International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 176-180, Jan-Mar 2012

Antimicrobial activity of plant extracts of *Ocimum tenuiflorum*

Archana Sharma*, Anju Meena and Rishikesh Meena**

Department of Botany Vedic P.G.Girls College Raja Park Jaipur-302004, Rajasthan, India.

****Department of Botany Govt. Birla College Bhawani Mandi-326502, Distt.Jhalawar,Rajasthan,India.**

***Corres. Author:drarchanasharma11@gmail.com,*
****rishi_1180@yahoo.com***

Abstract:-The present investigations evaluate the antimicrobial activity of *Ocimum tenuiflorum* against selected gram positive, gram negative bacteria and fungal strains. In this study, the crude ethyle acetate and methanol extract of *O.tenuiflorum* (stem, root and leaves) was assed for their antimicrobial activity using disc diffusion method. The activity was performed against common pathogenic bacterial (*Pseudomonasaeruginosa*,*Proteus vulgaris*,*Staphylococcus saprophyticus* and *Escherichia coli* and fungal strains **(***Aspergillus niger* and *candida albicans*). The extracts of *O.tenuiflorum* were found to be more or less active against almost all tested pathogenic strains. The inhibition zone ranged from 6mm-28mm and activity index ranged from 0.17-1.47mm**.** The most susceptible bacteria and fungi are *E.coli* (IZ=17mm and AI=0.89) and *A.niger* (IZ=7mm and AI=0.19mm) respectively.
The leaf extract of *Ocimum tenuiflorum* was found to be most active that exhibited more or less similar activity against all the pathogen tested, however both extracts methanol extracts exhibited comparatively high activity. The significant potential of *Ocimum tenuiflorum* extract concludes that it could serve as a source of natural antimicrobial agents.
Key words: *Ocimum tenuiflorum,* human pathogenic strains, ethyl acetate and methanol extract of *Ocimum tenuiflorum*.

Introduction

The family Labiateae is one of the largest families, which comprises the larger proportion of medicinal plant species. *Ocimum* is one of the important genera of family Labiateae. Ocimum species often referred to as the "king of the herb. *Ocimum tenuiflorum* is an important medicinal herb belonging to family Lamiaceae. It is commonly known as 'Shyama tulsi.
Recently, due to indiscriminate use of commercial antimicrobial drugs, multiple drug resistance human pathogens have developed [1, 2, 3].

This situation has interest for searching new antimicrobials from natural sophisticated traditional medicine system that have given rise to some important drugs still in use today [4,5.] Plant metabolites and plant based drugs appear to be one of the better alternatives as they are know to have minimal toxicity and cost effective in contrast to synthetic agents. Numerous plants have been screened for anti-infective properties as the probability of finding diverse chemistries have been implicated to serve as leads for the new anti-infective drugs as well as for the discovery of novel antimicrobial chemotherapeutic agents [6,7,8.]Thus, antimicrobial research is geared

toward the discovery and development of novel antibacterial and antifungal agents [9].
Therefore, in the present investigation, the antimicrobial potential of Ocimum *tenuiflorum* root, stem and leaf extracts has been evaluated against common pathogens.

The systematic screening of plant species with the purpose of discovery of new bioactive compounds is a routine activity in many laboratories. In particular, the search for components with antimicrobial activity is gaining increasing importance in recent times, due to growing world wide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms [10]. Hence, there is a constant need for new and effective therapeutic agents. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

Materials and methods

Collection of plant material

Fresh plants or plant parts were collected randomly from Botany Department University of Rajasthan Jaipur. Fresh plant material was washed under running tap water, air dried, homogenized to fine powder, and stored in tightened light-protected containers.

Preparation of the extracts

The plant materials viz. stem, root and leaves of *Ocimum tenuiflorum* were air dried and powdered using motor and pestle. The coarsely powdered materials were successively extracted with ethyle acetate,methanol extracts and ether in soxhlets apparatus for 24 hrs. After 24 hrs.It was filtered through 4 layers of muslin cloth and centrifuged at 5000 x g for 10 min. The supernatant was collected and the solvent was evaporated. The crude extract diluted with 5% of DMSO to make the final volume one-tenth of the original volume and stored at 4 °C in air tight bottles for further studies.

Preparation of Inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring loopful bacterial cells from the stock cultures to Erlenmeyer flask of nutrient broth that were incubated with agitation for 24 hrs at 37°C. The bacterial cultures of gram positive and gram negative bacteria were maintained on nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one liter distilled water). These micro-organisms were allowed to grow at 35°C-37°C temperature. A fresh inoculum of test microorganism in saline solution was prepared from a freshly grown agar slant before every antibacterial assay by adjusting the concentration of micro-organism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm, transmittance used bacteria was 40%.

Test microorganisms

The bacterial strains studied are *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Pseudomonas aeuriginosa* and *Proteus vulgaris* and fungal strains *Aspergillus niger* and *Candida albicans*. These test organisms were clinical isolates obtained from patients diagnosed for having bacterial and fungal infections and procured from the Durlabh ji Hospital Jaipur. Microorganisms were maintained at 4 °C on nutrient agar slants.
Each of the microorganisms was freshly cultured prior to susceptibility testing by transferring them into a separate sterile test tube containing nutrient broth and incubated overnight at 37°C. A microbial loop was used to remove a colony of each bacterium and fungus from pure culture and transfer it into nutrient broth.

Preparation of media

The growth media employed in the present study included nutrient agar and nutrient beef: Beef extract-3.0g; peptone-5.0g; agar-15.0g;distilled water-1000ml.

Nutrient broth is composed of with out agar.The medium was adjusted to pH7.4 and sterilized by autoclaving at 15 lbs pressure and 121°C temperature for 15 min.

Preparation of inoculum

Each organism was recovered for testing by sub culturing on fresh media. A loop ful inoculum of each bacterium and fungus was suspended in 5ml of nutrient broth and incubated overnight at 37 °C. These over night cultures were used for the study.

Antimicrobial activity

Antimicrobial assay of the methanol and acetate were performed against tested pathogenic strains by agar disc diffusion method [11], using streptomycin and ketoconozole as standard antibiotic. Standard size Whatman filter paper disc (6.0 mm diameter) were sterilized in an oven at 140°C for one hour, saturated with three plant extracts such as root, stem, and leaf. These were air dried at room temperature to remove any residual solvent that might interfere with the determination of activity. The discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with test bacteria (using saline solution) and air dried to remove the surface moisture.

Table No. 1

Plant parts used		*Micro organism used*											
		Pseudomonas aeruginosa		*Proteus vulgaris*		*E.Coli*		*Staphylococcus saprophytic*		*A.niger*		*Candida albicans*	
		I.Z.	A.I.	I.Z.	A.I.	I.Z.	A.I.	I.Z.	A.I.	I.Z.	A.I.	I.Z.	A.I.
Root	**Methanol**	11	0.78	19	1.3	13	0.81	21	0.65	8	0.24	10	0.29
Stem		15	0.68	22	1.3	17	0.89	24	0.68	7	0.19	9	0.25
Leaf		16	0.94	28	1.5	23	0.92	26	0.81	12	0.34	7	0.21
Root	**Ethyl acetate**	10	0.60	15	0.7	11	0.28	18	0.56	6	0.17	8	0.26
Stem		12	0.65	17	1	17	0.89	19	0.62	7	0.19	6	0.16
Leaf		14	0.68	18	0.7	18	0.63	22	0.64	8	0.24	12	0.31
Control		I.Z.		I.Z.		I.Z.		I.Z.		I.Z.		I.Z.	
Streptomycin		19		32		27		21		-		-	
Ketoconozole		-		-		-		-		28		28	

IZ = Inhibition Zone, AI = Activity Index, - = Not detected

The thickness of the agar medium was kept equal in all the pertriplates and the standard discs (streptomycin 2 µg/ml and ketoconozole 2 µg/ml) were used as a control. Before incubation, the petriplates were placed for one hour in a cold room (5°C) to allow the diffusion of the compounds from the disc into the medium. Plates were incubated at 37°C for 20-24 hours after which the zone of inhibition or depressed growth could be easily measured. The diameter of inhibition zone (mm) was measured. All the experiments were done in five replicates and the activity index was calculated for each of these.

$$\text{Activity index (A.I.)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Results and discussion

Medicinal plants have provided a source of inspiration for novel drugs compounds as since plant derived medicines have made large contributions to human health. The traditional healer make Use of water primarily as a solvent but alcoholic extracts of these plants were certainly much better and powerful. This may be due to the better solubility of active components in organic solvents [12, 13].

Plants have a great potential of producing natural drugs that have been the source of most of the active ingredients of medicines, as they are non toxic, having no side effects and easily available [14]. Several works have been documents on the pharmalogical screening of the plant extracts which have been exploited as the source of innumerable therapeutic agents [15, 16,17].

The antimicrobial efficacy of *Ocimum tenuiflorum* plant extracts (root, stem and leaf) against bacterial and fungal strains was evaluated by the agar well diffusion method via determination of the surrounding zones of inhibition (Table1). The extracts of *O.tenuiflorum* were found to be more or less active against almost all tested pathogenic strains. The inhibition zone ranged from 6mm-28mm and activity index ranged from 0.17-1.47mm**.** The most susceptible bacteria and fungi are *E.coli* (IZ=17mm and AI=0.89) and *A.niger* (IZ=7mm and AI=0.19mm) respectively.

The leaf extract of *Ocimum tenuiflorum* was found to be most active that exhibited more or less similar activity against all the pathogen tested, however both extracts methanol extracts exhibited comparatively high activity. Lowest activity was notice on *Pseudomonas aeruginosa* (IZ=10mm and AI=0.60) and *A.niger* (IZ=6mm and AI=0.17mm) as compared to standard antibiotics.

Among bacterial pathogens, gram positive bacterial were found to be more susceptible than gram negative bacterial strains. The antibacterial activity may be also due to the presence of several metabolic toxins or broad-spectrum antibiotics. Several metabolites from herb species, including alkaloids, tannins and sterols have been associated with anti microbial activity [18].The sites and number of hydroxyl group on the phenol components may increase in the toxicity against the microorganisms. [19] Suggested that the antimicrobial properties of tannins might be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins, their complexity with polysaccharides and their ability to modify the morphology of microorganism. Several reports have been shown that bioactive compounds isolated from plant extracts have inhibitory effect on pathogens strains. [20,21,22,23].

The results of present study supports the traditional usage of plant and *Ocimum tenuiflorum* plant extracts which posses compounds with antibacterial properties

that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens and further work may be carried out for pharmacological evaluation.

Conclusion:

The present investigation revealed that the extracts of Ocimum *tenuiflorum* root, stem and leaf have potent antimicrobial activity which explains its use in traditional system of medicines. The extracts of *Ocimum tenuiflorum* were found to be more or less active against almost all tested pathogenic strains. Hence, *Ocimum tenuiflorum* can be employed as a source of natural antimicrobials that can serve as an alternative to conventional medicines.

Acknowledgements:

The authors are thankful to the head of Botany Department, Vedic P.G. Girls College Rajapark Jaipur, for providing all necessary facilities for the present research work . The author is thankful to Santokbha Durlabh Hospital, Jaipur India for providing tested microorganism for antimicrobials activity.

References:

1. Giamarellos-Bourboulis E.J., Grecka P., Dionyssiou-A. and Giamarellou H. In vitro interaction of gamma-linolenic acid and arachidonic acid with ceftazolineon multiresistant Pseudomonas aeruginosa. Lipids.1999.34,151-152.
2. Marchese A. and Schito ,G.C. resistance patterns of lower respiratory tract pathogens in Europe. International Journal of Antimicrobial Agents. 2001.16(Suppl.1), 25-29.
3. Westh H.,Zinn C.S. and Rosdahl V.T. An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolated from 15 hospitals in14 counteries. Micro.Drug Resist., 2004.10:169-176
4. De Smet P.A.G.M. The role of plant –derived drugs and herbalmedicines in healthcare. Drugs. 1997.54,801-840.
5. Cowan M.M. Plant products as antimicrobial agents Clinical Microbiol.Rev.., 1999.12, 564-582.
6. Naqvi S.A.H., Khan M.S.Y. and Vohora S.B. antibacterial, antifungal and anthelmintic investigations on Indian medicinal plants. Fitoterpia. 1991 LXII3, 221-228.
7. Taylor R.S.L., Edel F., Manandhar N.P. and Towers G. H. N. Antimicrobial activities of Southern Nepalese medicinal plants.J. Ethano - pharmacol.,1996. 50,2: 97-102
8. Davis, J. Inactivation of antibiotics and the dissemination of resistance genes. Science. 1994. 264**:** 375-382.
9. Paz, E.A., Fernandez, M.P., Ferriera, J., Moyna, P., Soubes, M., Vazquez, A., Vero, S. and Zunino, L. Screening of Uruguayan medicinal plants for antimicrobial activity. Journal of Ethno-pharmacology.1995. 45**:** 67-70.
10. Shrinvasan D., Nathan S., SureshT. and Perumalsamy O.,Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J.Ethnopharmacol. 2001.74, 217-220.
11. Gould J.C. and Bowie J.H. the determination of bacterial sensivity to antibiotics edinb. Med.J. 1952. 59:178-199.
12. Yoganarasimhan, S.N. Medicinal plant of Indian Tamilnadu.2000.11:64-65,230
13. Kumar, B.S.A., Lakshman, K., Tirupathi,M.S. Jayaveera, K.N., Narayanasamy V.B., Sabemulla, K. and Nandeesh, R..Free radical scavenging and antibacterial activities of Armycard power (An Ayurvedic formulation).European Bulltien of Drug Research., 2009. 17:5-9.
14. Bandow J.E., Brotz H.,Leichert L.I.O.,Labishinski H. and HEcker, M.proteomic approach to understanding antibiotic action Amicro. Agents. Chemother.,2003.47:948-955.
15. Yoshikazu S. Murata H.,Nakanishi T.,Inatomi Y.Inhibitory effect of plant extracts on production of virotoxins by enterohemorrhagic Escherichia coli O157:H7J.Health Sci.2001.47,473-477.
16. Herrera R.M.,PerezM., Martin Herrera D.A.,Lopez-GraciaR.,Rabanal R.M.Antimicrobial activity of extracts from plant endemic to the Canary Island Phytother.Res.1996.10,364-365.
17. Natarajan E.,SenthilkumarS.,Francis Xavier T. and Kalaisevi V.antibacterial activities of leaf extracts of Alangium salviifolium J.Trop.I Med.Plants.2003.4:9-13.
18. Leven, M.D., Vanden, B.D.A., Marten,T., Vilientmick , A. and Lomweas,E.C. Screening of

higher plants for biological activity.Planta Med. 1979.36:311-312.

19. Yao J.and Mollering R .AntibacterialagentsIn Murray P. Baron E. Pfaller, M. ,Tenover, F., YolkenR. (eds.) Manul of clinical microbiology. ASM. Washing, DC., 1995.pp 1281-1290.
20. Mimica-Dukic N., Kujundzic S.,Sokovic M, and Couladis M.Essential oil composition and antifungal activity of Foeniculum vulgare Mill obtained by different distillation conditions. Phytother Res.2003.17 (4):368-71.
21. Meng,J.C., Zhu, Q.X.and Tan, R.X. New antimicrobial and sesquiterpenes from Soroseris hookeriana subsp.erysimoides.Planta Medica, 2000.66, 541-544.
22. Balasaraswathi R.,Sadasivam S.,Ward M.and Walker J.M. An antiviral protein from Bouganvillea spectabilis roots, purification and characterization Phytochem. 1998.47(8):1561-1565.
23. Sharma A., Meena S., and Barman N. Efficacy of ethyle acetate and ether extract of Terminalia chebula Retz against some human pathogenic strains. International Journal of Pharmatech Research. 2011. 3(2):724-727.

*****