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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *Boerhaavia diffusa* LINN

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ABSTRACT

Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value In the present study was planned to conduct phytochemical screening and antimicrobial activity of *Boerhaavia diffusa* Linn. The results of this study clearly indicate that the preliminary phytochemical analysis of *Boerhaavia diffusa* revealed presence of flavonoids, phenolics, steroids, tannin, saponins, phlobatannin, terpenoids triterpenoids, carbohydrate, glycosides, steroids, alkaloids while protein and anthroquinones were absent. Quantitative analysis revealed that total phenol (153mg/gm), Tannin (16mg/gm), alkaloids (20mg/gm), saponin (41mg/gm) and flavonoids (60mg/gm) were present. The results reveal that extract of *Boerhaavia diffusa* were significantly effective against both bacteria species of *Escherichia coli* and *Staphylococcus aureus* and fungi organism as *Candida albicans*. Overall, the *Boerhaavia diffusa* are a source of antimicrobial activity that can be important in disease prevention and health preservation.

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INTRODUCTION

Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Bhagwati Uniyal, 2003). In recent times, focus on plant research has increased all over the world and a large body of evidence collected to show immense potential of medicinal plants used in various traditional systems (Ayurveda, Siddha and Unani) (Dahanukar *et al*., 2000).

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Ashis, 2003). The plant is bitter, astringent, cooling, anthelminitic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic emetic expectorant anti inflammatory febrifuge, laxative and tonic it is useful in all type of inflammation, strangury, leucorrhoea opthalmia, lumbago, myalagia, scabies, cardiac disorders, jaundice, anemia, dyspepsia, constipation, cough ,bronchitis and general debility (Prajapati *et al* ., 2006).

MATERIALS AND METHODS

Plant materials: The fully mature *Boerhaavia diffusa* leaves were collected in December 2015 from Nanjikkottai, Thanjavur district, Tamil Nadu, India.

Preparation of alcoholic extract:

The leaf of *Boerhaavia diffusa* plant was first washed well and dust was removed from the leaves. Leaf were washed several times with distilled water to remove the traces of impurities from the leaf. The leaf were dried at room temperature and coarsely powdered. The equal concentrations of leaf powder was extracted with 70% methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli, Staphylococcus aureus* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans was* spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

RESULTS AND DISCUSSION

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Boerhaavia diffusa* investigated and summarized in Table-1. The phytochemical screening *Boerhaavia diffusa* leaves showed that the presence of flavonoid, terpenoids, triterpenoids, polyphenolic, steroid, tannin, saponin, phlobatannin, Carbohydrate, glycoside, steroid, alkaloids while protein and anthroquinone were absent.

Quantitative analysis revealed that the *Boherhaavia diffusa* contain significant amount of phenols, alkaloids, saponin and terpenoids. Significant amount of total phenol (153mg/gm), Tannin (16mg/gm), alkaloids (20mg/gm), saponin (41mg/gm) and flavonoids (60mg/gm) was presented (Table 2). The above phytoconstituents were tested as per the standard methods. This is beause of the pharmacological activity of this plant is used to trace the particular compound.

Quantitative analysis

Quantitative analysis revealed that the *Boherhaavia diffusa* contain significant amount of phenols, alkaloids, saponin and terpenoids. Significant amount of total phenol (153mg/gm), Tannin (16mg/gm), alkaloids (20mg/gm), saponin (41mg/gm) and flavonoids (60mg/gm) was presented (Table 2). The above phytoconstituents were tested as per the standard methods. This is beause of the pharmacological activity of this plant is used to trace the particular compound.

Table 1: Phytochemical screening of *Boerhaavia diffusa*

S.No	Phytochemical analysis	colouration	Results
1	Tannin	Blue black	+
2	Phlobatannin	Appearance of red precipitate	+
3	Saponin	Appearance of Emulsion	+
4	Flavonoid	Appearance of Yellow colouration	+
5	Steroid	Appearance of violet of blue(or) green	+
6	Terepenoid	Two layer of Reddish brown ring	+
7	Triterpenoid	Reddish violet colour	+
8	Alkaloids	Creamy (or) white precipitated	+
9	Carbohydrate	Appearance of Red precipitated	+
10	Protein	Violet (or) pink	-
11	Anthroquinone	Rose pink	-
12	Polyphenol	Blue green	+
13	Glycoside	Appearance of brown ring	+

(+) Presence (-) Absence

Table 2:Quantitative analysis of *Boerhaavia diffusa*

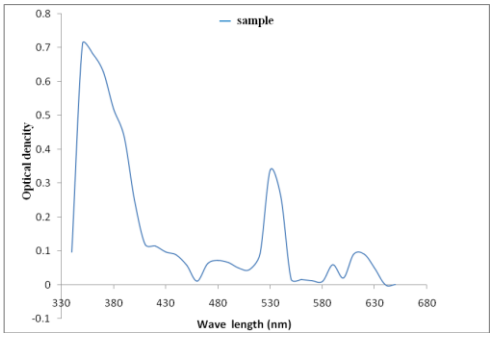
S.No	Phytochemicals	Method	Result (mg/g)
1.	Polyphenol	Edeoga *et al*.,(2005)	153mg
2.	Flavonoids	Boham and kocipai Abyazan (1994)	60 mg
3.	Alkaloids	Harbone (1973)	20 mg
4.	Saponin	Obadoni and ochuko (2001)	41 mg
5.	Tannin	Van-Burden and binson(1981)	16 mg

Qualitative Spectrophotometric Analysis

The UV-VIS profile of plant extract was taken at the 200 to 900 nm wavelength due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 360.00 nm in *Boerhaavia diffusa* respectively.

The application of standardized UV (or UV-Vis) spectroscopy has for years been used in analyses of flavonoids. The various flavonoid classes can be recognized by their UV spectra and UV-spectral characteristics of individual flavonoids including the effects of the number of aglycone hydroxyl groups, glycosidic substitution pattern, and nature of aromatic acyl groups have been reviewed. All the flavonoids contain at least one aromatic ring and consequently absorb UV light (Mabry *et al*., 1970). The typical UV-Vis spectra of flavonoids include two absorbance bands maxima in the range λ300~550nm and 470 to 670nm . Flavonoids composed of three rings structure (A, B, and C) with various substitutions. Changes in the substitution of the A-ring tend to be reflected in the band II absorption while alterations in the substitution of the B- and C-rings tend to be more apparent from band I absorption (Markham, 1982). Additional oxygenation (especially hydroxylation) generally causes a shift of the appropriate band to the longer wavelengths. Based on the UV-visible absorbance spectra, the flavonoids class can be predicted.

UV Vis. Spectrum of *Boerhaavia diffusa* Methanolic leaf extract



Antimicrobial activity

Boerhaavia diffusa extract was screened against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Boerhaavia diffusa* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 3. The inhibitory activities in culture media of the *Candida albicans* reported in Table 3 were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole.

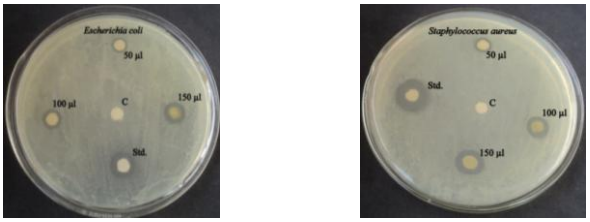
Table 3: Antimicrobial activity of *Boerhaavia diffusa* Methanolic leaf extract

Microbial Organism	**50µl**	**100 µl**	**150 µl**	**Std.**	**Control**
Escherichia coli (mm)	1.08± 0.07	2.23± 0.15	3.26± 0.22	7.32± 0.51	0
Staphylococcus aureus (mm)	1.52± 0.10	2..98± 0.20	4.17± 0.29	6.85± 0.47	0
Candida albicans (mm)	1.07± 0.07	2.13± 0.14	4.32± 0.30	6.91± 0.48	0

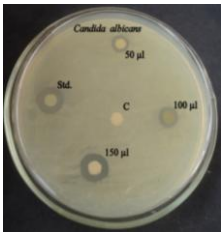
Values were expressed as Mean ± SD.
Bacterial standard - Chloromphenical
Fungal standard - Fluconazole

Fig 2: Antimicrobial activity of *Boerhaavia diffusa* Methanolic leaf extract

Escherichia coli ***Staphylococcus aureus***



Candida albicans



DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

Phytochemical study

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. Some isoflavones widely used in insecticides. They might also play a role in disease resistance. Some flavonoids such as quercetin and rutin, are known to support humam health by serving antiinflamatory, antihistaminic and antiviral agents (Okwu, 2004). Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids, such as glycyrrhizin and chrysin (Duraipandiyan, 2006) against HIV. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Del-Rio *et al*., 1997). Flavonoids have been referred to as nature's biological response modifiers, because of inherent ability to modify the body's reaction to allergies and virus and the showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Duraipandiyan, 2006).

Falodun *et al*. (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *et al*. (2006) examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al*. (2007) extracted eight bioactive compounds from dry leaf of *Cnidoscolus aconitifolius* using water and ethanol. Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al*. (2007) for its phytochemical properties.

Onwukaeme *et al*. (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycanthus angolensis*. Uma Devi *et al*. (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated for phytochemical analysis and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins (Vaghasiya and Chanda, 2007). Ayoola *et al*. (2008) investigated the phytochemical components of four medicinal plants used for the treatment of malaria in Southwestern Nigeria. *Ichnocarpus frutescens* leaf, stem and root were investigated (Mishra *et al*., (2009) for its phytochemical properties.

Vikas Kumar *et al*. (2009) examined leaves of *Paederia foefida* for the pharmacognotial and phytochemical studies. Aiyelaagbe and Osamundiemen (2009) was screened *Mangifera indica* for the chemically active compounds. Qualitative analysis was made for the active compounds present in the four important medicinal plants (Chitravadivu *et al*., 2009), *Acalypha indica*, *Cassia auriculata*, *Eclipta alba* and *Phyllanthus niruri*. The above studies are supported to our work.

Antimicrobial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al*., 2003).

Kesari *et al*. (2010) carried out the study on oil analysis and antimicrobial activity from seeds of elite genotype of *Pongamia pinnata*. The highest oil yield (33%) from seeds was recovered in n-Hexane. Physico-chemical properties of crude oil established suitability of P. pinnata for its use as a potential biofuel crop. The total mono unsaturated fatty acid (oleic acid 46%) present in seed oil was more in comparison to polyunsaturated fatty acid (33%) as analyzed by GC–MS. Seed oil also showed inhibition against the tested fungal and bacterial cultures. However, the efficacy of antimicrobial activity of the seed oil at four concentration levels (50%, 80%, 90% and 100%) against various pathogenic indicators was found to be concentration-dependent. The obtained results confirmed the use of seed oil from well characterized elite genotype of Pongamia as diesel fuel and in pharmaceuticals.

Gislene *et al*. (2000) evaluated the antimicrobial activity of plant extracts and phytochemicals with antibiotic susceptible and resistant microorganisms. In addition, the possible synergistic effects when associated with antibiotics were studied. Extracts from the following plants were utilized: *Achillea millifolium (yarrow), Caryophyllus aromaticus (clove), Melissa offficinalis (lemon-balm), Ocimun basilucum (basil), Psidium guajava (guava), Punica granatum (pomegranate), Rosmarinus officinalis (rosemary), Salvia officinalis (sage), Syzygyum joabolanum (jambolan) and Thymus vulgaris (thyme)*. The phytochemicals benzoic acid, cinnamic acid, eugenol and farnesol were also utilized. The highest antimicrobial potentials were observed for the extracts of *Caryophyllus aromaticus* and *Syzygyum joabolanum*, which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against antibiotic-resistant bacteria (83.3%). Sage and yarrow extracts did not present any antimicrobial activity. Association of antibiotics and plant extracts showed synergistic antibacterial activity against antibiotic-resistant bacteria. The results obtained with *Pseudomonas aeruginosa* was particularly interesting, since it was inhibited by clove, jambolan, pomegranate and thyme extracts. This inhibition was observed with the individual extracts and when they were used in lower concentrations with ineffective antibiotics. The above studies are supported to our work.

CONCLUSION

The results of this study clearly indicate that the preliminary phytochemical analysis of *Boerhaavia diffusa* revealed presence of flavonoids, phenolics, steroids, tannin, saponins, phlobatannin, terpenoids triterpenoids, carbohydrate, glycosides, steroids, alkaloids while protein and anthroquinones were absent. Quantitative analysis revealed that total phenol

(153mg/gm), Tannin (16mg/gm), alkaloids (20mg/gm), saponin (41mg/gm) and flavonoids (60mg/gm) were present. The results reveal that extract of *Boerhaavia diffusa* were significantly effective against both bacteria species of *Escherichia coli* and *Staphylococcus aureus* and fungi organism as *Candida albicans*

The results reveal that extract of *Boerhaavia diffusa* were significantly effective against both bacteria and fungi organism. The leaves of *Boerhaavia diffusa* are a newly discovered potential source of natural antimicrobial compounds. This report demonstrates for the first time that *Boerhaavia diffusa* inhibits the growth of different pathogens that can cause health problems. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases. However, further studies are needed to understand the origin of this activity. Particularly, major constituents of the leaves need to be tested for their antimicrobial activities. Overall, the *Boerhaavia diffusa* are a source of antimicrobial activity that can be important in disease prevention and health preservation.

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