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RESEARCH ARTICLE

Exploration of Antimicrobial Potential of Methanol and Water Extract of Seeds of *Swietenia Macrophylla* (Family: Meliaceae), to Substantiate Folklore Claim

ANUP MAITI, SAIKAT DEWANJEE, SUBHASH C MANDAL and S. ANNADURAI

For author affiliations, see end of text.

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ABSTRACT

The antimicrobial efficacy of methanol and water extracts of seeds of the plant *Swietenia macrophylla* (Family: Meliaceae) was evaluated against selected pathogenic bacterial strains (*Bacillus cereus* MTCC 430, *Klebsiella penumoniae* MTCC 109*, Pseudomonas aeruginosa* MTCC 424*, Escherichia coli* MTCC 443, *Staphylococcus aureus* MTCC 96*, Salmonella typhimurium* MTCC 98, *Micrococcus luteus* MTCC 106) and fungal stains (*Candida albicans* MTCC 183*, Cryptococcus albidus* MTCC 2661*, Aspergillus niger* MTCC 16404*, Aspergillus flavus* MTCC 1973). The antimicrobial activity was evaluated by disc diffusion and micro dilution assay methods. Streptomycin and gentamicin were used as standard antibacterial drugs whilst fluconazole was used as standard antifungal drug. Results of both assays ensured that the seeds possess significant antimicrobial activity in terms of antibacterial and antifungal activity. Results are comparable to that of standard drugs selected. It is also evident from results that methanol extract showed better activity than that of water extract.

Keywords: *Swietenia macrophylla, Antimicrobial activity, Disc diffusion assay, Minimum inhibitory concentration (MIC)*

Herbal medicines have been used since the dawn of civilization to maintain health and to treat disease. There is a tremendous historical legacy in folklore uses of plant preparations in medicines. Scientific studies on plants used in ethnomedicine led to the discovery of many valuable drugs [1]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [2,3]. The World Health Organization estimated that about 80 % of the world's population still believes in herbal drugs for their primary health care [4]. There are indiscriminate use of synthetic antimicrobial drugs for the treatment of infectious diseases and as a result drug resistance developed in human beings as well as in plant also [5,6,7]. Some times antibiotics cause adverse reaction like hypersensitivity, immunosuppression and allergic reactions [8]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from various sources, including medicinal plants [9, 10].

The plant *Swietenia macrophylla* (Family: Meliaceae) is a beautiful, lofty, evergreen large tree native to tropical America, Mexico and South America usually 30-40m in height and 3-4m in girth [11]. The seeds of *S. macrophylla* have been reported to have anti-inflammatory, antimutagenecity and antitumor activity [12]. Various phytochemicals have been isolated from the plant are swietenine, swietenolide [13], swietemahonin, khayasin, andirobin, augustineolide, 7-deacetoxy-7-oxogedunin, proceranolide and 6-O-acetyl swietonolide [14]. Paste of seeds of *Swietenia macrophylla* are traditionally used by the local healers of East Midnapore, West-Bengal, India for curing skin diseases and infections being caused by wounds. The present study was undertaken to explore the antimicrobial potential of methanol and water extract of seeds of *S. macrophylla* against some pathogenic bacteria like *Bacillus cereus* MTCC 430, *Klebsiella penumoniae* MTCC 109*, Pseudomonas aeruginosa* MTCC 424*, Escherichia coli* MTCC 443, *Staphylococcus aureus* MTCC 96*, Salmonella typhimurium* MTCC 98, *micrococcus luteus* MTCC 106 and fungi species namely *Candida albicans* MTCC 183*, Cryptococcus albidus* MTCC 2661*, Asper-*

Table 1. Antibacterial activity of the extract by disc diffusion assay

Sl. No.	Extract yield (%)	*Inhibition zone (mm)*				
		Bacillus cereus MTCC 430	*Staphylococcus aureus* MTCC 96	*Escherichia coli* MTCC 443	*Pseudomonas aeruginosa* MTCC 424	*Klebsiella pneumonia* MTCC 109
Extract (50 mg/ml)	Methanol (15.0)	24.10	28.00	29.60	15.00	15.60
Extract (50 mg/ml)	Water (12.7)	19.00	21.00	20.15	18.10	12.00
Streptomycin (10µg/disc)	——	32.00	30.00	29.00	28.00	20.00
Gentamicin (10µg/disc)	——	33.00	32.00	31.00	30.00	28.00

Values are mean of triplicates experiment
(5%) – percentage extract yield w/w was estimated as dry extract weight × 100

gillus niger MTCC 16404 and *Aspergillus flavus* MTCC 1973. These microorganism strains were clinical isolates collected from the Institute of Microbial Technology, Chandigarh, India and preserved as slant agar culture at 4^0C.

MATERIALS AND METHODS

Collection of plant material and extraction

Seeds of *Swietenia macrophylla* (Family: Meliaceae) were collected in the month Of December and January, from the villages of Midnapore (E), west-Bengal, India. The plant was authenticated by the Botanical Survey of India. A voucher specimen was deposited at our institute for future reference. The Seeds of *Swietenia macrophylla* were shade-dried, powdered and passed through 40-mesh sieve and stored in an airtight container for future use. Powdered seeds were extracted with 95% methanol with the help of soxhlet apparatus. Water extract was prepared by maceration for a period of 24 hours. The resulting extracts were evaporated under vacuum. The percentage yield of methanol and water extract was found 15% and 12.7% w/w respectively.

Disc Diffusion Assay

The agar diffusion method [15] was used to evaluate the antimicrobial activity of the subjected extracts. Inoculum of 100 µl suspension containing 10^8 CFU/ml of bacteria and 10^4 spores/ml of fungi were spread on Mueller Hinton Agar and potato dextrose agar medium respectively. The discs (9 mm in diameter) impregnated with 20 µl of 50 mg/ml (*i.e.* 0.5mg/disc) extracts were placed on seeded agar medium. Streptomycin (10µg/disc) and gentamicin (10µg/disc) were used as positive control for bacteria and fluconazole (10µg/disc) for fungi. Methanol was used as negative control. The experiments were conducted in triplicate and the test plates were incubated 24 hours at 37º C for bacteria and 28º C for fungi. The diameters of zone of inhibition measured in mm [16]

Micro Dilution Assay

The minimum inhibitory concentrations (MIC) were also determined for the subjected microorganisms for methanol extract. The MIC values of extract against pathogenic strains were determined by microdilution method [17] with some modifications. The inoculums of microorganisms were prepared from 6 hour broth (MHB/PDB) culture and suspensions were adjusted to 0.5 MCFarland standard turbidity [18]. The extract dissolved in DMSO 2.5% was first diluted to the highest concentration (1000 µg/ml) to be tested; there serial two fold dilutions were made in concentration range 0.48 to 1000 µg/ml in 10 ml sterile test tubes containing 2.5% DMSO. The 10 ml test tubes were prepared by dispensing 9 ml of MHB/PDB in each tube and 1 ml of inoculum (0.5 McFarland turbidity). A 1000 µl from plant extracts initially prepared at the concentration of 50 mg/ml has added into test tube. Then 1000 µl from their serial dilutions was transferred into ten consecutive tubes. The broth (MHB/PDB) without extracts, inoculums and DMSO 2.5% were also used as negative control. Antibiotics streptomycin and fluconazole were used as positive control. The tubes were covered with sterile sealer; contents of each tube were mixed on shaker at 300 rpm for 20 seconds and the incubation at appropriate temperature. Microbial growth was determined by using Ultraspec 200 UV/visible spectrophotometer and confirmed by plating 1 ml sample from clear plate on MHA/PDA. The MIC was interpreted as the lowest concentration of extracts that did not permit any visible growth when compared with that of control.

Table 2. Antifungal activity of the extract by disc diffusion assay

Test material	*Inhibition zone (mm)*			
	Cryptococcus albidus MTCC 2661	*Aspergillus flavus* MTCC 1973	*Aspergillus niger* MTCC 16404	*Candida albicans* MTCC 183
Extract- methanol (50 mg/ml)	36.33	23.46	29.68	31.32
Extract- water (50 mg/ml)	24.66	18.14	20.00	19.46
Fluconazole (10µg/disc)	28.00	21.00	22.00	25.00

Values are mean of triplicates experiment

Table 3. MIC and MBC values of the methanol extract against bacterial and fungal strains (µg/ml)

Bacterial Strains	*Extract*		*Streptomycin*	
	MIC	MBC	MIC	MBC
Gram positive				
Bacillus cereus MTCC 430	14.82	7×10^{-7}	4.00	2×10^{-9}
Staphylococcus aureus MTCC 96	30.18	3×10^{-6}	1.95	3×10^{-11}
Micrococcus luteus MTCC 106	15.12	4×10^{-7}	2.10	4×10^{-10}
Gram negative				
Klebsiella pneumoniae MTCC 109	7.95	2×10^{-8}	0.90	3×10^{-11}
Escherichia coli MTCC 443	16.20	4×10^{-7}	7.50	4×10^{-8}
Salmonella typhimurium MTCC 98	17.25	3×10^{-6}	1.25	2×10^{-11}
Pseudomonas aeruginosa MTCC 424	16.80	4×10^{-5}	2.65	4×10^{-9}
Fungal strains				Fluconazole
Aspergillus niger MTCC 16404	7.80	5×10^{-9}	0.48	3×10^{-8}
Cryptococcus albidus MTCC 2661	12.62	2×10^{-8}	3.90	2×10^{-10}
Candida albicans MTCC 183	3.90	6×10^{-7}	1.45	3×10^{-9}
Aspergillus flavus MTCC 1973	7.50	2×10^{-8}	1.95	3×10^{-8}

MIC- Minimum inhibitory concentration
MBC- Minimum bacterial concentration
MMC- Minimum mycotic concentration

RESULT AND DISCUSSION

The results of disc diffusion assay of both methanol and water extracts of the seeds of *Swietenia macrophylla* have been tabulated in table-1 and table-2. It is evident from table-1 that the both methanol and water extract was found to be active against the bacteria like *Escherichia coli* MTCC 443, *Staphylococcus aureus* MTCC 96, *Bacillus cereus* MTCC 430, *Klebsiella pneumonia* MTCC 109 and *Pseudomonas aeruginosa* MTCC 424. The results of disc diffusion assay of the crude extracts were compared with that of standard antibiotic Streptomycin (10µg/disc) and Gentamicin (10µg/disc) also recorded. Table-2 indicates that the extract is also potent for its antifungal efficacy. The extracts have shown profound antifungal activity with respect to fungal stains namely *Cryptococcus albidus* MTCC 2661, *Aspergillus flavus* MTCC 1973, *Aspergillus niger* MTCC 16404 and *Candida albicans* MTCC 183 and results are comparable to that of standard antifungal agent fluconazole (10µg/disc). Among bacteria *Escherichia coli* MTCC 443 and among fungi *Cryptococcus albidus* MTCC 2661 are most susceptible to the extracts. Results also proved that methanol extract has more effectiveness than that of water extract against subjected bacteria and fungal stains. Table-3 represents MIC and MBC/MMC values of the methanol extract against bacterial and fungal strains and results were compared with that of standard antibiotics streptomycin and fluconazole for bacteria and fungi respectively. From above results it can be concluded that the seeds of plant *Swietenia macrophylla* possess significant antimicrobial activity in term of antibacterial and antifungal effects. This antimicrobial property against bacteria and fungi surely is due to presence of some antimicrobial substances in seeds. Now our study will be directed to explore the lead compound responsible for aforementioned activity from this plant.

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REFERENCES

1. Shylesh BS, Padikkale J. In vitro cytotoxic and antitumor property of Emilia sanchifolia (L) DC in mice. *J Ethnopharmacol.* 2000; 73:495-500.
2. Saxena VK, Sharma RN. Antimicrobial activity of the essential oil of Lantana aculeata. *Fitoterapia.* 1999;70:67-70.
3. Ahmad I, Beg AZ. Antimicrobial and Phtytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol*. 2001;74:113-23.
4. World Health Organisation. Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram.* 1993;28:13–14.
5. Davis J. Inactivation of antibiotics and the dissemination of resistance genes. *Science.* 1994;264:375-82.
6. Loper JE, Henkels MD, Roberts RG. Evaluation of streptomycin, oxytetracycline, and copper resistance of Erwinia amylovoraisolated from pear orchards in Washington State. *Plant Dis.* 1991;75:287-90.
7. Service RF. Antibiotics that resist resistance. *Science.* 1995;270:724-27.
8. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol.* 1998;62:183-93.

9. Clark AM. Natural products as resource for new drugs. *Pharmaceut Res.* 1996;13:1133-1141.
10. Cordell GA. Biodiversity and drug discovery(-)a symbiotic relationship. *Phytochemistry.* 2000;55:463-80.
11. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, Vol-I, New Delhi, India, 1990; p. 397.
12. Guevera AP, Apilado A, Sakarai H, Kozuka M, Tokunda H. Anti-inflammatory, antimutagenecity and antitumor activity of mahagony seeds Swietenia macrophylla (Meliaceae). *Phill J of Sc.* 1996;125:271-78.
13. Guha Sircar SSG, Chakraborty T. Tetranortriterpenoid from Swietenia macrophylla. *J Ind Chem Soc.* 1951;28:207.
14. Mootoo BS, Allisha A, Motilal R, Pingal R, Ramlal A, Khan A, Reynolds WF, McLean S. Limonoids from Swietenia macrophylla and S. aubrevilleana. *J Nat Prod.* 1999;62(11):1514-17.
15. Murray PR., Baron EJ, Pfallar MA, Tenover FC, Yolke, RH. Manual of Clinical Microbiology, 6th edn Vol-6, ASM, Washington DC, 1995; p. 214-15
16. Mandal SC, Nandy A, Pal MP, Saha BP. Evaluation of antimicrobial activity of Asperagus recemosus Willd. root. *Phytother. Res.* 2000;14:118-19.
17. Zgoda IR, Porter JR. A convenient microdilution method for screening natural products against bacteria and fungi. *Pharm bio.* 2001;39:221-25.
18. National committee for clinical laboratory standard. Performance standards for antimicrobial susceptibility testing; Ninth informal supplement. Wayne, Pennsylvania: NCCLS; 1999; Document M 100-S9, Vol. 19, No.1, Table 21.

CURRENT AUTHOR ADDRESSES

Anup Maiti, Research scholar, Division of Pharmacognosy and Phytotherapy Research, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India. E-mail: anup_phytochem@yahoo.com (Corresponding author)

Saikat Dewanjee, Lecturer, Division of Pharmacognosy and Phytotherapy Research, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India.

Subhash C Mandal, Reader, Division of Pharmacognosy and Phytotherapy Research, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India.

S. Annadurai, Lecturer, Department of Pharmacy, Christian Medical College, Vellore 632 004, Tamil Nadu, India.