Research Article
CODEN (USA): IJPLCP

[Das, 3(10): Oct., 2012]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Antimicrobial activity study of ethanolic extract of *Boerhaavia diffusa* whole plant

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Abstract

The search for antimicrobial agents has continued to be concentrated on the lower plants, fungi and bacteria. Less research has focused on higher plants although identified plant compounds like emetine, berberine quinine still find specialized uses. Secondary metabolites of higher plants serve as defense agent against microorganisms. Some screenings have yielded good result and few of these have proceeded to give active antimicrobial compounds like polygodial, anethol, himejima, crytolepine, etc. The present work is carried out on the Boerhaavia diffusa whole plant on the basis of the literature obtained from the Ethno medicinal documentation. The common name is Punarnava and it is found wild throughout India mostly in Himalayan valleys. Keeping in view the tremendous ethno medicinal use of *Boerhaavia diffusa* it was aimed to scientifically validate the antimicrobial property of *Boerhaavia diffusa* whole plant.

Key-Words: Antimicrobial study, *Boerhaavia diffusa*, Minimum inhibitory concentration, Punarnava

Introduction

In developing countries, the use of traditional remedies is common practice and a large number of plants are used. In India, the most accessible health care provider is the traditional medical practitioner who has in his possession a quantity of effective herbal remedies[1]. Ethno medicine provides a source of information about these plants, the investigation of which reveals a reservoir of pharmacological substances with anti-inflammatory and antianginal properties[1,2]. India is rich in medicinal plants. The local inhabitants of eastern India mostly depend on plants for their survival and for treatment of diseases; this medical knowledge is stored in stored in the memory of herbal healers[3,4]. As per the literature, the medicinal plant *Boerhaavia diffusa* is used to treat different aliments like wound, inflammations and in hypertension[5]. Based on the wound healing and anti-diarrheal activity present study was carried out to evaluate both anti-bacterial and antifungal activity of aforementioned plant[6,7]. Since ethanol is the solvent, which brings out most of the compounds present in any plant material, the present investigation was carried out using ethanol extract of the root of the plant.

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Boerhaavia diffusa (Nyctaginaceae family) is an herbaceous perennial plant, native of India and Brazil, where it was used for centuries as a medicinal plant by indigenous populations[5]. The root of B. diffusa contains alkaloids (punarnavine), rotenoids (boeravinones A-F), flavonoids, amino acids, lignans (liriodendrons), ß-sitosterols and tetracosanoic, esacosanoic, stearic and ursolic acids[4,5]. The root of B. diffusa is used for the treatment of many diseases, such as liver disorders (jaundice, hepatitis, etc.), gastro-intestinal disorders (as laxative), renal disorders (for calculations, cystitis and nephritis), and for the treatment of anaemia and of menstrual syndrome the drug has recently been used as an adjuvant in an anticancer therapy[1,8]. Despite the wide therapeutically use of B. diffusa, there are still no scientific data in the literature which clearly (i) demonstrate the existence of an antimicrobial activity and (ii) explain the mechanism of its action. Since B. diffusa is largely used in Ayurveda medicine and the scientific data that explain such use are deficient, the purpose of our study was: (i) to evaluate the antimicrobial activity of the ethanolic extract of the whole part of *Boerhaavia diffusa*.

Material and Methods

Collection of plant material

The whole parts of *Boerhaavia diffusa* L. was obtained from Patna, India. The taxonomical identification was done by The Botanical Survey of India, Shibpur,

Howrah, West Bengal. The voucher specimens (CHN/26b/2012/Tech.II/709) are preserved at Department of Pharmaceutical Technology, NSHM Knowledge Campus for further studies.

Preparation of extract

The dried coarsely powdered plant (400g) was moistened with 25% ammonia solution and allowed to stand overnight. It was then exhaustively extracted with 95% ethanol using a soxhlet extractor. The extract was concentrated under controlled temperature to syrupy mass by vacuum distillation. The syrupy mass (30g) was treated with concentrated Hydrochloric acid. After shaking and filtering, the acidic filtrate was washed with portions of benzene, made basic (pH 10) with ammonia solution and then extracted with portions of chloroform. The combined chloroform extracts were washed with water, dried over anhydrous sodium sulphate and evaporated to yield the alkaloidal fraction (2g, 1% w/w).

Microorganisms used for antimicrobial study

The four micro-organisms namely *Escherichia coli* strains 871, 2142 and C1 (Gram negative bacteria), *Bacillus subtilis* UC564 (Gram-positive bacteria), *Staphylococcus aureus* 15, ML296 and ML329 (Gram positive bacteria), *Salmonella typhi* DI (Gram negative bacteria) have been used for the study of the anti-microbial activity of the ethanolic extract of the whole parts of *Boerhaavia diffusa*.

Determination of Zones of inhibition by Disc Diffusion method

Pure Ofloxacin as a standard antibiotic for comparison of the results was taken. Two sets of four dilutions each of fruit extract of *Boerhaavia diffusa* (250, 500, 1000, 2000 and 4000 µg/ml) and Ofloxacin (25, 50, 100 and 200 µg/ml) were prepared in sterile Mc Cartney bottles. The extracts and standard drugs were dissolved in DMSO. Sterile nutrient agar plates were prepared and incubated at 37 0C for 24 hours to check for any sort of contamination. Four sterile filter paper discs (Whatman No.1) of 6 mm diameter were soaked in four different dilutions of crude extract and placed in appropriate position on the surface of the flooded plate, marked as quadrants at the back of the petridishes. The petridishes were incubated at 37^0C for 24 hours and the diameters of zone of inhibition measured in mm. Similar procedure was adopted for the pure Ofloxacin and the corresponding zone of diameters was compared accordingly.

Results and Discussion

The results of determination of MIC values of the ethanolic extract of the whole plant of *Boerhaavia diffusa* have been tabulated in Table A. It is evident that the methanol extract is very active against the bacteria causing diarrhea at low concentrations. The results of zone of inhibition of the crude whole plant extract and its comparison with standard antibiotic Ofloxacin is recorded in Table B. The following results show that the extract possessed some antimicrobial activity against most of the tested organisms, depending upon the nature of the active ingredients present in the extracts and their capacity for diffusion into agar medium. Minimum antimicrobial inhibition activity concentration of the ethanolic extract of the whole part of *Boerhaavia diffusa* found to be more active using different concentrations (2000 and 4000 µg/ml) showed significant inhibitory activity against *Bacillus subtilis* UC564, *Staphylococcus aureus* strains, *Salmonella typhi* DI and *Escherichia coli* strains respectively which has been showed in Table A. The ethanolic extract of *Boerhaavia diffusa* shows significant antimicrobial activity at 2000 µg/ml and 4000 µg/ml. The zone of inhibition against E. coli871, 2142, C1 was found at higher dose, whereas the zone of inhibition against the other three experimented microbes viz. B. subtilis UC564, S. aureus 15 ML296, ML329, and S. typhi DI was found at 2000 µg/ml leading to an idea that this is the minimum inhibitory concentration of the drug.

Acknowledgement

The author is very much thankful to The Botanical Survey of India, Shibpur, Howrah, West Bengal for the identification of plants and also thankful to the Principal, Department of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata for providing the facility in the institute.

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Table A: Determination of MIC of the ethanolic extract of *Boerhaavia diffusa* against four major microorganisms

Name of the Bacteria	No. of strains	Growth in nutrient agar media containing different concentration of extract in µg/ml							
		* 0	50	100	250	500	1000	2000	4000
Escherichia coli	3	+	+	+	+	+	+	+-	-
Bacillus subtilis	1	+	+	+	+	+	+-	-	-
Staphylococcus aureus	3	+	+	+	+	+	+	-	-
Salmonella typhi	1	+	+	+	+	+	+-	-	-

* CONTROL, + GROWTH, - NO GROWTH

Table B: Determination of the ZOI (mm) produced by the ethanolic extract of the whole plant of *Boerhaavia diffusa* and its comparison with Ofloxacin

Name of the Bacteria	No. of strains	Ethanolic extract (µg/ml)				Ofloxacin (µg/ml)			
		500	1000	2000	4000	25	50	100	200
Escherichia coli	3	0	0	0	18.0	13.0	16.0	19.0	24.0
Bacillus subtilis	1	0	0	13.0	19.5	15.0	18.0	20.5	23.0
Staphylococcus aureus	3	0	0	10.5	16.0	14.5	16.0	19.5	25.5
Salmonella typhi	1	0	0	14.5	18.0	12.5	15.0	18.0	23.0

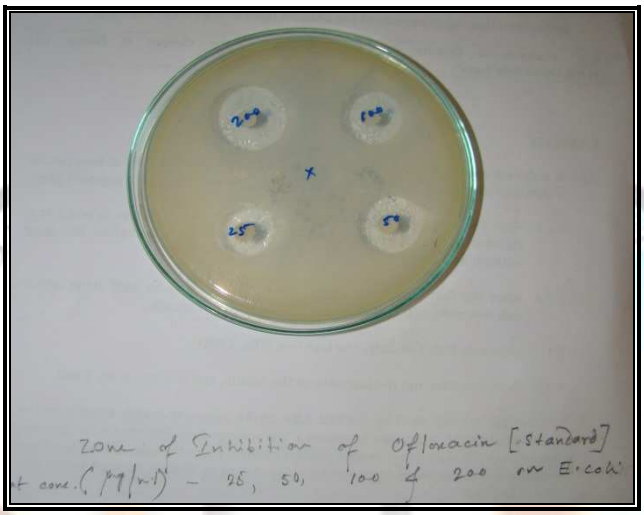

Fig. 1: Zone of inhibition of Ofloxacin (Standard) at conc. - 25, 50, 100 & 200 (µg/ml) on *Escherichia coli*

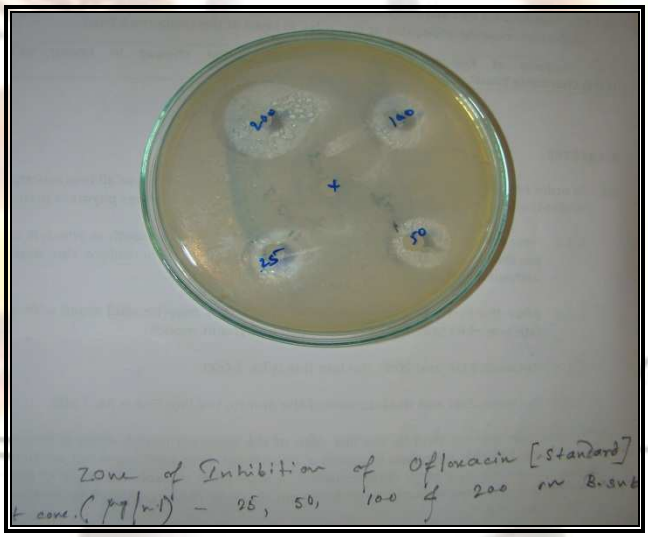

Fig. 2: Zone of inhibition of Ofloxacin (Standard) at conc. - 25, 50, 100 & 200 (µg/ml) on *Bacillus subtilis*