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Antimicrobial Spectrum and Phytochemical Study of *Walsura trifoliata* (A. Juss.) Harms. (Meliaceae) Bark Extracts

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Abstract: Indigenous uses of *Walsura trifoliata* (A. Juss.) Harms. (Meliaceae) bark in different parts of Eastern Ghats of Southern Peninsular India, curing skin allergies, astringency and diarrhoeia is wide spread. The objective of the present study was to evaluate the antimicrobial and phytochemical activity of bark extract against pathogenic microorganisms. Successive petroleum ether, methanol, benzene and aqueous extracts of *Walsura trifoliata* bark were tested for their phytochemical constituents, antibacterial and antifungal activity. The methanol and aqueous extracts were found to be most effective against most of the tested organisms. The present findings significantly conform the uses of *Walsura trifoliata* in the indigenous systems of medicine to treat various diseases like skin allergies, astringency and diarrhoea.

Key words: *Walsura trifoliata*, phytochemical constituents, antibacterial activity, antifungal activity

INTRODUCTION

Plants play a vital role in existence and survival of man. Medicinal Plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Nostro *et al*., 2000). It is estimated that there are about 250,000 species of higher plants and the majority of them have not been examined for their pharmacological activities. The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Ram *et al*., 2000; Nagalakshmi *et al*., 2001; Jayasinghea *et al*., 2002; Chowdhury *et al*., 2003; Mishra *et al*., 2005; Lavanya *et al*., 2006) and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi (Taylor *et al*., 1995). The plant is well reputed in traditional system of medicine and used by tribal peoples to treat various diseases i.e., skin allergies, astringent and diarrhoeia (Pullaiah and Rani, 1999). This, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits. Thus there is an increased need for the development of alternative antipathogenic substances. One possible approach is to screen local medicinal plants in search of suitable chemotherapeutic antibacterial and antifungal substances.

MATERIALS AND METHODS

Plant Materials and Extraction

Walsura trifoliata (A. Juss.) Harms. bark were collected from the evergreen forest of Seshachalam hills ranges of Tirumala hills of Eastern Ghats, Chittoor district of Andhra Pradesh, in October 2004. The ethnobotanical information regarding the drug-yielding plant was recorded using the standard

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methods of Schultes (1960), Croom (1983) and Hamilton (1995). The information on name, part used, purpose, mode of administration, etc. was recorded in the field notebooks as well as audiotapes. The sample specimens were collected in bulk quantities for analysis. Based on the folk evidences regarding the effective utilization for different skin allergies, astringent and diarrhoeia, the samples were collected and screened for antimicrobial properties. The voucher specimens (No. 2089) were identified with the help of regional floras (Gamble, 1935; Pullaiah and Rani, 1999) and deposited MMK Herbarium, Vijayawada. The plant material (bark) were shade dried, powdered (about 60 g) and successively extracted with different solvents (250 mL) using a soxhlet apparatus for 6 h. The extracts were filtered and concentrated under reduced pressure, below 40°C to dryness. Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors (Kapoor *et al*., 1969; Rizk and Bashir, 1980; Fadeyi *et al*., 1989; Odebiyi and Sofowora, 1990). The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

Microorganisms and Media

Bacillus subtilis, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus griseus*, (gram positive) *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas fluorescence*, (gram negative) *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Penicillium chrysogenum* (fungi) were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The test organisms were sub-cultured at 37°C and maintained on nutrient agar media for bacteria and sabouraud agar medium for *Candida albicans*.

Antimicrobial Activity

The antimicrobial activity of the ethanol extracts of each sample was evaluated by using disc diffusion method (Bauer *et al*., 1966). Petriplates containing 20 mL of respective medium was seeded with selected microbial strains. Five milliliters of nutrient broth was inoculated with a loop (6 mm) of bacteria/yeast and incubated at 35°C for 6 h. One milliliter of broth was taken at 0.6 optical density (at log phase, ca.108 cells mL^{-1}) and inoculated the nutrient agar (sterile) and transferred to 180×20 mm petri dishes. The sterile Whatmann No. 1 filter paper discs of 6 mm diameter were impregnated with 1000-5000 μg of concentrated plant extracts and placed on the surface of the freshly inoculated medium. Standard antibiotic discs viz., Ampicillin, Tetracy-cline, Gentamycine and Clotrimazole were obtained from Hi-Media, Mumbai, were used as positive controls. Ethanol and water alone served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The media were incubated for 24 h at 37°C and the diameters of the inhibition zones were recorded. Three independent trials were conducted for each concentration.

RESULTS AND DISCUSSION

The methanol, aqueous, petroleum and benzene extract of bark of *Walsura trifoliata* were tested and results shown in Table 1. Different extracts inhibited the growth of 12, 10, 3 and 3 of the 14 used microorganisms, respectively. Methanol extracts of *Walsura trifoliata* exhibits significant activity against *Staphylococcus aureus*, *Bacillus cereus* and moderate inhibition on *Bacillus coagulans* and *Candida albicans*, less activity against *Aspergillus flavus*. However, aqueous extracts also exhibited significant activity against *Bacillus cereus*, *Bacillus coagulaus* and *Escherchia coli*, moderate inhibition on *Bacillus subtilis*, over all when aqueous extract showed more significant activity. The petroleum ether and benzene extracts showed less inhibitory activity when compare with the above two extracts. The petroleum ether showed activity against *Escherchia coli*, *Aspergillus niger* and *Pseudomonas fluorescence* and where as Benzene extracts were against *Bacillus lichenigormis*, *Bacillus cereus* and *Staphylococcus epidermidis*.

Table 1: Antimicrobial activity of *Walsura trifoliate* bark extracts[a]

Microorganisms	P	M	B	A	Standards[b]
Bacteria					
Bacillus subtilis (G +) NCIM 2063	-	-	-	20 (8)	NT
Bacillus licheniformis (G +) MTCC 1483	-	13 (5)	8 (19)	12 (8)	16
Bacillus coagulans (G +) NCIM 2030	-	9 (13)	-	26 (9)	14
Bacillus cereus (G +) MTCC 1305	-	14 (20)	11 (6)	27 (5)	14
Staphylococcus aureus (G +) NCIM 2079	-	14 (31)	-	-	16
Staphylococcus epidermidis (G +) NCIM 2493	-	12 (20)	7 (12)	13 (23)	13
Staphylococcus griseus (G +) MTCC 1540	-	11 (24)	-	10 (19)	14
Escherichia coli (G -) NCIM 2065	33 (20)	13 (10)	-	26 (8)	14
Proteus vulgaris (G -) NCIM 2857		7 (10)	-	-	13
Pseudomonas fluoresence (G -) MTCC 748	7 (17)	8 (12)	-	11 (3)	10
Fungi					
Aspergillus flavus MTCC 1884	-	5 (20)	-	14 (5)	7
Aspergillus niger MTCC 1785	23 (12)	-	-	-	NT
Candida albicans MTCC 1637	-	10 (35)	-	14 (3)	9
Penicillium chrysogenum MTCC 1996	-	11 (20)	-	-	8

[a]: Values (zone of inhibition in mm) are the mean of three replicates; minimum inhibitory concentration (mg mL^{-1}) are reported in parentheses, when determined. P: Petroleum ether extract; M: Methanol extract B, benzene extract; A: Aqueous extract; (all tested at 25 mg mL^{-1}); -: No inhibition; NT: Not Tested; [b]: Ampicillin (10 mg mL^{-1}) for *Bacillus*, *Staphylococcus* species, Tetracycline (30 mg mL^{-1}) for *E. coli*, Gentamycine (10 mg mL^{-1}) for *Proteus* and *Pseudomonas* species and Clotrimazole (25 mg mL^{-1}) for fungal species

Table 2: Phytochemical screening of petroleum ether (P), methanol (M), benzene (B), aqueous (A) extracts of *Walsura trifoliata* bark extracts[a]

Constituents	P	M	B	A
Alkaloids	+	+++	-	+++
Anthocyanidins	-	-	-	-
Carboxylic acids	++	+	+	-
Catechols	-	+	++	+
Coumarins	-	++	-	-
Fatty acids	+	++	-	-
Fixed oils	++	++	-	+
Flavonoids	-	-	-	-
Glycosides	++	-	-	-
Phenols	-	+	++	++
Proteins	-	-	-	+
Quinines	-	-	-	-
Resins	-	++	-	+
Saponins	+++	++	+	+++
Steroids	+++	++	+	-
Tannins	+++	-	+	+++
Triterpenoids	-	-	-	+
Volatile oils	+	-	-	+

- : Not detected; +: Low concentration; ++: High concentration; +++: Very high concentration

Phytochemical screening of these extracts showed the presence of alkaloids, carboxylic acids, fatty acids, phenols, saponins and steroids (Table 2). Catechols, coumarins, proteins, tannins, volatile oils were observed in low concentrations. We suspect that tannins found in the in the Phytochemical analysis of the extracts could be responsible for the antibacterial activity. Tannins have astringent actions, which form the basis for their therapeutic applications (Edward *et al*., 1970). If was found that plants which contain tannins posses antimicrobial activity (Trease and Evans, 1989).

The results showed antimicrobial activates agreeing with comparable results of previous researches using extracts other plants species like *Azadirachta indica* (Ram *et al*., 2000) *Chukrasia tabularis* (Nagalakshmi *et al*., 2001), *Toona ciliata* and *Amoora rohituka* (Chowdhury *et al*., 2003) *Aglaia spectabilis* (Lavanya *et al*., 2006) etc. The effectiveness may be due to the cumulative action of different compounds present in the plant parts (Bai, 1990). They include alkaloids, flavonoids, triterpenoids and other compounds of phenolic nature and are classified as active antimicrobial compounds (Rojas *et al*., 1992). This study revealed that the bark extracts of

Walsura trifoliata had antimicrobial activities to certain pathogens with a broad spectrum result to standard antibiotics. The results obtained also provide support to the uses of the plants in traditional medicine. However the toxicological analysis of the active compounds is necessary in order to assess its tolerance in the human body when administered.

CONCLUSION

Based on the results of the present study it can be concluded that the methanol and aqueous extract of *Walsura trifoliata* bark has exhibited significant activity. The above results indicate that the bark extracts may be used in the treatment of skin allergies, astringency and diarrhoeia, caused by microorganisms tested. Further studies with purified active compounds may be useful to evaluate the actual antibacterial properties of this plant. In addition toxicological experiments must also be undertaken to ensure the safe use.

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REFERENCES

Bai, D., 1990. Traditional Chinese materia; A respect and prospect. Plant Medica, 56: 5002.

Bauer, A.W., M.D.K. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by standard single disc diffusion method. Am. J. Clin. Pathol., 45: 493-496.

Chowdhury, R., M. Choudhury, H. Mohammad and A. Rashid, 2003. Antimicrobial activity of *Toona ciliata* and *Amoora rohituka*. Fitoterapia, 74: 155-158.

Croom, E.M., 1983. Documenting and evaluating herbal remedies. Econ. Bot., 37: 113-127.

Edward, P.C., E.T. Varrd and R.B. Lynn, 1970. A Textbook of Pharmacognosy. Henry Kinston, London.

Fadeyi, M.Q., A.E. Adeoye and J.D. Olowokudejo, 1989. Epidermal and phytochemical studies in the genus *Boerha6ia* (Nyctanginaceae) in Nigeria. Int. J. Crude Drug Res., 29: 178-184.

Gamble, J.S., 1935. Flora of Presidency of Madras. Vol. 1. BSI, Calcutta.

Hamilton, A., 1995. The People and Plants Initiative. In: Ethnobotany: A Methods Manual, Martin, G.J. (Ed.). WWF International. Chapman and Hall, London.

Jayasinghea, U.L.B., C.P. Jayasooriyaa, B.M.R. Bandarab, S.P. Ekanayakec, L. Merlinid and G. Assantee, 2002. Antimicrobial activity of some Sri Lankan Rubiaceae and Meliaceae. Fitoterapia, 73: 424-427.

Kapoor, L.D., A. Singh, S.L. Kapoor and S.N. Srivastava, 1969. Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. Lloydia, 32: 297-302.

Lavanya, T.M., K. Sri Rama Murthy, N. Srinivasa Reddy and K.R.S. Sambasiva Rao, 2006. Phytochemical and antimicrobial study of *Aglaia spectabilis* leaf extracts. J. Trop. Med. Plants, 7: 163-168.

Mishra, V., N. Parveenb, K.C. Singhalc, Nizam and U. Khana, 2005. Antifilarial activity of *Azadirachta indica* on cattle filarial parasite *Setaria cervi*. Fitoterapia, 76: 54-61.

Nagalakshmi, M.A.H., D. Thangaduri, D. Muralidhar Rao and T. Pullaiah, 2001. Photochemical and antimicrobial study of *Chukrasia tabularis* leaves. Fitoterapia, 72: 62-64.

Nostro, A., M.P. Germano, A. Amgelo, V.A. Marino and M.A. Cannatelli, 2000. Extraction methods and bioautography for evolution of medicinal plant antimicrobial activity. Lett. Applied Microbiol., 30: 379-388.

Odebiyi, A. and A.E. Sofowora, 1990. Phytochemical Screening of Nigerian medicinal plants. Part III. Lloydia, 41: 234-246.

Pullaiah, T. and S.S. Rani, 1999. Trees of Andhra Pradesh. Regency Publications, New Delhi.

Ram, M.S., G. Ilavazhagan, S.K. Sharma, S.A. Dhanraj, B. Suresh, M.M. Parida, A.M. Jana, K. Devendra and W. Selvamurthy, 2000. Anti-microbial activity of a new vaginal contraceptive NIM-76 from neem oil (*Azadirachta indica*). J. Ethnopharmacol., 71: 377-382.

Rizk, A. and M. Bashir, 1980. A chemical survey of sixty plants. Fitoterapia, 53: 35-44.

Rojas, A., R. Hernandaz, P. Miranda and R. Mata, 1992. Screening for antimicrobial of crude drug extracts and pure natural products from Mexican medicinal plants. J. Ethnopharmacol., 35: 275-285.

Schultes, R.E., 1960. Tapping our heritage of ethnobotanical lore. Econ. Bot., 14: 257-262.

Taylor, R.S., N.P. Manandhar and G.H.N. Towers, 1995. Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol., 46: 153-159.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. Balleire Tindal, London.