Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2010; 3(2): 18-21

ISSN: 0975 4873

Research Article

Antimicrobial Activity of *Artemisia abrotanum* and *Artemisia pallens*

Suresh J*, Vasavi Reddy.A, Dhanya Rajan, Ihsanullah.M, Mohd. Nayeemmullah Khan.

Department of Pharmacognosy, JSS College of Pharmacy, S.S. Nagra, Mysore -570015, Karnataka.

ABSTRACT

In the present study, the ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* were screened for their antibacterial activity against gram positive organisms such as *Bacillus subtilis*, *Bacillus stearothermophilus*, *Micrococcus luteus* and gram negative organisms such as *Klebsiella pneumoniae*, *Pseudomonas cepacia and Salmonella typhi*. These extracts were also assessed for their antifungal activity against *Candida albicans*, *Saccharomyces cerevisiae*, *Trichosporon beigelii*. The antimicrobial activity of ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* were evaluated by cup plate method using different dilutions such as 10 mg/ml, 20 mg/ml and 30 mg/ml. These extracts showed maximum activity at 30mg/ml. Among the strains, the maximum zone of inhibition was noted against *Pseudomonas cepacia* (28.6mm) and *Bacillus stearothermophilus* (27.6mm) by *Artemisia abrotanum* and *Artemisia pallens* respectively. Both the plants extract showed the maximum antifungal activity against *Trichosporon* beigelii (17mm). Similarly *Artemisia abrotanum* was effective against *Saccharomyces cerevisiae (17mm)*. These results indicate that the ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* shown both antibacterial and antifungal activity.

KEY WORDS: *Artemisia abrotanum* (AA), *Artemisia pallens (*AP), Gram-positive, Gram-negative bacteria, fungal strains, MIC**.**

INTRODUCTION

Artemisia abrotanum commonly known as "Southern wood" traditionally considered as antiseptic, astringent, emmenagogue, expectorant, febrifuge, stomachic, stimulant, tonic, antiinflammatory, vermifuge and spasmolytic. It is used for treating upper respiratory tract diseases. *Artemisia pallens* commonly known as "Davana" has been traditionally used in Indian folk medicine for the treatment of diabetes mellitus, wound healing, immunomodulating, anthelmintic, antipyretic and wound healing[1,2]. Conventionally available synthetic antibacterial drugs are associated with undesirable side effects and resistance problem. There were no reports about the antibacterial and antifungal activity of these two medicinal plants. Hence, in the present study the ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* were screened for their antibacterial and antifungal activities.

MATERIALS AND METHODS

Plant materials

The aerial parts of *Artemisia abrotanum* were collected from Cinchona village, Ootacamund, The Nilgiris, The plant species was identified by Dr. Suresh Baburaj, Survey of Medicinal Plants and Collection Unit, Ootacamund, Tamilnadu, India. Further, the plant materials were dried under shade and after optimum drying, coarsely powdered and stored in well closed container till further use.

Method of extraction

The coarsely powdered aerial part of *Artemisia abrotanum* and the aerial parts of *Artemisia pallens* were extracted with 95% ethanol by cold maceration and the marc was again extracted with ethanol. The process was repeated four times and the filtrates were combined, distilled and evaporated.

Chemicals and media:

Dimethyl sulfoxide (DMSO) was purchased from Ranbaxy laboratories Ltd., Mohali. Nutrient agar, Nutrient broth, Sabourad's Dextrose agar and Sabourad's Dextrose broth were obtained from Hi-Media Pvt. Ltd., Mumbai.

Microorganism:

The bacterial and fungal strains were used in the study. The test organisms were *Bacillus subtilis* (NCIM 2063), *Bacillus stearothermophilus* (NCIM 2235), *Micrococcus luteus (*NCIM 2103), *Klebsiella pneumonia* (NCIM 2057), *Pseudomonas cepacia* (NCIM 2106), *Salmonella typhi* (NCIM 2312), *Candida albicans* (NCIM 3471), *Saccharomyces cerevisiae*(NCIM 3193) and *Trichosporon beigelii(*NCIM 3326). The strains were procured from National Collection of Industrial Microorganisms (NCIM) and National Chemical Laboratory, Pune.

**Author for Correspondence*
Email: sureshjoghee@yahoo.com

Table 1: Antibacterial activity of the ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* by cup plate method

S.No	Organisms	Diameter of Zone of Inhibition in mm							
		Artemisia abrotanum Conc (mg/ml)				*Artemisia pallens* Conc (mg/ml			
		10	20	30	STD	10	20	30	STD
GRAM POSITIVE STRAINS									
1	*Bacillus subtilis*	00	00	00	15.0	00	00	00	15.0
2	*Bacillus stearothermophilus*	--	18.5	20.6	24.8	21.0	24.5	27.6	30.0
3	*Micrococcus luteus*	--	19.3	20.1	23.4	22.2	24.2	26.2	31.4
GRAM NEGATIVE STRAINS									
1	*Klebsiella pneumoniae*	--	12.3	14.4	20.4	21.1	24.3	27.4	32.3
2	*Pseudomonas cepacia*	24.6	26.6	28.6	31.4	10.1	17.3	19.6	24.8
3	*Salmonella typhi*	10.1	11.1	13.0	15.7	10.9	20.1	18.0	20.1

Preparation of micro organism

One loop-full of microorganism was inoculated into 100 ml of sterile medium and incubated for 24 h at 37º C for bacterial culture and for 48 h at 27º C for fungal culture. After 24 h / 48 h of incubation 1 ml of broth containing the microorganism was added into 9 ml of peptone water. Ten fold serial dilutions were made in the range of 10^{-1} to 10^{-8}. 100 µl of the dilutions ranging from 10^{-5} to 10^{-8} were spread on the sterile nutrient agar/SDA plates and kept at 37º C or 27º C for 24 h / 48 h. The numbers of colony forming units were counted and numbers of microorganisms in each ml of stock culture were calculated.[3, 4]

Standard drugs

Penicillin (1mg/ml) diluted with dimethylsulphoxide was used as standard antibacterial drug. Amphotericin - B (500 µl/) in sterile water was used as standard antifungal drug**.** Dimethylsulphoxide was used as a control for the study.

Screening of antibacterial and antifungal activity by cup plate method

The antibacterial and antifungal activity of ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* were evaluated by cup plate method using different dilutions viz., 10 mg/ml, 20 mg/ml and 30 mg/ml. Sterilized nutrient agar plates were prepared under aseptic conditions. Six mm diameter holes were made in the agar plates using a sterile borer. 0.1 ml of the test organisms was spreaded on agar plates. Samples, standard drug and the solvent control (DMSO) were added into each hole separately. The plates were maintained at +4º C for 1 h to allow the diffusion of solution into the agar medium. The plates were incubated at 37ºC for 24 h for bacteria and 28ºC for 48h for fungi. The zone of inhibition was measured using antibiotic zone reader.[5, 6, and 7]

Determination of Minimum Inhibitory Concentration:

Minimum Inhibitory Concentration was determined by two-fold serial dilution method. A series of test tubes were prepared containing the same volume of media inoculated with the test organism (the inoculums may vary from 10^3 to 10^6 cells per milliliter). Drug was added to the tubes in a stepwise dilution by a factor of 2 (two fold serial dilution) that is if the concentration of drug in the first tube is 500 mg/ml, in the second tube it will be 250 mg/ml and in the third 125 mg/ml and so on. Cultures were incubated at 24 h for bacteria at 37ºC and 48 h for fungi at 27ºC. One tube was left without drug to serve as a positive control for the growth of the organism. Tubes are inspected visually to determine the growth of the organism indicated by turbidity.[7]

RESULTS AND DISCUSSION

The ethanolic extract of AA was found to be effective against various bacteria as indicated by the zone of inhibition. Maximum inhibition was obtained against *Pseudomonas cepacia* (28.6 mm) followed by *Klebsiella pneumoniae* (26.4 mm), *Micrococcus luteus* (25.4 mm), *Salmonella typhi* (22.0 mm) and *Bacillus*

Table2: Antifungal activity of the ethanolic extract of *Artemisia abrotanum* and *Artemisia pallens* by cup plate method:

Sl.No	Organisms	Diameter of Zone of Inhibition in mm							
		Artemisia abrotanum Conc (mg/ml)				*Artemisia pallens* Conc (mg/ml)			
		10	20	30	STD	10	20	30	STD
1	*Candida albicans*	05	15	16	19	00	05	16	23
2	*Saccharomyces cerevisiae*	03	16	17	26	00	09	10	24
3	*Trichosporon beigelii*	03	15	17	21	03	16	17	26

Table 3: Antibacterial and antifungal activity of the *Artemisia abrotanum* and *Artemisia pallens* by two fold serial dilution method:

S.No	Organisms	MIC in µg/ml	
		AA	AP
	Gram positive strains		
1	*Bacillus stearothermophilus*	250	250
2	*Micrococcus luteus*	500	500
	Gram negative strains		
1	*Klebsiella pneumoniae*	250	500
2	*Pseudomonas cepacia*	500	125
3	*Salmonella typhi*	125	250
	Fungal strains		
1	*Candida albicans*	250	1000
2	*Saccharomyces cerevisiae*	125	500
3	*Trichosporon beigelii*	125	500

stearothermophilus (20.6 mm) at a concentration of 30 mg/ml.The ethanolic extract of AP was found to be effective against various bacteria as indicated by zone of inhibition. Maximum inhibition was obtained against *Bacillus stearothermophilus* (27.6 mm) followed by *Klebsiella pneumoniae* (27.4 mm), *Micrococcus luteus* (26.2 mm), *Salmonella typhi* (20.1 mm), *Pseudomonas cepacia* (19.6 mm) at a concentration of 30mg/ml. The results are shown in figure table 1.

The ethanolic extract of AA *was* found to be effective against various fungi as indicated by the zone of inhibition. Maximum inhibition was obtained against T. *beigelii* (17 mm) followed by *S. serevisiae* (17 mm) *and C. albicans* (16 mm). The ethanolic extract of AP was found to be effective against inhibiting the growth of various fungi as indicated by zone of inhibition. Maximum inhibition was obtained for *T. beigelii* (17 mm) followed by *C.albicans* (16 mm), *S. cerevisiae* (10mm). The results are shown in figure table 2.

The ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens* were found to be effective against all bacterial strains used in the study at a concentration of 10 to 30 mg/ml except *Bacillus subtilis*, which was found to be ineffective against the ethanolic extracts of *Artemisia abrotanum*. The ethanolic extracts (AA) showed maximum inhibition against *Pseudomonas cepacia* and the *Artemisia pallens* was found to be more effective against *Bacillus stearothermophilus*. And the MIC results showed that the *Salmonella typhii* has the minimum MIC value against AA and *Pseudomonas cepacia* against AP (Table 1, Table 3).

The ethanolic extract of *Artemisia abrotanum* and *Artemisia pallens* was found to be effective against all the fungal strains used in the study. Both AA and AP extracts were found to be effective for *Trichosporon beigelii*. AA was also effective against *Saccharomyces cerevisiae*, *Saccharomyces cervisiae* and *Trichosporon beigelii* has the minimum MIC against both AA and AP (Table 2, Table 3). This activity may be attributed to the presence of terpenoids and flavanoids present in both the plant species.

Human pathogenic microorganisms, phytopathogens, are prone to developing drug resistance to decrease substantially the effectiveness of those pesticides (Rosenberger and Meyer, 1981). There is an urgent need, therefore, to work towards the development of safer antimicrobial agents, that are expected to be renewable, non-petrochemical, naturally ecofriendly and easily obtainable.

As an evolutionary process, plants on which insects, microorganisms and mammals are feeding, usually acquire self defending capabilities by producing a variety of secondary metabolites such as alkaloids, terpenoids, steroids and aromatic compounds, which are presumably unpleasant or even toxic to the enemy. Inside the tissue of nearly all the healthy plants, there are a lot of microorganisms called “endophytes”. Endophytes are mutualistic to their host, at least some of them are thought to be making returns for the nutrition from the plant by producing special substances such as secondary metabolites to prevent the host from successful attack of fungi, pest and mammals. As a matter of fact, metabolites of endophytes were reported to inhibit a number of microorganisms (Fisher *et al*., 1984; Gurney *et al*., 1993).

The terpenoids may interfere with the electron transport chain or oxidative phosphorylation or dissolve the cytoplasmic membrane due to their lipophilic properties (Parcha *et al*., 2003). Triterpenoids, certain flavonoids with antimicrobial and antifungal activities and biocides with insecticidal activity, have been reported (Sharma *et al*., 1989). Antimicrobial activity of methanolic extracts of aerial parts of *Artemisia diffusa, A. oliveriana, A. scoparia and A. turanica* against *B. subtilis, S. aureus, E. coli, P. aeruginosa* and *C. albicans* has been reported (Ramezani *et al*., 2004). Also, two flavones isolated

from *Artemisia giraldii* have shown antibiotic activity against *S. aureus, S. lutea, E. coli, P. aeruginosa, Proteus sp., A. flavus* and *T. viride* (Zheng et al., (1996).

CONCLUSION

In the present study, both the plant extracts and essential oils showed significant antimicrobial activity against Gram +ve and Gram –ve bacteria and fungi by cup plate and two fold serial dilution techniques and this activity may be attributed to the presence of terpenoids and flavanoids present in both the plant species.

ACKNOWLEDGEMENT

The authors sincerely thank Dr. H.G. Shivakumar, Principal, J.S.S. College of Pharmacy, Mysore, for his support and encouragement. Our gratitude goes to J.S.S. University, Mysore, for providing all the necessary facilities.

REFERENCE

1. Bjork L, Hedner T, Remberg P, Sterner O. Ethno botanical use of southern wormwood, *Artemisia abrotanum* L. in Europe, A critical review of its therapeutic use from ancient to modern medicine. Ethnopharmacologia 2 002b.
2. Asolkar LV, Kakkar KK, Chakre OJ. Glossary of Indian medicinal plants with active principles. New Delhi: Publications & Information Directorate, CSIR, New Delhi, 1965 -1981; 92-97.
3. Qadrie Zl, Jacob B, Anandan R, Rajkapoor B, Rahamathulla M. Anti-bacterial activity of ethanolic extract of *Indoneesiella echioides* (l) Nees. Evaluated by the filter paper disc method. Pak J Pharm Sci 2009; 22(2):123-5.
4. Hugo WB, Russell AD. Pharmaceutical Microbiology. Edn 4, Oxford: Blackwell Scientific Publications, London, 1987, 265–68.
5. Yashphe J, Segal R, Breuer A, Erdreich-Naftali G. Antibacterial activity of *Artemisia herba-alba*. J Pharm Sci 1979; 68(7): 924-5.
6. Khan A, Rahman M, Islam S. Antibacterial, antifungal and cytotoxic activities of tuberous roots of *Amorphophallus campanulatus*. Turk J Biol., 2007; 31:167-172.
7. Manisha V, Neha S, Satish S. Antimicrobial Activity of Stem Bark Extracts of *Nyctanthes arbortristis* Linn. (Oleaceae). IJPPR, 2009; 1(1):12-14.
8. Parcha V, Gahlot M, Bajaj BK. Antimicrobial activity of essential oil of Dysoxylum malbaricum. Indian. Perfum. 2003; 47(2): 183-187.
9. Sharma OP,Sharma PD.Chemical composition of fruits and stem essential oils of *Lantana camara*. J.Sci.Ind. 1989; 48:471-475.
10. Ramezani M, Fazli-Bazzaz BS, Saghafi- Khadem F, Dabaghian A. Antimicrobial activity of four Artemisia species of Iran. Fitoterapia.2004; 75: 201-203.
11. Zheng WF, Tan RX, Yang L, Liu ZL. Two flavones from *Artemisia giraldii* and their antimicrobial activity. Planta Med. 1996; 62(2):160-2.
12. Rosenberger DA, Meyer CW. Post harvest fungicides for apples: development of resistence to benomyl, vinclozolin and iprodione. Plant. Dis.1981; 65, 1010—1013.
13. Fisher PJ, Anson AE, Petrini O. Antibiotic activity of some endophytic fungi from ericaceous plants. Bot. Helv.1984; 94: 249—253.
14. Gurney K A, Mantle PG. Biosynthesis of 1-N-Methyl-albonourism by an endophytic Streptomycin sp. Isolated from perennial ryegrass. J.Nat.Prod.1993; 56: 1194-1198.