INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

In Vitro Antibacterial Potential of *Boerhaavia diffusa*

Y L Ramachandra[1*], C. Ashajyothi[1] and S. Padmalatha Rai[2]

[1]Department of P.G. Studies and Research in Biotechnology, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Karnataka, India.

[2]Division of Biotechnology, Manipal Life Sciences Center, Manipal University, Manipal, Karnataka, India.

ABSTRACT

The present study aimed at evaluating the *in vitro* antibacterial activity of petroleum ether, chloroform and methanol crude extracts of aerial and root parts from *Boerhaavia diffusa* plant against six microorganisms like, *E.coli* ATCC 69314, *K. pneumoniae* NCIM 2719, *P. aeruginosa* NCIM 2200, *A. tumefaciens* NCIM 2943, *S. aureus* NCIM 2080 and *B. subtilis* MTCC 441, Which includes Gram-negative and Gram-positive bacteria, by using agar well plate method. Among the three extracts methanol crude extract of aerial part of plant exhibited strong antibacterial activity compared to petroleum ether extract and chloroform extract. The zone of inhibition is varying between 13.99 ± 0.22mm to 19.46 ± 0.26mm. The most susceptible bacteria were E.coli (7.76 ± 0.43mm). Methanol extract of root showed maximum activity against *S. aureus* (17.49+0.42mm), *P. aeruginosa* (15.61 ± 0.42 mm) and *K. pneumoniae* (15.22 ± 0.40mm). All the three extracts of root showed less activity against *E. coli*.

Keywords: Antibacterial activities; Agar well diffusion; Aerial parts extract; Root extract.

INTRODUCTION

Medicinal plants are the local heritage with global importance. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with the body's own defense. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians[1].

The Ayurvedic medicine predominantly uses plant drugs, and therefore, it can be defined as the phytotherapy comprehended in a very sophisticated way. One of the most typical exemplary plant of the Ayurvedic medicine is *Boerhaavia diffusa* Linn[2]. *Boerhaavia diffusa* (Spreading Hogweed in English), belonging to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (known also under its traditional name as *Punarnava*) and of Brazil (known as *Erva tostão*). *B. diffusa* is up to 1m long or more, having spreading branches. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes. The leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous above and usually white underneath. The shape of the leaves varies considerably ovate - oblong, round, or subcordate at the base and smooth above. The genus *Boerhaavia* has several species, and is distributed in the tropical, subtropical, and temperate regions of the world[3]. It is found in Australia, China, Egypt, Pakistan, Sudan, Sri Lanka, South Africa, USA and in several countries of the Middle East. The root, leaves, aerial parts or the whole plant of *B. diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine (daily used by millions of people in India, Nepal, Sri Lanka and indirectly through it being the major influence on Unani, Chinese and Tibetan medicines). The root is mainly used to treat gonorrhea, internal inflammation of all kinds, dyspepsia, edema, jaundice, menstrual disorders, anaemia, liver, gall bladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers[4], then as a diuretic documented in Indian Pharmacopoeia[5], digestive aid, laxative and a menstrual promoter. The root powder, when mixed with *mamira* (*Thalictrum*

foliolosum), is used to treat eye diseases. It cures corneal ulcers and night blindness[6], and helps restore virility in men. People in tribal areas use it to hasten child birth[7]. The juice of *B. diffusa* leaves serves as a lotion in ophthalmia. It is also administered orally as a blood purifier and to relieve muscular pain[8].

The present study was undertaken to investigate antibacterial activity of *B. diffusa* against some enteric pathogens. The crude extracts of aerial and root parts of plant were tested for the potential antibacterial property.

METHOD

Plant material and preparation of the extract

The leaves stem bark and roots of *B. diffusa* were collected from in and around the Davanagere, Shivamogga and Kuvempu University campus. The fresh plant materials were shade dried, powdered mechanically (sieve no.10/44). 200g of powdered material was soaked in 100ml of petroleum ether, chloroform and methanol separately for 48 h. It was filtered by using Whatman no.1 filter paper. The solvent was distilled out completely from the filtrate under the reduction pressure in Rota vapour.

Phytochemical screening

The preliminary phytochemical analysis of petroleum ether, chloroform and methanol extracts was carried out using the methods as described in[9-12].

Test organisms

The standard bacterial strains were obtained from the Department of Microbiology, P.G. Center, Kuvempu University, Tolahunase, Davangere, and Karnataka, India. Four strains of Gram-negative bacteria *Escherichia coli* ATCC 69314, *Klebsiella pneumoniae* NCIM 2719, *Pseudomonas aeruginosa* NCIM 2200 and *Agrobacterium tumefaciens* NCIM 2943 and two strains of Gram-positive bacteria *Staphylococcus aureus* NCIM 2080 and *Bacillus subtilis* MTCC 441 were used. The organisms were maintained on nutrient agar slants at 4°C and subculture in to nutrient broth by a picking off technique for 24 hours before use[13].

Preparation of culture medium and inoculation

Nutrient agar (Hi Media, India) was used as the bacteriological medium. The media were sterilized by autoclaving at 120 °C for 20 minutes. Under aseptic conditions, in the laminar air flow, 15 ml of culture medium was dispensed into pre-sterilized petridishes to yield a uniform depth of 4 mm. After solidification of the medium, the microbial cultures were inoculated by spread plating technique.

Antibacterial assay

The extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100mg/ml. Pure DMSO was taken as the negative control and Ciprofloxacin 30mg/ml as the positive control, supplied by Cipla Ltd. Wells were prepared in the agar plates using a sterile cork borer of 6.0mm diameter. The 30µl, 40µl and 50µl of the petroleum ether, chloroform, methanol crude extracts and of aerial and root parts of *B. diffusa* whole plants and 30µl control compound was introduced into the well. The plates were incubated aerobically at 35°C and examined after 24 hours. The plates were examined for microbial growth inhibition and the Inhibition Zone Diameter (IZD) measured to the nearest millimeter and compared with those produced by the commercial antibiotic Ciprofloxacin.

Statistical analysis

The results of the experiment are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple pair wise comparison tests to assess the statistical significance.

RESULTS

All the extracts were subjected to preliminary qualitative phytochemical evaluation. The phytochemical profiles of various solvent extracts from plant used in this study are presented in Table 1. The analysis revealed the presence of alkaloids, phenol, glycosides, steroids, carboxylic acid, reducing sugar, flavonoids, saponins, tannins, proteins, triterpenoids, quinines, carbohydrates and sterols. In particular, methanol extract of aerial and root parts of plant showed positive for most of the secondary metabolites except triterpenoids, quinines.

Antibacterial activity of different extracts from aerial parts of *B. diffusa*

In the present investigation, the antibacterial activity of the Petroleum ether, chloroform and Methanol crude extracts from aerial parts of *B. diffusa* plant were determined against six microorganisms. The result revealed that petroleum ether and methanol extracts except the chloroform extract showed significant zone of inhibition against bacteria like *S. aureus* (16.83 ± 0.12mm, 19.46 ± 0.26mm), *P. aeruginosa* (14.91 ± 0.48mm, 18.3 2± 0.29mm), *K. pneumoniae* (12.57 ± 0.30mm, 16.91 ±. 0.27mm) and *B. subtilis* (12.05 ± 0.39mm, 14.95 ± 0.43mm) respectively. The moderate activity was shown against *A. tumefaciens* (11.96 ± 0.51mm) and minimal inhibitory concentrations of the extract were determined against *E. coli* (08.2 ± 0.25mm). The chloroform extract showed significant zone of inhibition against *S. aureus* (13.99 ± 0.38mm) and *B. subtilis* (12.57 ± 0.40 mm). The moderate activity was shown against *A. tumefaciens* (12.56 ± 0.29mm) and minimal inhibitory concentrations of

the extract were determined against *E. coli* (7.76 ± 0.43mm).

Antibacterial activity of different extracts from root of *B. diffusa*

The result revealed that methanol extract of root showed maximum activity against *S. aureus* (17.49 ± 0.42mm), *P. aeruginosa* (15.61 ± 0.42 mm) and *K. pneumoniae* (15.22 ± 0.40mm), compared to methanol extract, the Petroleum ether and chloroform extracts showed moderate activity against *B. subtilis* (11.53 ± 0.46mm, 9.11 ± 0.40mm) and *A. tumefaciens* (9.31 ± 0.21mm, 8.15±0.44mm) respectively. All the three extracts showed less activity against *E. coli* (7.89±0.27mm, 5.95±0.06mm, 9.17±0.17mm) respectively. The data is presented in Table 2.

DISCUSSION

Numerous naturally occurring antimicrobials are present in animal and plant tissues (14) where they probably evolved as part of the defense mechanisms of the host against microbial invasion. There are many plants that demonstrate antimicrobial activity (15) and these plants have found application in the food industry as antibacterial and antifungal agents (16). Extracts of leaves of *Boerhaavia diffusa, Alchornea cordifolia*, and *Bridellia micrantha* were investigated for antibacterial activity against *Helicobacter pylori*, *Salmonella typhi*, *Salmonella enteritidis*, *Shigella* (17). Plant extracts of *Boerhaavia diffusa, Ocimum sanctum, Jatropha gossypifolia, Azadirachta indica, Solidago virgaurea*, and *Commelina benghalensis* were used to investigate antibacterial activity against six bacterial strains *Pseudomonas testosteroni, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus subtilis, Proteus morganii, Micrococcus flavus*.

Abo and Ashidi reported antimicrobial potential of *B. diffusa, Bridelia micrantha, Alchornea cordifolia* and sourced from traditional healers through an ethnobotanical survey of anti-infective plants in Egbado South in Ogun State, Nigeria (18). On the basis of the report, Abo and Ashidi (18) in the present study pet ether, chloroform and methanol crude extracts of aerial parts, root of *B. diffusa* were tested against both Gram positive and Gram negative bacteria by agar well method. The result revealed that, the zone of inhibition of Petroleum ether extract (16.83 ± 0.12mm), chloroform (13.99 ± 0.22mm), methanol extract (19.46 ± 0.26mm), the zone of inhibition is varying between 13.99 ± 0.22mm to 19.46 ± 0.26mm. These data indicated that methanol crude extract of aerial part of plant exhibited strong antibacterial activity compared to petroleum ether extract. The chloroform extract of both aerial and root extract shown relatively less antibacterial activity.

CONCLUSION

In the present study the antibacterial activity of *B. diffusa* may be attributed to individual or synergistic effect of phytoconsitituents present in it. The petroleum ether and methanol extracts of aerial parts and root of *B. diffusa* whole plant exhibited significant wide spectrum of antibacterial activity against both Gram's positive and Gram's negative bacteria. The present results will form the basis for selection of plant species for further studies which aimed at the isolation and structural elucidation of antibacterial active constituents from the plant has been initiated.

Table 1: Table shows phytochemical detection of bioactive components in *B. diffusa*

TEST	Aerial parts			Root		
	Pet. Ether Extract	Chloroform Extract	Methanol Extract	Pet. Ether Extract	Chloroform Extract	Methanol Extract
Alkaloids	+	-	+	+	-	+
Phenols	+	-	+	+	-	+
Glycosides	+	+	+	-	+	+
Steroids	-	-	+	-	-	+
Carboxylic acids	-	+	+	+	+	+
Reducing sugars	+	-	+	+	-	+
Triterpenoids	-	-	-	-	-	-
Flavonoids	+	-	+	+	-	+
Saponins	+	-	+	+	-	+
Tannins	-	-	+	-	+	+
Proteins	-	+	+	-	+	+
Carbohydrates	+	+	+	+	-	+
Quinines	-	-	-	-	-	-
Sterols	-	-	+	-	-	+

Table 2: Table shows antibacterial activity of petroleum ether, chloroform and methanol extracts of *B. diffusa*

Extracts ↓	Bacterial Strains →	*S. aureus* NCIM 2080	*P. aeruginosa* NCIM 2200	*K. pneumoniae* NCIM 2719	*B. subtilis* MTCC 441	*A. tumefaciens* NCIM 2943	*E. coli* ATCC 69314
Petroleum ether	Aerial parts	16.83 ± 0.12	14.91 ± 0.48	12.57 ± 0.30	12.05 ± 0.39	9.40 ± 0.26	7.75 ± 0.30
	Root	10.76 ± 0.44	9.01 ± 0.31	11.25 ± 0.47	9.22 ± 0.27	9.76 ± 0.25	7.89 ± 0.27
	Ciprofloxacin (50µg)	17.95 ± 0.37	19.01 ± 0.25	18.79 ± 0.86	20.31 ± 0.33	16.80 ± 0.78	4.49 ± 0.32
Chloroform	Aerial parts	13.99 ± 0.38	12.56 ± 0.29	10.82 ± 0.50	12.56±0.40	8.85 ± 0.22	7.76 ± 0.43
	Root	9.12 ± 0.38	9.11 ± 0.46	11.01 ± 0.33	9.11 ± 0.40	8.15 ± 0.44	5.95 ± 0.06
	Ciprofloxacin (50µg)	17.95 ± 0.3	19.01 ± 0.25	18.79 ± 0.86	20.31 ± 0.33	16.80 ± 0.78	15.89 ± 0.18
Methanol	Aerial parts	19.46 ± 0.26	18.32 ± 0.29	16.91 ± 0.27	14.95 ± 0.43	11.96 ± 0.51	8.21 ± 0.25
	Root	17.49 ± 0.42	15.61± 0.42	15.22 ± 0.40	13.91 ± 0.31	12.02 ± 0.54	9.17 ± 0.17
	Ciprofloxacin (50µg)	17.95 ± 0.37	19.01 ± 0.25	18.79 ± 0.86	20.31 ± 0.33	16.80 ± 0.78	15.89 ± 0.18

The values are the mean of three experiments ± S.E.
*p< 0.001 vs. Standard antibiotic (Tukey's pairwise comparison test)

REFERENCES

1. Chapter in a book: Samuelsson G. Drugs of natural origin: a textbook of pharmacognosy. 4th ed. Stockholm, Swedish Pharmaceutical Press; 1999
2. Lad V. The complete Book of Ayurvedic Home Remedies. New York: Three Rivers Press; 1999
3. Heywood VH. Flowering Plants of the World. Oxford University Press, London, UK 1978; 69–70.
4. Kirtikar KR and Basu BD. Indian Medicinal Plants. Vol. III. 2nd ed. Lalit Mohan Basu, Allahabad, Uttar Pradesh, India. 1956;2045–2048.
5. Chopra GL. Angiosperms. Systematic and Life Cycle S Nagin & Co; Jalandhar, Punjab, India 1969;361–365.
6. Gupta RBL, Singh S and Dayal Y. Effect of *punarnava* on the visual acuity and refractive errors. Indian Journal of Medical Research. 1962;50:428–434.
7. Shah GL, Yadav SS and Badinath V. Medicinal plants from Dahana Forest. Journal of Economic and Taxonomic Botany 1983; 141
8. CSIR. The Wealth of India: Raw Materials Vol. VII B. CSIR, New Delhi, India 1988; pp174.
9. Harborne JJ. Phytochemical Methods: A guide to modern techniques of plant analysis, 2nd ed,Chapman and Hall, New York; 1984; pp 85
10. Trease GE, Evans WC. Pharmacognosy 13th Ed, ELBS Publication, Delhi; 1989; pp171.
11. Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy ,23rd ed, Nirali Prakashan, Pune. 1998; 106-114
12. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments, 13th ed, 2005;Nirali
13. Aneja KR. Experiments in Microbiology, Plant Pathology and Biotechnology, 4th ed, New Age International Ltd, New Delhi, India; 2003;196-197.
14. Smid EJ and Gorris LGM. Natural antimicrobials for food preservation. In Handbook of Food Preservation, Marker Dekker Inc., New York, 1999; 285 -308.
15. Beales N. Food ingredients as Natural Antimicrobials, CCFRA Review No 31, Campden & Chorleywood Food Research Association, United Kingdom; 2002
16. Lanciotti R, Gianotti A, Patrignani N, Belleti N, Guerzoni NE and Gardini F. Use of natural aroma compounds to improve shelf life and safety of minimally processed fruits. Trends Food Sci. Technol, 2004;15:201-208.
17. Adeleye Adeyemi A, Ezekiel Omonigbehin, Smith Stella, Odusola Oluwatosin, and Sobande Jumoke. Antibacterial activity of extracts of *Alchornea cordifolia* (Schum and Thonn) Mull. Arg., *Boerhaavia diffusa* (L)and *Bridellia micranthaI* (Hoscht) Baill, used in traditional medicine in Nigeria on *Helicobacter pylori* and four diarrhoeagenic bacterial pathogens. Journal of Biotechnology. 2008;720; 3761–3764.
18. Abo KA and Ashidi JS. Antimicrobial screening of *Bridelia micrantha, Alchornea cordifolia* and *Boerhavia difussa*. African Journal of Medicine and Medical Sciences 1999;28(3-4):167-169.