EVALUATION OF ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACTS OF WHOLE PLANT OF *Borreria hispida* (Linn)

A.Kottai Muthu, Penugonda sravanthi , D.Satheesh Kumar, A. Anton smith, R. Manavalan

Department of pharmacy, Annamalai University, Annamalai Nagar-608 002, India

ABSTRACT:

The objective of this study was to evaluate the antibacterial activity of various extracts of whole plant of *Borreria hispida* (Linn). The extract was prepared from the whole plant of *Borreria hispida* by hot continuous percolation method in Soxhlet apparatus with various solvents (Pet. Ether, ethyl acetate, Methanol). All the extracts of *Borreria hispida* were tested for antibacterial efficacy against *Bacillus subtilis* NCIM 2063, *Bacillus pumilus* NCIM 2327, *Staphylococcus aureus* NCIM 2079, *Pseudomonas aeruginosa* NCIM 2036, *Escheria coli* NCIM 2065, *Klebsiella pneumonia* NCIM 2957. All the three extracts were comparable with standard drug (Ciprofloxacin). The methanolic extract was found to be the most effective anti bacterial activity against the entire organism tested. The minimum Inhibitory concentration of methanolic extract of *Borreria hispida* was found to the range 250 mcg/ml to 50 mg/ml on tested all the test organisms. This study scientifically supports the usage of whole plant as a remedy for various superficial bacterial and fungal infections in traditional medicine.

Key words: Whole plant of *Borreria hispida*, antibacterial activity, hot continuous percolation.

INTRODUCTION

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection[1,2,3,4]. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections[5].

The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistance microorganisms[6]. More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal and antimicrobial activities, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies.[7, 8]

Santo et al, [9] repoted that the World Health Organization has indeed recognized medicinal plants as the best source for obtaining a variety of synthetic drugs. No doubt, some studies have identified and isolated the main active ingredients in the plants responsible for this antimicrobial activity [10, 11]. However, the study on medicinal plants will allow for the demonstration of their physiological activity and also catalyze many pharmacological studies that will lead to the development of more toxicity and high sensitivity especially towards the emerging microbial agents. [12]

Borreria hispida is belongs to the family Rubiaceae. It is widely distributed in throughout India, up to 900m in hills and on all dry lands as a weed. The seed of *Borreria hispida* is used as PPAR-alpha gene

expression, antioxidant redox status, protein metabolism in STZ diabetic rats .Potential role of *Borreria hispida* in ameliorating cardiovascular risk factor[13]. The literature survey showed that no study has been done on antibacterial activity of *Borreria hispida.* Therefore, we were interested in studying the antibacterial activities of the various extracts of whole plant of *Borreria hispida* (Linn).

MATERIAL AND METHODS

Plant materials

The whole plant of *Borreria hispida* (Linn), were collected from Naaserath, Tuticorin District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Borreria hispida* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[14] for 24 hrs. The mark was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Micro Organisms Used

The following bacterial strains were obtained from National Chemical Laboratory, Pune, India, and used to study the antibacterial activity of various extracts of whole plant of *Borreria hispida* (Linn) [15].

Bacilllus subtilis NCIM 2063, *Bacillus Pumilus* NCIM 2327, *Stapylicoccus aureus* NCIM 2079, *Pseusomonas aeruginosa* NCIM 2036, *Escherichia coli* NCIM 2065, *Klebsiella pneumonia* NCIM 2957.

Evaluation of Antibacterial Activity:

Filter paper disc diffusion method

The test solutions of variuos extracts were prepared by using sterile dimethyl formamide as solvent. Ciprofloxacin (100mcg/ml) was taken as the standards for antibacterial activity.

Antimicrobial activity was tested by using the filter paper disc diffusion method[16], employing 24 hours cultures of the above mentioned organisms. The test organism were seeded into sterile nutrient agar medium by uniformly mixing one ml of inoculum with 20 ml sterile melted nutrient agar cooled to 48-50^{0}c in a sterile petridish. The medium was allowed to solidify.

All the three extracts (test) and standard drugs as well as blank were impregnated in whatmann filter paper disc and placed on solidified medium in the petridish and the petridishes were left undisturbed for two hours at room temperature. The petridishes were then incubated at 37^{0}C for 24 hours and the zone of inhibition was measured.

Minimum Inhibitory (mm) concentration

The methanolic extract exhibited maximum antibacterial activity when compared with other two extracts were further tested against all the organisms for evaluation of its antibacterial efficiency at different concentration (250 mcg/ml, 500 mcg/ml, 1 mg/ml, 10 mg/ml, 50 mg/ml) by using the filter paper disc diffusion method. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the filter paper disc.

RESULT AND DISCUSSION

The antibacterial activity of the various extracts of whole plant of *Borreria hispida* (L) was studied against gram positive and gram negative bacteria shown in Table 1. The methanolic extract of *Borreria hispida* was found maximum antibacterial activity than that of other two extracts like petroleum ether and ethyl acetate extracts. The minimum inhibitory concentration was determined by using methanolic extract of *Borreria hispida*. The minimum inhibitory concentration obtained with different concentration of the methanolic extract and the standard drug are presented in table 2.The minimum inhibitory concentration of the methanolic extract of *Borreria hispida* range from 250 mcg/ml to 50 mg/ml on tested bacteria. Significant antibacterial activity was found with the methanolic extract at a concentration of 250mcg/ml as compared with standard drug ciprofloxacin (100 mcg/ml).

Table 1: Antimicrobial activity of different extracts of *Borreria hispida (Linn)*

Test Micro organisms	**Zone of Inhibition (mm)**				
	Pet.ether extract (100mg/ml)	**Ethyl acetate extract (100mg/ml)**	**Methanol extract (100mg/ml)**	**Ciprofloxacin standard (100mcg/ml)**	**Blank DMF**
Bacillus subtilis	7±0.5	9±0.5	15±1	19±0.5	0
Bacillus pumilus	7±1	10±1	16±0.5	21±1	0
Staphylococcus aureus	6±0.5	9±1	15±0.5	19±0.5	0
Pseudomonas aeruginosa	7±1	9±0.5	16±1	19±1	0
Escherichia coli	7±1	10±1	18±1	20±0.5	0
Klebsiella pneumoniae	6±0.5	10±0.5	17±1	19±1	0

* Zone are mean ± SD for n =3

- No zone of inhibition

Table 2: Minimum inhibitory concentration value of Methanolic extract of *Borreria hispida (Linn)* on the selected microorganisms

Test Micro organisms	**Zone of Inhibition (mm)**					
	250 mcg/ml	**500 mcg/ml**	**1 mg/ml**	**10 mg/ml**	**50 mg/ml**	**Standard***
Bacillus subtilis	8	9	10	12	14	19
Bacillus pumilus	6	7	9	11	13	21
Staphylococ cus aureus	7	8	9	12	14	19
Pseudomonas aeruginosa	8	9	10	12	14	20
Escherichia coli	7	10	11	13	16	19
Klebsiella pneumoniae	6	9	11	12	15	20

* Standard for Antibacterial-ciprofloxacin

The active principles of Borreria hispida are responsible for antibacterial activity. Hence it can be concluded that the methanolic extract of *Borreria hispida* possess a powerful antibacterial action against all the bacteria were tested. This also stands as a scientific support for the usage of this plant for treating fever and in traditional medicine.

CONCLUSION

The results of the above study revealed that the methanolic extract of *Borreria hispida* was exhibit antibacterial activity which might be helpful in preventing the progress of various diseases and can be used in alternative system of medicine. Further, these findings could be used to develop suitable dosage forms such as cream, ointment, and lotion as per the requirement of the treatment.

REFERENCES

1. Graybill, J. R. (1988). Systemic fungal infections: diagnosis and treatment. I. Therapeutic agents. Infectious Disease Clinics of North America. 805-825.
2. Ng, P. C. (1994). Systemic fungal infections in neonates. Arch Dis of Childhood. 71: F130-F135.
3. Dean, D. A. and Burchard, K. W. (1996). Fungal infection in surgical patients. Am J Surg. 171: 374-382.
4. Gonzalez, C. E., Venzon, D., Lee, S., Mueller, B. U., Pizzo, P. A and Walsh, T. J. (1996). Risk factors for fungemia in children infected with human immunodeficiency virus: a case-control study. Clin Infect Dis. **23:** 515-521.
5. Sieradzki K, Wu SW and Tomasz A. (1999). Inactivation of the methicillin resistance gene mecA in vancomycin-resistant *Staphylococcus aureus*. Micro. Drug Resist. **5(4)**: 253-257.
6. Davis, P. H. Flora of Turkey and East Eagean Island, Edinburg. Edinburg University Press. 1982; 7: 947.

7. Reynolds JEF, Martindale-the extra pharmacopeia (31st ed), Royal Pharmaceutical Society of Great Britain, London 1996.
8. Lis-Balchin M and Deans SG. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. Journal of Applied Bacteriology, 1997; 82: 759-762.
9. Santos, P.R.V., Oliveria, A.C.X; Tomassini,T.C.B. Controle microbiogico de products fitoterapicos. Rev. Farm. Bioquim. 1995; 31:35-38.
10. Oloke JK, Kolawo BO, Erhun WO. The antibacterial and antifungal activities of certain compounds of *Aframomum malegueta* Fitoterapia 1988; 59: 384-8.
11. Carson CF, Riley TV. Antimicrobial activity of the major components of The essential oil of *Melaleuca alternifolia.* J. Appl. Bacteriol 1995;76:264-9.
12. Fabricant DS, Famsworth NR: The value of plant used in traditional medicine for drug discovery. Environmental Health Perspectives Supplements, 2001; 109: 69-75.
13. Vasanthi, Hannah R PhD; Mukherjee, Subhendu MS; Lekli, Istvan MFarm; Ray, Diptarka MS; Veeraraghavan, Gayathri PhD; Das, Dipak K PhD. Potential Role of Borreria hispida in Ameliorating Cardiovascular Risk Factors. Journal of Cardiovascular Pharmacology:2009. 53 ; 6: 499-506
14. Harborne J.B. (1984) Phytochemical methods 11 Edn. In Chapman &, Hall. New York: 4-5.
15. S. Umadevi, G.P. Mohanth, V. Chelladurai, P.K. Manna, R. Manavalan, Antimicrobial and Antifungal activity of Andrographics ectiodes Jour. Of Nat. Rem. Vol. 3 / 2 2003 Pageno 185 – 188.
16. B.P. Saha, Kalali Saha, Pulak K.Mukherjee, Subash C.Mandal and M.Pal, Antibacterial activity of Leucas lavandulaefolia Rees, Indian Drugs 1995; 32(8), 402-404.