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IN VITRO ANTIMICROBIAL POTENTIAL OF BOERHAVIA DIFFUSA LEAF EXTRACT ON PATHOGENIC ORGANISM

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ABSTRACT

Boerhavia diffusa belonging to the family of the Nyctaginaceae is mainly a diffused perennial herbaceous creeping weed of India. The present study deals with the investigation of phytochemical analysis and evaluation of the antimicrobial activity of the aqueous, acetone+water and methanolic extract of the *Boerhavia diffusa* leaf extract. The result revealed the presence of phytic acid, saponins, phenolics, trypsin inhibitor and tannin in leaf extracts. The leaf extract of *Boerhavia diffusa* possesses antimicrobial activity as the zone of inhibition was observed in gram negative bacterial strains. The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antimicrobial activity of various solvent extracts of *Boerhavia diffusa* (Family: Nyctaginaceae) leaves.

KEYWORDS: Antibacterial, *Boerhavia diffusa*, inhibition zones, medicinal plants, Nyctaginaceae.

INTRODUCTION

Infectious diseases pose serious problems to health and they are main causes of morbidity and mortality worldwide.[33] The clinical efficiency of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide many opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and immediate need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.[24] Therefore, scientists are increasingly turning their attention to for medicine, looking for new leads to develop better drugs against microbial infections.[2] The

increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity.[8]

Present days, secondary plant metabolites (phytochemicals), previously with unknown biological activities, have been extensively investigated as a source of medicinal agents.[17] Thus, it is anticipated that phytochemicals with adequate antibacterial efficiency will be used for the treatment of bacterial infections.[1] Since immortal, man has used various parts of the plants in the treatment and prevention of various ailments.[31] About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine. *Boerhavia diffusa* is one of the most widely used plants. It is widely distributed in the tropics and subtropics.[9] It has a long history of uses by indigenous and tribal people and in Ayurvedic or natural herbal medicines.[10,11] *Boerhavia diffusa* (Spreading Hogweed in English), belongs to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (Punarnava in Hindi).

The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18th century.[18] *Boerhavia 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.[30] The main chemical ingredients of this plant include alkaloids (punarnavine), rotenoids (boeravinones A to F) and flavones.[7] *B. diffusa* possess a rotenoid named boeravinone G, which has been shown to be a very powerful antioxidant and genoprotective agent. Alkaloids in medicinal plants are reported for their antimicrobial and antimalarial activity.[4] *B. diffusa* leaf contains a large number of phytochemicals such as tannin, phytic acid, phenolics, saponins and trypsin which are considered as secondary metabolites and essential for their anti-oxidant activity.[29]

Pharmacological studies have demonstrated that *B. diffusa* possesses diuretic action,[25] anti-inflammatory,[3] antifibrinolytic,[14] anticonvulsant[21] and hepatoprotective activities.[5,23] *B. diffusa* is used as an Ayurvedic medicine in India and Unani medicine in Arab countries for the treatment of diabetes, stress, dyspepsia, abdominal pain, inflammation, jaundice, enlargement of spleen and congestive heart failure.[7-22] It has also been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers and nephritic syndrome.[20-28]

The aim of the present study was to evaluate the preliminary phytochemical screening and antibacterial potential of different solvent extracts of *B. diffusa* leaves on Gram-negative microorganism of medical importance.

MATERIALS AND METHODS

Plant Material and Preparation of the extract

B. diffusa plant was collected, authenticated and deposited in the department itself. The leaves were washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored in sterile containers for further use. A 20 g of dried powdered leaves sample was taken dissolved in 100 ml of different solvent (methanol, water, acetone + water) for 48 hours on rotator shaker. Then the solvents were filtered through filter paper to remove free extractable substances. The filtrate was concentrated by drying at room temperature for several days untill dried leaves sample were obtained.

Chemical Analysis

Trypsin inhibitor (TI) activity was determined following the standard procedures as described by Kakade *et al*.,[15] as modified by Hammerstrand *et al*.,[12] The total phenolic content (TPC) of each sample was estimated using the Folin– Ciocalteu colorimetric method according to Mallick and Singh· [19] Phytic acid was estimated following Wilcox *et al*.,[32] Tannins were measured as tannic acid equivalents Swain *et al*.,[30] Saponins were determined using the standard procedure as described by Harbone *et al*.,[13]

ANTIMICROBIAL ACTIVITY ASSAY

The strains used for the present study were *E.coli*.

A. Disc Diffusion Assay

Antimicrobial activity of *B. diffusa* phytochemicals were determined by disc diffusion method. Species of bacteria *E.coli* were spread on nutrient agar medium. Different concentration of different solvent of leave extract were loaded onto the disc of 4mm diameter in the ratio of 100%, 50% and 25% and control (pure culture) using PBS. The plates were then incubated at 37^0C for 24h. The zone of inhibition was measured with the help of standard scale.

B. Minimum inhibitory concentration (MIC)

Micro dilution method was used to evaluate the MIC.[33] MIC has been explained to be the lowest concentration, which can attain complete growth inhibition. The nutrient broth (100ul) of each bacterium was loaded on sterilized micro plates. 50ul of the leave extracts were added to each disc and then incubated at 37^0C for 24 hrs. The disc which had no bacterial growth was regarded as MIC.

RESULT AND DISCUSSION

Phytochemical activity

Present study showed the presence of different phytochemicals in *B. diffusa* leaf extract in different solvent extract (Methanol, Water and Acetone+ water) as mentioned in different literature. The *B. diffusa* leaf contains a large number of phytochemicals such as tannin, phytic acid, phenolics, saponins and trypsin which are considered as secondary metabolites and essential for their anti-oxidant activity.[29]

The phytic acid concentration varies widely in different solvent extract. The highest concentration was found in water extract (1.04±0.03mg/gm) and lowest in acetone+water extract (0.04±0.05mg/gm). The highest concentration of tannin was found in methanolic extract (1.76±0.04mg/gm) and lowest concentration was found in acetone+water extract (0.44±0.02mg/gm). The highest saponin content was present in methanol (2.87±0.04 mg/gm) and lowest concentration was found in acetone+water extract (0.03±0.04). The highest concentration of trypsin was found in methanolic extract (0.56±0.04 mg/gm) and lowest concentration was found in acetone+water extract (0.21±0.04mg/gm). The total phenolics contents also vary in different samples. The highest concentration was again found in methanolic extract (1.88±0.04mg/gm) and lowest concentration was found in acetone+water extract (1.08±0.04mg/gm). (Table 1).

Table 1: Phytochemical Activity Of *Boerhavia Diffusa* Leaf Extract

Sno.	Methanolic extract(mg/gm)	Water extract(mg/gm)	Acetone+water (mg/gm)
Phytic acid	0.85±0.04	1.04±0.03	0.04±0.05
Tannin	1.76±0.04	0.85±0.03	0.44±0.02
Saponins	2.87±0.04	0.88±0.05	0.03±0.04
Trypsin inhibitor	0.56±0.04	0.33±0.04	0.21±0.04
Phenolics	1.88±0.04	1.94±0.04	1.08±0.04

Significant at ($P \leq 0.05$). All values are the average of three determinations. Means± standard deviation (SD)

Antibacterial activity

The result of antibacterial activity of *Boerhavia diffusa* leaf extract was evaluated against gram negative bacteria *E. coli*. The antimicrobial activity was determined by using disc diffusion method. Among different extract of leaves methanol extracts of *Boerhavia diffusa* showed maximum antimicrobial activity. It shows 100% activity against *E.coli*. Methanolic extract inhibited the growth of tested microorganisms with large zones of inhibition diameter 5.0cm. In water extract a clear zone of inhibition was also observed with 4.0cm diameter. The moderate activity was shown against *E coli* by Acetone+water extract of *B. diffusa* showed the minimum antimicrobial activity against tested organism. Zone of inhibition was observed with 2.6cm respectively. *E. coli* displayed the highest susceptibility to methanolic extract. These data indicated that methanol crude extract of leaf exhibited strong antibacterial activity compared to aqueous extract and acetone +water extract.

The following results show that the extract possessed some antimicrobial activity against gram negative bacteria, depending upon the nature of the active ingredients present in the extracts and their capacity for diffusion into agar medium. The encouraging results indicate that the aqueous and methanolic leaf extracts of *B. diffusa* might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications. With this study we found that methanolic extract provides more consistent antibacterial activity.

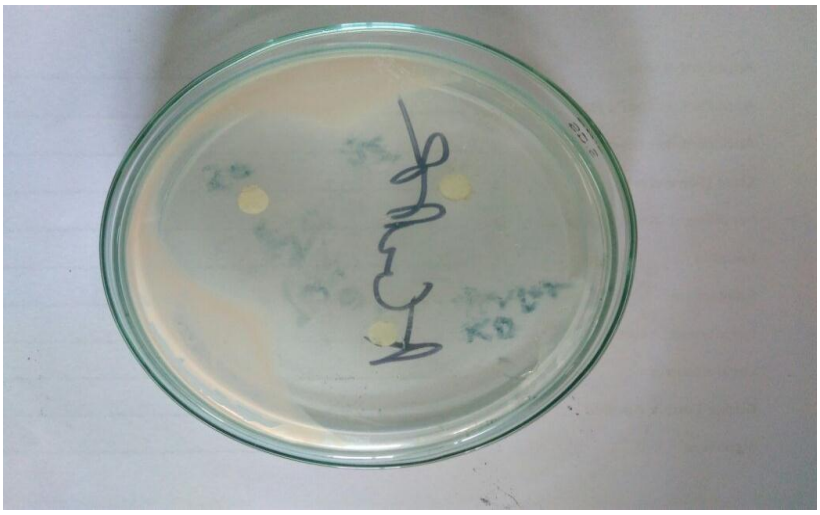

Fig 3: Antibacterial Activity of Methanolic Leaf Extract Of *Boerhavia diffusa*

Fig 4: Antibacterial Activity of Aqueous Leaf Extract of *Boerhavia diffusa*

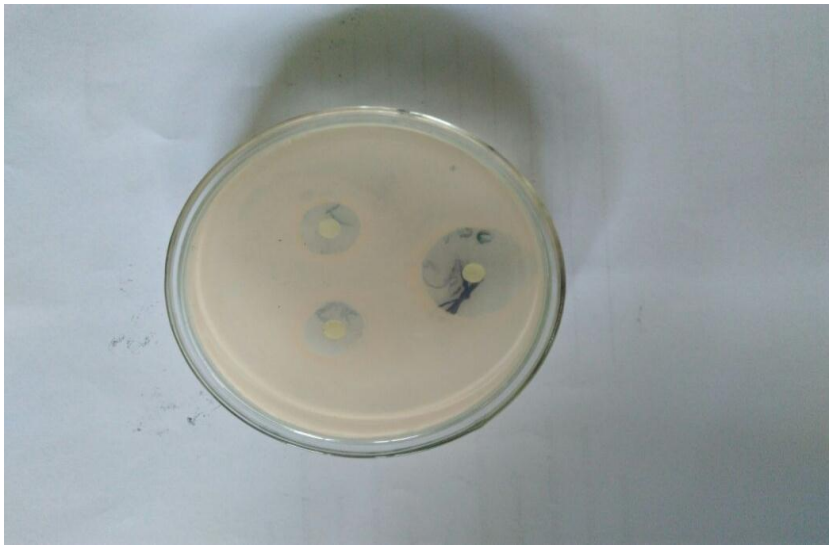

Fig 5: Antibacterial Activity of Acetone+Water Leaf Extract of *Boerhavia diffusa*

CONCLUSION

Boerhavia diffusa is a plant of repute in traditional as well as ethnobotanical systems of medicine in various parts of world. It contains diverse chemical compounds which have shown therapeutic activities, for example, diuresis, anticancer, hepatoprotection, and immunomodulation. Pharmacological studies showed that it possesses antiinflammatory activities, which makes it more suitable in the treatment of inflammatory renal diseases. It also exhibits antioxidant effects as oxidative injury underlies many of the diseases. Our research concludes presence of many phytoconstituents in *B. diffusa* leaf extracts which can provide various useful biological activities to this medicinal plant. Extracts of *B. diffusa* showed no adverse side effects or toxicity, so can be used for a long period of time. On

evaluating the results we can conclude that among solvents methanol is recommended as best for isolation of these phytochemicals.

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