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PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *BOERHAAVIA DIFFUSA*

R. Kiruthika and P. Venkatalakshmi*

PG and Research Department of Biochemistry, S.T.E.T. Women's College, Sundarakkottai, Mannargudi -614016.

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***Corresponding Author**
P. Venkatalakshmi
PG and Research Department of Biochemistry, S.T.E.T. Women's College, Sundarakkottai, Mannargudi -614016.

ABSTRACT

Boerhaavia diffusa is one of the Rasayana herbs in Ayurveda. It has been used as a nephroprotective herb in the traditional system of medicine. In the present study steps have been taken to carry out preliminary phytochemical and physicochemical analyses of whole plant of *B.diffusa*. *In vitro* antibacterial and antifungal properties of the plant extract have also been evaluated.

KEYWORDS: Antibacterial, Antifungal, *Boerhaavia diffusa*, Phytochemical

INTRODUCTION

Plants with medicinal properties are the gift of the nature to mankind and are in use for centuries in the traditional systems of medicine. The associated health hazards of allopathic medicine can be minimized by the use of herbal medicine. India is one of the world's leading biodiversity centers which harbor innumerable plant species with medicinal values.

Boerhaavia diffusa is an herbaceous member of the family Nyctaginaceae. It is widely distributed in the tropics and subtropics. It is a perennial creeping weed, prostrate or ascending herb, up to 1m long or more having spreading branches. It has a long history of uses by indigenous and tribal people and in ayurveda. The root, leaves and aerial parts or the whole plant of *Boerhaavia diffusa* have been employed for the treatment of various disorders in the ayurvedic system of medicine. The root is mainly used to treat gonorrhea, internal inflammation, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder, and kidney disorders, abdominal pain, abdominal tumours and cancers.[1]

It cures corneal ulcers and night blindness and helps restore virility in men. People in tribal areas use it to hasten childbirth. The juice of *Boerhaavia diffusa* leaves serve as a lotion in ophthalmia. It is also administered orally as a blood purifier and to relieve muscular pain. It has been reported to have adaptogenic.[2] antioxidant,[3] hepatoprotective.[4] antiatherogenic,[5] immuno modulatory,[6] and nephro protective.[7] potentials. In the present study, attempts have been made to evaluate the antibacterial and antifungal activities of *Boerhaavia diffusa in vitro*.

MATERIALS AND METHODS

Collection and extraction of plant material

The whole plant of *Boerhaavia diffusa* was collected from Mannargudi, Thiruvarur (Dt), Tamil Nadu. The collected plant material was carefully identified with the regional flora. The plants were air dried under shade for 10-15 days. Then the dried material was ground to fine powder and stored in air tight bottles. The plant powder was used for further analyses. Ethanol and aqueous extracts were prepared according to the methodology of Indian pharmacopoeia.[8] The coarse powder was subjected to soxhlet extraction separately and successively with ethanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure, at controlled temperature (40°C- 50°C) and put in air tight container, stored in refrigerator till used.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis was carried out using the aqueous and ethanolic extracts as per the standard methods.[9]

Determination of physicochemical constants[10]

The procedures recommended in Indian Pharmacopoeia were followed for determining loss on drying at 110°C, total ash, acid insoluble ash, water soluble ash, and alcohol and ethanol extractive values.

In vitro Antimicrobial activity

Antibacterial activity

Preparation of Bacterial inoculums

Fresh microbial inoculum was prepared and used in the present study. The nutrient broth was prepared, poured into the tubes and sterilized. The pure microbial cultures were collected from the Microbiology department, S.T.E.T Women's College, Mannargudi and inoculated in

the tubes using inoculation needles or loops. The tubes were incubated at 37°C for 24 hours.

Preparation of nutrient agar medium

Beef extract - 3g
Peptone - 5g
NaCl - 5g
Agar - 5g
Distilled water - 1000ml

The above ingredients were weighed and poured into the conical flask with distilled water. Then pH of the medium was adjusted to 6.8 using a p^H meter by the addition of either acid or alkali. The flask was sterilized using auto clave at 121°C, 15 lbs pressure for 15 minutes and allowed to cool. This medium was used for the experiment.

Disc diffusion method[11]

The antibacterial activity of the extract was tested against the selected bacterial strains, the 20 ml of sterilized nutrient agar medium was poured into each sterile petriplate and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then 0.5cm discs were prepared and impregnated with test extracts of different concentrations, dried and slightly pressed in to the media. The plates were incubated at 37°C for 24 - 48 hours. After incubation period the results were observed and measured the diameter of inhibition zone around each disc.

Antifungal activity

Preparation of Fungal inoculums

The young microbial inoculum was prepared and used in the entire research period. The Potato Dextrose Agar (PDA) broth were prepared and poured into tubes and sterilized. The pure microbial cultures were collected from Microbiology Department, S.T.E.T women's College, Mannargudi and inoculated in the tube using inoculation needles or loops. The tubes were incubated at 37°C for 24 hours.

Preparation of potato dextrose agar medium

Potato infusion - 200g/l
Dextrose - 20g/l
Agar - 15g/l

Distilled water - 1000ml
PH - 6.8

200gm of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation 20g of dextrose was mixed with potato infusions 15g of agar was added as solidifying agent. These constituents were mixed and autoclaved and ued for the experiment.

Disc diffusion method[11]

In the freshly prepared and sterilized potato dextrose agar medium, a pinch of ampicillin was added and mixed well. Then 200ml of medium was poured into each petriplate and allowed to solidify. The test fungal culture was evenly spread over the appropriate media by using sterile cotton swab. Then 0.5cm discs were prepared and impregnated with test extracts of different concentrations, dried and slightly pressed in to the media. Then these plates were incubated at 27°C for 48-72 hours. After incubation period the results were observed and the diameter of incubation zones were measured around each well.

RESULTS AND DISCUSSION

Medicinal plants have been used in the treatment of various diseases from the time immemorial. The use of plants as the source of medicine lies deep in the history of mankind. Herbal drugs are considered to be forefather's gift to mankind.[12] Medicinal plants have curative properties due to the presence of various phytochemicals. Recently there has been an upsurge of interest in the therapeutic potential of traditional plants for various ailments. Based on the detailed literature review, in the proposed study, an ayurvedic rasayana plant *Boerhaavia diffusa* was selected to provide scientific evidence for its traditional medicinal claim.

Preliminary phytochemical analysis

Medicinal plants produce bioactive compounds for the purpose of protection against predators. These bioactive compounds have immense therapeutic potential in curing human ailments. So the identification of bioactive compounds in plants their isolation, purification and characterization by various analytical methods is important. In the present study preliminary phytochemical analysis of aqueous and ethonolic extracts of *B.diffusa* revealed the presence of various phytochemicals (Table 1). Physicochemical characters of the herbal extract also proved the genuineness of the herbal drug (Table 2).

Table 1: Phytochemical Screening of ethanolic and aqueous extracts of *B. diffusa*

S.No	Name of the Test	Ethanol Extract	Aqueous Extract
1.	Alkaloid	+	+
2.	Carbohydrate	+	+
3.	Glycosides	-	+
4.	Saponin	+	+
5.	Phytosterols	-	+
6.	Fixed oil and fats	+	+
7.	Resin	+	-
8.	Phenol	+	+
9.	Tannin	-	-
10.	Flavanoids	+	+
11.	Protein and amino acid	+	+
12.	Steroid	+	+
13.	Triterpenes	+	+
14.	Gums and mucilage	-	-
15.	Coumarins	+	+
16.	Chlorogenic acid	+	+

(+) = Indicate Presence; (-) = Indicate Absence.

Table 2: Physicochemical characters of powdered sample of *Boerhaavia diffusa*

S.No.	Parameters	Percentage yield (w/w)
1.	Moisture content	0.54
2.	Total ash	50.00
3.	Acid insoluble ash	23.20
4.	Water soluble ash	25.50
5.	Alcohol soluble extractive	7.50
6.	Water soluble extractive	9.00

Anti microbial activity

Infectious diseases have become the leading cause of morbidity and mortality worldwide. The harmful side effects of the synthetic drugs and their high cost produced a gradual revival of interest in the use of medicinal and aromatic plants as antimicrobial agents in developed as well as developing countries. Consequently plant research has been intensified now days to develop potential antimicrobial drugs of plant origin. Numerous experiments have been carried out to screen natural products for antimicrobial property.[13,14,15,16,17,18] In the present study, anti microbial activity of the ethanolic extract of *B.diffusa* was carried out against *Escherichia coli, Streptococcus pyogens, Aspergillus niger and Candida albicans* (Table 3, 4). Maximum inhibitory activity was exhibited by the extract at a concentration of 200µg/ml. Anti microbial activity of the ethanolic extract was compared with standard antibiotic chloramphenicol and clotrimazole.

Table 3: Antibacterial activity of ethanolic extract of *Boerhaavia diffusa*

S.No	Microorganisms	Zone of Inhibition in mm					
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	200 µg/ml	Chloramphenicol 50 µg/ml
1	*Escherichia coli*	7	9	10	20	22	15
2	*Streptococcus pyogenes*	9	12	11	13	15	17

Table 4: Antifungal activity of ethanolic extract of *Boerhaavia diffusa*

S.No	Microorganisms	Zone of Inhibition in mm					
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	200 µg/ml	Clotrimazole 50 µg/ml
1	*Candida albicans*	5	7	7	8	13	10
2	*Aspergilus niger*	5	10	8	13	19	13

CONCLUSION

The results of the present study have provided evidence for the antimicrobial activity of *B.diffusa*. Further *in vivo* experiments are to be carried out to justify the traditional medicinal claim of the plant.

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