal plants. Pak. J. Biol. Sci., 7: 1559-1562.

Falah, S., T. Suzuki and T. Katayama, 2008. Chemical constituents from *Swietenia macrophylla* bark and their antioxidant activity. Pak. J. Biol. Sci., 11: 2007-2012.

Hossain, M.S., M.A. Hossain, R. Islam, A.H. Alam, Kudrat-e-Zahan, S. Sarkar and M.A. Farooque, 2004. Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2-aminophenol and their coordination complexes with Magnesium (Mg-II). Pak. J. Biol. Sci., 7: 25-27.

Kadota, S., L. Marpaung, T. Kikuchi and H. Ekimoto, 1990a. Constituents of the seed of *Swietenia mahagoni* JACQ. I. isolations, structures and ^{1}H and ^{13}C nuclear magnetic resonance signal assignment of new tetranortriterpenoids related to swietenine and swietenolide. Chem. Pharm. Bull., 38: 639-651.

Kadota, S., L. Marpaung, T. Kikuchi and H. Ekimoto, 1990b. Constituents of the seeds of *Swietenia mahagoni* JACQ. II. structures of swietemahonin A, B, C, D, E, F and G and swietemahonolide. Chem. Pharm. Bull., 38: 894-901.

Kadota, S., L. Marpaung, T. Kikuchi and H. Ekimoto, 1990c. Constituents of the seed of *Swietenia mahagoni* JACQ. III. structure of mahonin and secomahoganin. Chem. Pharm. Bull., 38: 1495-1500.

Khan, A., M. Rahman and M.S. Islam, 2008. Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*. Indian J. Pharmacol., 40: 41-44.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, J.E. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine Shrimp: A convenient general bioassay for active plants constituents. J. Med. Plant Res., 45: 31-34.

Nikkon, F., Z.A. Saud, H. Rahman and M.E. Haque, 2003. *In vitro* antibacterial activity of the compound Isolated from chloroform extract of *Moringa oleifera* lam. Pak. J. Biol. Sci., 6: 1888-1890.

Rahman, M.M., M.I.I. Wahed, M.H. Biswas, G.M. Sadik and M.E. Haque, 2001. *In vitro* antibacterial activity of the compounds of *Trapa bispinosa* Roxb. Science, 1: 214-216.

Rios, J.L., M.C. Recio and A. Villar, 1988. Screening methods for natural products with antimicrobial activity: A review of the literature. J. Ethnopharmacol., 23: 127-149.

Saad, M.M.G., T. Iwagawa, M. Doe and M. Nakatani, 2003. Swietenialides, novel ring D opened phragmalin limonoid orthoesthers from *Swietenia mahogany* JACQ. Tetrahedron, 59: 8027-8033.

Shen, J., X. Shen, H. Jiang D. Li, J. Chen, Q. Chen, G. Li, J. Chen, J. Yue, M. Chen and X. Wang, 2005. *Swietenia mahagony* extract shows agonistic activity to PPAR and gives ameliorative effects on diabetic db/db mice. Acta Pharmacol. Sin., 26: 220-222.

Van Wagenen, B.C., R. Larsen, J.H. Cardellina II, D. Randazzo, Z.C. Lidert and C. Swithenbank 1993. Ulosantoin, a potent insecticide from the sponge *Ulosaruetzleri*. J. Org. Chem., 58: 335-337.

Zhao, G., Y. Hui, J.K. Rupprecht, J.L. McLaughlin and K.V. Wood, 1992. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. J. Nat. Prod., 55: 347-356.