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In vitro antimicrobial activity of *Ageratum houstonianum* Mill. (Asteraceae)

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ABSTRACT

The present study was conducted in three different solvent extracts of leaves of *Ageratum houstonianum* (Mill.) (Asteraceae) and were screened against reference cultures, clinical isolates and fungal strains under *in vitro* condition. Among three solvent extracts of *A. houstonianum* tested against selected reference cultures, ethyl acetate extract was found to exhibit the highest activity (19 mm) against *P. aeruginosa*, followed by a second higher activity (18 mm) against *S. typhi*. In the case of *K. pneumoniae*, moderate inhibition was observed by ethyl acetate extract. The minimum inhibitory concentration for *P. aerugenosa* and *S. typhi* was found as 0.312 mg/ml of ethyl acetate extract. In the case of clinical isolates also, ethyl acetate extract of *A. houstonianum* was found to show higher activity (17 mm) against methiciline Resistant *S. aureus* (ICMR-5) which is found to be better than commercially available streptomycin, followed by a second higher activity using hexane extract (16 mm) against the same clinical isolate, whereas methanol extract showed only a moderate activity. Streptomycin was used as the positive control.

Introduction

Medicinal plants have been used for a wide variety of purposes for many years in India and all over the world. Several ethnomedicinal plant species of Ghana have been identified and their usage documented (Dokosi, 1998; Mshana *et al.*, 2000; Agbovie *et al.*, 2002; Anonymous, 2004). They have been used as antibacterial, antifungal, antiviral and antiprotozoan agents and for the general treatment of skin diseases, dermatitis, burns, diarrhoea, fever, wounds, cuts, sores, coughs and localized skin swellings. Due to the possible toxicities of the synthetic antimicrobial products, increasing attention has been directed towards the natural resources from plant based medicine, including essential oils and various extracts of plants have provoked interest as sources for the treatment of many infectious diseases (Naimiki, 1990).

Particularly, the antimicrobial activities of plant products and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Reynolds, 1996; Lis-Balchin, 1997). In addition to its use as an ornamental, *A. houstonianum* and the closely related *A. conyzoides* have been used in folk medicine (Johnson, 1971).

A study recently conducted by the group (Ndip *et al.*, 2007) indicated that crude extracts of another species of the same genus *Ageratum*, *Ageratum conyzoides* exhibited potent *Helicobater pylori* activity. Medicinal plants generally contain compounds which may be potential natural antibacterial agents and which may serve as an alternative effective source for the treatment of common bacterial infections. Herbal medicine has been widely used and formed an integral part of primary health care in many countries (Liu, 1987; Desta, 1993; Anesini and Perez, 1993).

In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. Therefore, a search for new antifungal and antibacterial drug is extremely necessary (Fortes *et al.*, 2008). More phytochemical and pharmacological studies are necessary in order to test popular indications and search for new pharmaceuticals (Almeida *et al.*, 2006). Among pathogenic fungi, the dermatophytes have the ability to invade keratinized tissues of animals and humans and cause a disease, dermatophytosis which is the commonest human contagious fungal disease (Esquenazi *et al.*, 2004; Sidat *et al.*, 2006). The aim of the present study was to evaluate the antimicrobial activity of different solvent extracts of *A. houstonianum* against microorganisms including fungi, bacteria and clinical isolates.

Materials and Methods

Plant Collection and Extraction

Healthy, disease free plants of *A. houstonianum* (leaves) were collected from Javadhu hills, Tiruvannamalai district, Tamilnadu, India. The species were identified and authenticated by the taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamilnadu, India. A voucher specimen was deposited at the departmental herbarium. Freshly collected leaf material was washed thoroughly, shade dried in open air and grounded into powder. The leaf powder (3 kg) was sequentially soaked in (9 L) of hexane, ethyl acetate and methanol for 72 h each with intermittent shaking. After 72 h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate

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was then air dried to yield 16.8, 57.9 and 24.2 g of hexane, ethyl acetate and methanol crude extracts respectively. All the crude extracts obtained were stored at 4°C in air tight containers until assay.

Microbes used and preparation of bacterial inoculums

The following test organisms were commonly used to test antimicrobial activity using disc diffusion method and minimum inhibitory concentration of the extracts: *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio fischeri* MTCC 1738, *Klebsiella pneumoniae* ATCC 15380, *Yersinia enterocolitica* MTCC 840, *Erwinia amylovora* MTCC 2760, *Salmonella typhi* MTCC 733, *Enterobacter aerogens* MTCC 111, *Proteus vulgaris* MTCC 1771 and *Candida albicans* MTCC 227. In addition, the clinical isolates (Table 3) were obtained from the Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHB) (Himedia) for 24 h at 28°C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 10^4/ml cfu/ml.

Disc diffusion method

Antimicrobial activity was carried out using disc-diffusion method (Murray *et al*. 1995). Petri plates were prepared with 20 ml of Mueller Hinton Agar (MHA) (Hi-media, Mumbai, India). The 24 h grown test cultures were swabbed on the solidified media and allowed to dry for 10 min. The tests were conducted using three different concentrations of the crude extract (5mg, 2.5mg and 1.25mg per disc). The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Streptomycin (10µg /disc) was used as positive control. The plates were incubated for 18 h at 28°C and then zone of inhibition was recorded in millimeters.

Minimum Inhibitory Concentration using broth micro dilution method

Based on the maximum zone of inhibition against microbes, four microbes (*P. aerugenosa* ATCC 27853, *K. pneumoniae* ATCC 15380, *S. typhi* MTCC 733 and *C. albicans* MTCC 227) were selected to find out minimum inhibitory concentration (MIC) using crude extracts. The MIC was performed according to the standard reference method (NCCLS, 2002) using 96 well plates. The extracts were dissolved in water with 20% dimethyl sulfoxide (DMSO). The initial concentration of the extract was 1mg/ml. The initial test concentration was serially diluted two-fold. Each well was inoculated with 5 ml of fungal suspension and incubated at room temperature for 24 h. The antimicrobial agent streptomycin was used as positive control. Minimum Inhibitory Concentration (MIC) was defined as the lowest extract concentration, showing no visible microbial growth after incubation time.

Results and Discussion

The present study was to evaluate the antimicrobial activity of solvent (hexane, ethyl acetate and methanol) extracts from the leaves of *A. houstonianum* tested against selected reference cultures (Table 1) and clinical isolates (Table 3). Ethyl acetate extract of *A. houstonianum* was found to exhibit the highest activity (19 mm) against *P. aeroginosa* followed by second highest activity (18 mm) in *S. typhi* by ethyl acetate extract. Methanol extract also inhibited the growth of *P. aeroginosa* which is on par with ethyl acetate extract. All the three extracts could inhibit the growth of *P. aeroginosa*. But none of these extracts inhibited the growth of *Enterobacter aerogens*. The minimum inhibitory concentration for *P. aerugenosa* and *S. typhi* was found as 0.312 mg/ml of ethyl acetate (Table 2). In the cases of *P. vulgaris* and *C. albicans* only moderate inhibition zone was observed in all the three extracts. Overall hexane extract did not show much activity when compared to other two extracts.

As on date, various works have been done by the scientists from various parts of the world on the antimicrobial activity of the Indian medicinal plants (Perumal, 1999; Jeevan, 2004; Rios and Recio, 2005; Mukherjee, 2006; Olaleye, 2007), and many solvents have been employed by various researchers in their studies and they have given varied results in each case. Presence of phytoconstituents in plants, namely, flavonoids, alkaloids, tannins and triterpenoids produced exciting opportunities for the expansion of modern chemotherapies against wide range of microorganisms (Lutterodt *et al*., 1999). No synergetic effect was observed when different concentrations of extracts from lemon balm, clove and eugenol were combined with ampicillin to inhibit the growth of *K. pneumoniae* and *E. aerogenes* (Gislene *et al.*, 2000). Some of the extracts of phytochemicals tested were active against *B. subtilis*. On the other hand, *P. aeruginosa*, which is also resistant to different antibiotics, had its growth inhibited by the extracts from clove, jambolan, pomegranate and thyme (Saxena *et al.*, 1994).

Based on the antibacterial activity in reference cultures, they were screened for the activity against clinical isolates (Table 3). Even in the case of clinical isolates, ethyl acetate extract of *A. houstonianum* showed higher activity (17 mm) against methiciline Resistant *S. aureus* (ICMR-5) which is found to be better than commercially available streptomycin, followed by a second higher activity using hexane extract (16 mm) against the same clinical isolate, whereas methanol extract showed only a moderate activity. But no extracts showed inhibition zone against ciprofloxacin resistant strain *E. coli* (ICMR-24). All the three extracts could inhibit the growth of *Enterococcus durans* moderately.

Similar kind of work was carried out by Akinyemi *et al.* (2005) in which water and ethanol extracts of the some of the shredded plnats such as *Terminalia avicennioides, Phylantus discoideus* and *Bridella ferruginea* were investigated for antibacterial activity. In an *in vitro* anti-methicillin resistant *Staphylococcus aureus* test (MRSA) the minimum inhibition concentration (MIC) 30.6 to 43.0 and 45.4 to 71.0 µg/kg were recorded for ethanol and water extracts. The minimum bactericidal concentration (MBC) was higher for both extracts. Studies carried out by Salvat *et al.* (2004) showed that methanolic extracts of *Lantana balansae* strongly inhibits the activity of clinical pathogen *S. aureus.* Thirty nine native plant species were collected from the provinces of Chaco and Forosa, in northern Argentina, and were screened for antimicrobial activity and minimal inhibitory concentration (MIC) against *P. aeroginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Enterococcus faecium* showed high activity against *P. aeroginosa* and *Staphylococcus* aureus. Muzagadial isolated from *W. ugandensis* was found to show potent antifungal activity, which is due to cell membrane damage in susceptible fungi (Taniguchi *et al.*, 1998). However, it requires an identification of the active principle from the crude extract.

References

1. Agbovie, T., Amponsah, K., Crentsil, O.R., Dennis, F., Odamtten, G.T and Ofusohene-Djan, W., 2002. Conservation and Sustainable Use of Medicinal Plants in Ghana

Ethnobotanical Survey. http://www.unepwcmc.org/species/plants/ghana.
2. Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C and Fasure, K.A., 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for antimethicillin resistant *Staphylococcus aureus* activity BMC Complement Altern Med 5, 6.
3. Almeida, C.F.C.B.R., Amorim, E.L.C., Albuquerque, U.P and Maia, M.B., 2006. Medicinal plants popularly used in the Xing´o region—A Semi-arid location in Northeastern Brazil. Journal of Ethnobiology and Ethnomedicine 15, 1-7.
4. Anesini, C and Perez, C., 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity. J Ethnopharmacol 39, 119-128.
5. Anonymous, 2004. Evaluation of the Evidence Base for Using Plants in Medicine. UK, NhS. Available from, http://www.nelh.nhs.uk/hth/herbal.asp.
6. Desta, B., 1993. Ethiopia traditional herbal drugs part II: antimicrobial activity of 63 medicinal plants. J Ethnopharmacol 42, 129-139.
7. Dokosi, O.B., 1998. Herbs of Ghana. pp. 1-746, 1st Edition, Ghana Universities Press, Accra.
8. Esquenazi, D., Alviano, C.S., DeSouza, W and Rozental, S., 2004. The Influence of Surface Carbohydrates During *In Vitro* Infection of Mammalian cells by the Dermatophyte *Trichophyton rubrum.* Research in Microbiology 155, 144–153.
9. Fortes, T.O., Alviano, D.S., Tupinamb, G., Padron, T.S., Antoniolli, A.R., Alviano, C.S and Seldin, L., 2008. Production of an Antimicrobial Substance against *Cryptococcus neoformans* by *Paenibacillus brasilensis* Sa3 Isolated from the Rhizosphere of Kalanchoe *brasiliensis*. Microbiological Research 163, 200-207.
10. Gislene, G.F., Nascimento, J.L., Paulo, C.F., Giuliana. L and Silva, 2000. Antibacterial Activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology 31, 247-256.
11. Jeevan, A., 2004. *In vitro* Antimicrobial Activity of Certain Medicinal Plants from Eastern Ghats, India, used for skin diseases. J Ethnopharmacol 3, 353-357.
12. Johnson, M.F., 1971. A monograph of the genus Ageratum L. (Compositae), Ann. Missouri Bot. Gard 58, 6-88.
13. Lis-Balchin, M and Deans, S.G., 1997. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. Journal of Applied Bacteriology 82, 759-762.
14. Liu, C.X., 1987. Development of Chinese medicine based on pharmacology and therapeutics. J. Ethnopharmacol 19, 119-123.
15. Lutterodt, G.D., Ismail, A., Basheer, R.H and Baharudin, H.M., 1999. Antimicrobial Effects of *Psidium guajava* extracts as one mechanism of its antidiarrhoeal action. Malaysian J Med Sci 6, 17-20.
16. Mshana, N.R., Abbiw, D.K., Addae-Mensah, I., Adjanohoun, E., Ahyi, M.R.A., Ekpera, J.A., Enow-Orock, E.G., Gbile, Z.O., Noamasi, G.K., Odei, M.A., Odunlami, H., Oteng-Yeboah, A.A., Sarpong, K and Tachie, A.A., 2000. Traditional Medicine and Pharmacopoeia; Contribution to the Revision of Ethnobotanical and Floristic Studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity (OAU/STRC), Accra, pp. 1-920.
17. Mukherjee, D., 2006. Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds. J Ethnopharmacol 106, 1-28.
18. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., 1995. Manual of Clinical Microbiology, 6th ed. ASM, Washington, DC.
19. Naimiki, M., 1990. Antioxidants/antimutagens in foods.CRC Critical Reviews in Food Science and Nutrition 29, 273-300.
20. National Committee for Clinical Laboratory Standards NCCLS 2002. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous fungi. Approved Standard M38-A. National Committee for Clinical Laboratory Standards, Wayne, Pa
21. Ndip, R.N., Mackay, W.G., Farthing, M.J.G and Weaver, L.T., 2003. Culture of *Helicobacter pylori* from clinical specimens: a review of microbiological methods. J Pediat Gastroenterol Nutr 9, 1030-1040.
22. Olaleye, M.T., 2007. Cytotoxicity and Antibacterial Activity of Methanolic extract of *Hibiscus sabdariffa*. J Med Plant Res 1, 009-013.
23. Perumal, R., 1999. Preliminary screening of ethnomedicinal plants from India. J Ethnopharmacol 66, 235-240.
24. Reynolds, J.E.F., 1996. Martindal the extra pharmacopeia. Pharmaceutical Society of Great Britain 31st ed.
25. Rios, J.L and Recio, M.C., 2005. Effects of Two Medicinal Plants *Psidium guajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) Leaf Extracts on Rat Skeletal Muscle Cells. J Ethnopharmacol 22, 80-84.
26. Salvat, A., Antonacci, L., Fortunato, R.H., Suarez, E.Y and Godoy, 2004. Antimictobial Activity in Methanolic Exracts of Several Plant Species from Northern Argentina. Phytomedicine, 11, 230-234.
27. Saxena, G., McCutcheon, A.R., Farmer, S., Towers, G.H.N and Hancock, R.E.W., 1994. Antimicrobial Constituents of *Rhus glabra*. J. Ethnopharmacol 42, 95-99.
28. Sidat, M.M., Correia, D and Buene, T.P., 2006. Tinea Capitis Among Rural School Children of the District of Magude, in Maputo province, Mozambique. Mycoses, 49, 480–483.
29. Taniguchi, M., Yano, Y and Tada, E., 1998. Mode of Action of Polygodial, an Antifungal Sesqueterpene dialdehyde. Agricultural and Biological Chemistry 52, 1409-1414.

Table 1 Antimicrobial activity of crude extracts of *Ageratum houstonianum* against selected reference microbes

Name of Microbe	Zone of Inhibition (mm)									
	Hexane (mg/disc)			Ethyl acetate (mg/disc)			Methanol (mg/disc)			S
	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0	
Reference cultures										
ATCC 25923, *S.aureus*	10	12	14	10	11	12	10	10	12	15
ATCC 27853, *Pseudomonas aerugenosa*	15	17	18	16	18	19	14	16	18	18
MTCC 1738, *V. fischeri*	10	11	13	10	12	14	9	10	12	16
ATCC 15380, *Klebsiella pneumoniae*	11	13	15	13	15	16	11	13	15	15
MTCC 840, *Yersinia enterocolitica*	10	11	13	9	10	12	10	11	13	20
MTCC 2760, *Erwinia amylovora*	9	10	10	10	13	14	9	10	10	20
MTCC 733, *S. typhi*	10	12	14	14	16	18	10	11	12	18
MTCC 111, *Enterobacter aerogens*	-	-	-	-	-	-	-	-	-	20
MTCC 1771, *Proteus vulgaris*	10	13	15	11	12	14	10	12	14	19
MTCC 227, *Candida albicans*	12	14	16	12	14	17	10	12	15	16

'S' Streptomycin (10 µg/disc); '-' No inhibition

Table 2 Minimum Inhibitory Concentration (MIC) of crude extracts of *Ageratum houstonianum* (mg/ml)

Bacteria	Hexane (mg/ml)	Ethyl acetate (mg/ml)	Methanol (mg/ml)	Streptomycin (µg/ml)
ATCC 27853, *P. aerugenosa*	> 0.312	0.312	> 0.312	> 5
ATCC 15380, *K. pneumoniae*	0.625	< 0.625	0.612	5
MTCC 733, *S. typhi*	> 0.625	> 0.312	> 0.625	> 5
MTCC 227, *C. albicans*	0.625	0.625	> 0.625	> 5

Table 3 Antimicrobial activity of crude extracts of *Ageratum houstonianum* against selected clinical isolates

Name of Microbe	Zone of Inhibition(mm)									
	Hexane (mg/disc)			Ethyl acetate (mg/disc)			Methanol (mg/disc)			S
Clinical isolates	1.25	2.5	5.0	1.25	2.5	5.0	1.25	2.5	5.0	
ESBL *Klebsiella* (ICMR-6)	-	-	-	-	10	-	-	9	-	14
*Staphylococus aureus [*Methiciline Sensitive *S. aureus]*	9	10	11	10	12	13	9	10	10	15
MRSA *[*Methiciline Resistant *S. aureus]* ICMR – 5	12	15	16	13	15	17	12	14	16	15
ICMR-24 *[E.coli]* Cipro R	-	-	-	-	-	-	-	-	-	-
ICMR-19 *Acetobacter baumanii* (Carbapenem R)	10	11	12	10	12	14	9	11	12	16
ESBL *[Klebsiella pneumonia]*	-	-	-	-	-	-	-	-	-	12
Enterococcus durans (P 502)	12	13	15	12	15	16	10	12	15	16

'S' Streptomycin (10 µg/disc); '-' No inhibition