Evaluation of antibacterial activity of *Boerhaavia diffusa* L. leaves

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The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antimicrobial activity of various solvent extracts of *Boerhaavia diffusa* L. (Family: Nyctaginaceae) leaves. The antimicrobial activity of different solvent extracts of *B. diffusa* L. leaves were tested against the Gram-positive and Gram-negative bacterial strains by observing the zone of inhibition. The Gram-positive bacteria used in the test were *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis* and *Micrococcus luteus,* and the Gram-negative bacteria were *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii, Klebsiella pneumoniae, Proteus vulgaris, Serratia marcescens, Shigella flexneri and Vibrio cholerae.* It was observed that ethanol, methanol, chloroform, ethylacetate and aqueous extracts showed activity against Gram-positive and Gram-negative bacteria. The ethanol extract of *B. diffusa* L. leaves showed more activity against Gram-positive (e.g. *S. aureus*, zone of diameter 11 mm) and Gram-negative bacteria (e.g. *E. coli*, zone of diameter 9 mm) when compared to other solvent extracts except *V. cholerae*. The results confirmed the presence of antibacterial activity of *B. diffusa* L. leaves extract against various human pathogenic bacteria.

Key words: Antibacterial, *Boerhaavia diffusa* L., infectious diseases, inhibition zones, medicinal plants, Nyctaginaceae

INTRODUCTION

Infectious diseases pose serious problems to health and they are main causes of morbidity and mortality worldwide.[1] 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.[2] Therefore, scientists are increasingly turning their attention to for medicine, looking for new leads to develop better drugs against microbial infections.[3] 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.[4]

Present days, secondary plant metabolites (phytochemicals), previously with unknown biological activities, have been extensively investigated as a source of medicinal agents.[5] Thus, it is anticipated that phytochemicals with adequate antibacterial efficiency will be used for the treatment of bacterial infections.[6] Since immortal, man has used various parts of the plants in the treatment and prevention of various ailments.[7] About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine. *Boerhaavia diffusa* is one of the most widely used plants.

B. diffusa Linn. is an herbaceous member of the family Nyctaginaceae. It is widely distributed in the tropics and subtropics.[8] It has a long history of uses by indigenous and tribal people and in Ayurvedic or natural herbal medicines.[9,10] Pharmacological studies have demonstrated that *B. diffusa* possesses diuretic action,[11] anti-inflammatory,[12] antifibrinolytic,[13] anticonvulsant[14] and hepatoprotective activities.[15,16]

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.[17-20] It has also been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers and nephritic syndrome.[21-24]

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 several Gram-positive and Gram-negative microorganisms of medical importance.

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MATERIALS AND METHODS

Plant Material

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 50 g of dried powdered leaves sample was taken and treated with petroleum ether. The treated sample was dissolved in 150 ml of ethanol, methanol, diethyl ether, chloroform, ethyl acetate and water, respectively. All the preparations were kept in shaker for 3 days. 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.

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, anthroquinones, saponins, triterpenoids, flavonoids, carbo hydrates, alkaloids, phytosterols, glycosidal sugars, tannins, phenols and furanoids using the method of Harborne.[25]

Test Organisms

The strains used for the present study were *E.coli*, *Pseudomonas aeruginosa*, *Salmonella typhii*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Serratia marcescens*, *Shigella flexneri*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus faecalis* and *Micrococcus luteus*.

Agar Disc Diffusion Method

This method was adopted to determine the antibacterial activity of leaves extract against the test organisms.

The Muller Hinton Agar (MAA) was (3.1 g/100 ml) weighed and dissolved in 100 ml of distilled water in a sterile conical flask. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into the sterile petri plate. The disc was saturated with 50 µl of the extract and was allowed to dry. The disc was placed on MAA plate swabbed with the culture of microorganisms.

The plate was incubated at 37°C for overnight. The microbial growth was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of leaves (*B. diffusa*) extracts.

RESULTS AND DISCUSSION

The results of qualitative analysis of phytochemical present in the ethanol, ethylacetate, diethyl ether, methanol, chloroform and aqueous extracts of *B. diffusa* leaves were presented in Table 1. Proteins and aminoacids were extracted in all solvents used except chloroform. Quinones were absent in aqueous extract. Saponins were absent in methanol and diethylether extracts. Triterpenoids were extracted in ethanol solvent alone. Flavonoids were found in all solvent extracts except ethyl acetate. Alkaloids were absent in ethanol and methanol extracts. Sterols were absent in aqueous extract. Glycosides were present in all solvent extracts. Tannins were not found in ethanol and methanol solvent extracts. Furanoids were extracted in ethyl acetate and chloroform solvents. Phenols were absent in aqueous extract. Carbohydrates were found in ethanol, ethyl acetate and chloroform extracts.

In the present study, preliminary phytochemical analysis revealed the presence or absence of various phytochemicals qualitatively in ethanol, methanol, diethylether, ethyl acetate, chloroform and aqueous extracts of *B. diffusa* [Table 1]. Previous studies reported the presence of flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins in *B. diffusa*.[26-28] The present study also correlated with the aforesaid studies. These pytochemicals present in leaves extracts might be responsible for the antibacterial activity. It is not surprising that there are differences in the antimicrobial effects of different solvent extracts due to the phytochemical

Table 1: Qualitative analysis of phytochemicals in *Boerhaavia diffusa* leaves extracts

Extracts	Ethanol extract	Methanol extract	Diethyl ether extract	Ethyl acetate extract	Chloroform extract	Aqueous extract
Proteins and amino acids	+	+	+	+	-	+
Quinones	+	+	+	+	+	-
Saponins	+	-	-	+	+	+
Triterpenoids	+	-	-	-	-	-
Flavonoids	+	+	+	-	+	+
Alkaloids	-	+	+	+	+	+
Sterols	+	+	+	+	+	-
Glycosides	+	+	+	+	+	+
Tannins	-	-	+	+	+	+
Furanoids	-	-	-	+	+	-
Phenols	-	+	+	+	+	-
Carbohydrates	+	-	-	+	+	-

+: Present; -: Absent

Table 2: Inhibition zone diameter of *Boerhaavia diffusa* leaves extracts against gram-positive and gram-negative bacteria

Bacteria	Zone diameter (mm)					
	Ethanol extract	Methanol extract	Chloroform extract	Diethyl ether extract	Ethyl acetate extract	Aqueous extract
Gram negative						
Escherichia coli	9	-	10	-	8	8
Pseudomonas aeruginosa	11	-	8	-	9	-
Salmonella typhii	10	-	8	-	7	10
Klebsiella pneumoniae	10	9	10	11	7	8
Proteus vulgaris	7	9	8	10	9	-
Serratia marcescens	7	9	9	11	8	-
Shigella flexneri	10	8	7	-	9	8
Vibrio cholerae	-	-	8	-		-
Gram positive						
Staphylococcus aureus	11	10	11	9	8	9
Bacillus subtilis	8	7	8	-	-	8
Streptococcus faecalis	13	8	12	-	7	6
Micrococcus luteus	8	-	11	-	7	9

properties and differences among species.

Their antibacterial potency was assessed by the presence or absence of inhibition zones and zone diameters (mm). The aqueous extract of *B. diffusa* showed the antimicrobial activity against Gram-positve bacteria like *S. aureus, B. subtilis, S. faecalis and M. luteus,* and Gram-negative bacteria like *E. coli, S. typhii, K. pneumoniae and S. flexneri* [Table 2].

Ethanol extract showed inhibitory an effect on Gram-positive bacteria like *S. aureus, B. subtilis, S. faecalis, M. luteus* and all Gram-negative bacteria selected for the present study. Methanol extract showed inhibitory effect against all Gram-positive bacteria selected for the present study except *M. luteus* and Gram-negative bacteria like *K. pneumoniae, P. vulgaris, S. marcescens* and *S. flexneri*.

Diethyl ether extract showed inhibitory effect on Gram-positive bacteria like *S. aureus* and Gram-negative bacteria *P. aeruginosa, K. pneumoniae, P. vulgaris, S. marcescens* and *S. flexneri*. Ethylacetate extract showed inhibitory effect on all Gram-positive bacteria selected for the present study and all Gram-negative bacteria except *V. cholerae*. Chloroform extract showed inhibitory effect on all Gram-positive bacteria selected for the present study except *B. subtilis* and all Gram-negative bacteria except *S. flexneri.*

Comparison of controls with the solvent extracts of *B. diffusa* leaves revealed that the leaves extracts are more effective towards pathogenic organisms. The results of present antimicrobial study revealed that ethanol, chloroform and water extract showed more activity towards human pathogenic organisms except *V. Cholerae*.

Stainer *et al.*[29] stated that the some of the solvent extracts that were ineffective in their study did not possess antibiotic properties or the plant extracts might have contained antimicrobial constituents just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in some solvents. The present study also agreed with above said statement and the antibacterial activity of *B. diffusa* leaves. The results were correlated with the other medicinal plants antimicrobial activities.[30,31]

CONCLUSION

Based on the results of the present study it is concluded that the *B. diffusa* leaves have potent antibacterial activity against various Gram-negative and Gram-positive bacteria which might be due to the phytochemicals present in the leaves. Also, there is further scope to study the identification and purification of active compound(s) involved in this antibacterial activity of *B. diffusa*.

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