EVALUATION OF *ALTERNANTHERA BETTZICHIANA* (REGEL) NICOLS FOR ANTIMICROBIAL ACTIVITY

J.C.Hundiwale*[1], M.V.Kulkarni[1], A.U.Hivrale[1], D.A.Patil[2], R.G.Mali[3]

1. Dept. of Life Sciences, North Maharashtra University, Jalgaon (M.S.)
2. Dept. of Botany, S.S.V.P. Sanshta's LK Dr. P.R. Ghogrey Science College, Dhule (M.S.)
3. Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Dist. Jalgaon (M.S.)

Summary

The present study evaluates the antimicrobial activity of the different extracts of *Alternanthera bettzichiana.* The antimicrobial activity of various extracts of *A. bettzichiana* was performed by using agar disc diffusion method and was tested against various microorganisms *viz.*, *Bacillus subtillis, Staphylococcus aureus, E. coli, Salmonella typhi Aspergillus niger and Aspergillus flavus*. The results of the study showed that aqueous extract possess mild activity and while alcoholic extract shown moderate activity against the tested organisms except the fungal strains. Among the test bacteria, *Bacillus subtillis* was found to be most sensitive to all the extracts. Minimum Inhibitory Concentration (MIC) was determined by broth dilution method by using some bacterial and fungal strains of microorganisms. Streptomycin and Cotrimazole (10µg/ml) were used as reference standard for antimicrobial screening.

Key words: *Alternanthera bettzichiana, Amaranthaceae,* antimicrobial activity, Cotrimazole, Streptomycin

Corresponding Author Address:
Mr. J.C.Hundiwale
Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda-425 107, Dist. Jalgaon (MS)
Phone: +91-2586-22366
Email: narsim16@rediffmail.com

Introduction

Infectious diseases are a serious problem world wide[1]. Although pharmaceutical industries have produced number of new antibiotics in last few decades, resistance to these drugs by microorganisms has increased. In general, microorganisms have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [2].

Historically, plants have provided a good source of anti-infective agents; emetine, quinine, berbine etc. remain highly effective instruments to combat against microbial infections[3]. Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants[4]. Many efforts have been done to discover new antimicrobial compounds from various sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systemic screening of them may result in the discovery of novel effective compounds[5]. The genus *Alternanthera*, a medicinally important member of family *Amaranthaceae* is reported to contain volatile constituents, essential amino acids, flavone glycoside and steroids[6]. *Alternanthera bettzichiana* (Regel) Nicols a horticulture species of many forms, is much branched, diffuse herbs, stem ribbed, green; young branches white pubescent with spike like minute flower[7] , reported as a wild edible material[8], utilized to purify and nourishing blood, antipyretic, as galactagogue and for wound healing. The parallel species of the same genus were also reported for their potential claims in certain viral diseases[09], as an immunomodulator[10], protective against cancer[11] and in treatment of diarrhoea[12]. The purpose of this study was to screen the various extracts prepared by successive extraction of *A. bettzichiana* that could be useful for the development of new tools as antimicrobial agent for the control of infectious diseases.

Materials and methods

Plant Material

Fresh samples of the plant *A. bettzichiana were* collected from the different parts of satpuda region of Chopda Tahsil, Jalgaon district in the month of September. The identity of this plant was confirmed by Dr.D.A.Patil (Head, Dept. of Botany, S.S.V.P.Santha's LK Dr. Ghogrey Science College, Dhule) and further authentificated from Botanical survey of India (BSI), Pune. A voucher specimen (JCH. 1) of the plant has been deposited at the Dept. of Biotechnology, school of life sciences, North Maharashtra University, Jalgaon.

Preparation of plant extract

Whole part of the plant material was dried for several days in shade and powdered with the help of an electric grinder. The dried powdered plant material was extracted successively with petroleum ether, chloroform and 90 % ethanol using soxhlet extraction apparatus. Aqueous extract was prepared by using cold maceration process. Then the extracts were filtered in hot condition and distilled on water bath. All the extract was finally dried at low temperature under reduced pressure in a rotary evaporator. The extractive values were found to 6.4 % (petroleum ether), 25.6 % (chloroform), 32.0 % (ethanol) and 19.2 % water with respect to the dry weight of the starting powdered material.

All the extracts were stored in the refrigerator and were subjected to the qualitative phytochemical screening for identification of active constituents using standard methods[13].

Microorganisms used

For the present study, the strains of microorganisms utilized includes gram positive bacteria *viz., Bacillus subtilis, Staphylococcus aureus* and gram negative strains of *E. coli and Salmonella typhi. In vitro* study against fungal strains such as *Aspergillus niger and Aspergillus flavus* was also carried out. All these microbial cultures were obtained from Dept. of Microbiology, North Maharashtra University, Jalgaon.

Antimicrobial activity

In vitro antibacterial and antifungal activities of the crude extract of the plant were determined by disc diffusion method[14]. Nutrient broth and agar, Vogel Johnson medium, McConkeys medium (Agar and broth) was used for culture of bacteria and Sabouraud medium (Agar and broth) was utilized for culture of fungi.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of the different extract was determined by broth dilution method[15] at concentration ranging from 10 to 200 µg/ml in dimethylformamide (DMF) [16] against the microorganisms used for the study.

Determination of zone of inhibition

The zone of inhibition was determined by agar disc diffusion method. The extract was dissolved in DMF at a concentration of 100, 300 and 500 µg/ml. Streptomycin (10 µg/ml) and Cotrimazole (10 µg/ml) were used as reference standard for the antibacterial and antifungal screening. Solvent control (DMF) was also maintained throughout the study.

Results and discussion

Preliminary phytochemical screening of all crude extracts of *A. bettzichiana* revealed the presence of an array of active constituents including alkaloids, steroids, cardiac and coumarin glycosides, proteins and sugars (Table I). The present study is focused to evaluate the antimicrobial potential of plant *A. bettzichiana*. Table II depicts the results of antimicrobial activity of the various extracts of *A. bettzichiana*. The results of zone of inhibition study revealed that the petroleum ether, chloroform, ethanol and aqueous extract posses antimicrobial activity in a concentration dependant manner against the test organisms and was also comparable with the standard drugs Streptomycin and Cotrimazole (10µg/ml) included in the study. As shown in table II, the extracts of *A. bettzichiana* displayed antimicrobial activity with the diameter of zone of inhibition ranging from 4.9 mm to 13.8 mm.

Alcoholic extract of *A. bettzichiana* exhibited significant antibacterial activity. In case of all these extracts MIC of 80 to 120 was observed against the tested strains of microorganisms and was determined by using broth dilution method. None of the extracts of *A. bettzichiana* exhibited any antifungal activity.

In general, commercial antibiotic drugs cause side effects such as liver, kidney and gastrointestinal tract toxicity[17]. On the other hand, herbal remedies often do not produce any side effects[18]. Therefore, alternative medicine has become a popular remedy to various types of ailments. Research on natural plant products has been well encouraged by the World Health Organization. Although natural products are known to control some infectious diseases, the use of plant secondary metabolites for antimicrobial studies has received less attention[19]. The finding and characterization of the active molecule may be interesting in the search for new efficacious and safe antimicrobial agent against variety of pathogenic organisms. In conclusion, *Alternanthera bettzichiana*s has revealed significant antimicrobial activity against the various test organisms used for the study, however present study warrants further detailed phytochemical and mechanism based studies on the plant in search of novel lead from the natural resources. This work is in progress in department and will be reported at a later date.

Table I. Preliminary phytochemical Analysis of *Alternanthera bettzichiana*

Chemical Class	Pet.Ether extract	Chloroform extract	Alcoholic extract	Aqueous extract
Test for Carbohydrates	-	-	-	+
Test for Gums	-	-	-	+
Test For Proteins	-	-	-	+
Test for Steroids	-	+	+	-
Test for Cardiac glycosides	-	-	+	+
Test for Coumarin glycosides	-	-	+	+
Test for Alkaloids	-	-	+	-

(+) Positive
(-) Negative

Table II. Antibacterial activity of various extracts of *Alternanthera bettzichiana*

Plant material	Conc. µg/ml	Zone of inhibition (mm)					
		Bacillus subtillis	*Staph. aureus*	*E.coli*	*Salmonella typhi*	*Aspergillus niger*	*Aspergillus flavus*
Petroleum ether extract	100 300 500	7.5 ± 0.6 8.7 ± 0.7 9.0 ± 0.6	7.0 ± 0.6 8.2 ± 0.8 9.5 ± 0.9	7.5 ± 0.5 7.9 ± 0.6 8.0 ± 0.7	7.9 ± 0.6 8.0 ± 0.8 8.4 ± 0.8	--	--
Chloroform extract	100 300 500	9.0 ± 0.8 10.4 ± 0.9 11.0 ± 1.0	7.0 ± 0.7 7.8 ± 0.6 10.5 ± 0.9	7.5 ± 0.6 8.2 ± 0.8 8.5 ± 0.7	8.5 ± 0.7 8.9 ± 0.9 9.5 ± 0.9	--	--
Ethanolic extract	100 300 500	11.0 ± 1.1 12.0 ± 1.0 13.8 ± 0.9	9.5 ± 0.8 10.6 ± 0.9 11.9 ± 1.1	8.0 ± 0.7 9.0 ± 0.8 9.7 ± 0.9	10.5 ± 1.0 11.5 ± 1.1 12.0 ± 1.1	--	--
Aqueous extract	100 300 500	5.5 ± 0.5 6.3 ± 0.5 7.4 ± 0.6	5.1 ± 0.4 6.0 ± 0.6 7.0 ± 0.7	-- -- --	4.9 ± 0.5 5.3 ± 0.5 6.0 ± 0.6	--	--
Standard streptomycin	10	15.0 ± 1.2	14.5 ± 1.3	15.0 ± 1.3	15.0 ± 1.4	NA	NA
Standard Cotrimazole	10	NA	NA	NA	NA	16.0 ± 1.2	18.0 ± 1.5

Each value is represented as Mean ± S.E.M. (n=3)
(--): No activity
NA: Not applicable

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References

1) Watson AJM, Diarrhoea. British Medical Journal 1992; 1304(4): 304.
2) Cohen ME. Epidemiology of drug resistance implications for a post-antimicrobial era. Science 1992; 257: 1050-1055.
3) Maurice MI, Angela R, and Chris OO. New antimicrobials of plant origin. J. Janick (ed.) 1999; Alexandra, VA: 457-462.

4) Farnsworth NR, Loub WD, Information gathering and data bases that pertinent to the development of plant-derived drugs in plants; the potentials for extracting Protein Medicines and other useful chemicals. Workshop proceedings; OTA-BP-F-23, U.S. Congress 1983. Office of Technology Assessment, Washington, D.C.:178-195.

5) Janovska D, Kubikova K, Kokoska L. Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. Czech. J. Food Sci 2003; l21: 107-111.

6) The wealth of India: Raw Materials, Vol.I A-J, CSIR, New Delhi, 2004:51.

7) Geeta C, Bhattacharyya UC. The genus Alternanthera (*Amaranthaceae*) in India. Bull. Bot. Surv. India 1994; 36:267-277.

8) Arinathan V, Mohan VR, John De Britto A and Murugan C. Wild edibles used by palliyars of the Western Ghats, Tamil Nadu. Ind. J. of Traditional Knowledge 2007; 6 (1): 163-168.

9) Bing-nan Zhou, Gabor Blasko and Geoffrey A Cordell. Alternanthin, A *C*- Glycosylated flavonoid from *Alternanthera philoxeroides*. Phytochemistry 1988; 27(11): 3633-3636**.**

10) Guerra RNM, Pereira HAW, Silveira LMS, Olea RSG. Immunomodulatory properties of *Alternanthera tenella* colla. Braz J Med Biol Res 2003; 9: 65-68.

11) Shridhar and Lakshminarayana. Lipid classes, fatty acids and tocopherols of leaves of twenty six edible plant species. J Agric Food Chem 1993; 44:41-61.

12) Burkill HM. The useful plants of west tropical Africa. Royal Botanic Gardens, Kew (K) 1985; 1: 247-249.

13) Khandelwal KR. Practical Pharmacognosy, 11th Ed., Nirali Prahashan. Pune 2001: 149-160.

14) Cruickshank R. Medical Microbiology: a guide to diagnosis and control of infection, 11th ed. E and S Livingston Ltd. Ediburg and London 1968: 888.

15) Hirano R, Sasamoto W, Matsumoto A. Vitaminol.J Nutr Sci 2001; 47: 357.

16) Vahanwala SJ, Golatkar SG, Rane JB, Pawar KB, Ambaye RY and Khaduse BG. Antimicrobial activity of *Couroupita guianensis* Aubl. Indian Drugs July 2000; 37 (7): 343-345.

17) Mali RG, Mahajan SG, Mehta AA. Evaluation of *Bauhinia variegate* Linn stem bark for anthelmintic and antimicrobial properties. Journal of Natural Remedies 2008; 8(1): 39-43.

18) Perry L M. Medicinal plants of East and Southeast Asia; attributed properties and uses, MIT press, Cambridge 1980: 73-81.

19) Shafiqur Rahman M, Nural Anwar M. Antimicrobial activity of the root of *Plumbago zeylanica*. Bangladesh J Microbiol 2007; 24: 73-75.