Evaluation of secondary metabolites and antibacterial effect of *Boerhavia diffusa* ***roots*** with chromatographic studies

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Abstract- India has a vast diversity of medicinal herbs. Around 3,000 years ago, these herbs were acknowledged and used as medicinal plants helpful for treating people. New scientific research has established that some plants and herbs have presence of many active compounds and possess specific pharmacological properties.*Boerhavia diffusa* is one of the renowned medicinal plants used to treat large number of human ailments as mentioned in Ayurveda. The plant in whole or its peculiar parts (aerial parts and roots) have a numerous medicinal properties. It shows antibacterial, hepato-protective, antistress and also in treatment of stress, abdominal pain, inflammation and jaundice. Various phytochemical, Pharmacological, experimental and clinical investigations were done on *Boerhavia diffusa* by many scientists to clearly understand the ancient ayurvedic usage of punarnava. The evaluation of secondary metabolites and antibacterial activities of punarnava root extract were investigated on pathogens using the well diffusion method. HPTLC analysis of *Boerhavia diffusa* extracts were also carried out in different solvent.

Key word: *Boerhavia diffusa*, Antibacterial activity, Phytochemical analysis and HPTLC.

Introduction

Boerhavia diffusa commonly known as punarnava in Sanskrit is an herbaceous plant of the family nyctaginaceae. The whole plant or its specific parts (roots, leaves and stem) are known to have a long history of use by indigenous people in India; it has many ethanobotanical uses and is medicinally used in traditional, ayurvedic system. Besides, the *Boerhavia diffusa* plant reported to possess many pharmacological and clinical properties (Awasthi and Verma, 2003). The leaves are used in ophthalmic diseases (Shah and Gopa, 1987), as analgesic, in dropsy and jaundice (Raj and Patel, 1978), in rheumatism (Rao, 1981). Dried leaves are used in dhoomapana (smoking) in treatment of bronchial asthama (Satheesh and Pari, 2004, Chude et al., 2001).

Boerhavia diffusa is used to revitalize and clean the liver (Rawat et.al.1997). According to Ayurveda, when the liver is unable to perform well it also leads to an

imbalance of Vata-Pitta-Kaphadoshes. This might lead to liver diseases like jaundice. Consumption of *Boerhavia diffusa* helps to correct the function of the liver by removing toxins from the liver cells. *Boerhavia diffusa* also helps to improve digestive fire due to its appetizer property. It helps to digest the food easily and reduce the burden of the liver. The hepatoprotective activity of roots showed marked protection of serum parameters in thioacetamide toxicity in rats. Furthermore, the aqueous extract of thin roots collected in summer has more activity suggesting the proper time and type of root collection for the most desirable Results. The investigation also validates the use of *Boerhavia diffusa* L. roots in hepatic ailments by the several tribes in India (Rawat et.al, 1997)

Presently, secondary plant metabolites (phytochemicals), previously with unknown biological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju et.al. 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficiency would be used for the treatment of bacterial infections (Balandrin et.al. 1985). *Boerhavia diffusa i*s one of the most widely used plants and secondary metabolites found in medicinal plants are one of the main sources of drugs and health products. Improving of the content and yield of secondary metabolites in medicinal plants has become increasingly important.

Antibacterial activity of *Boerhavia diffusa* were studied against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) compared to Amoxicillin. The present study provides a scientific rationale for the traditional use of *Boerhavia diffusa* in the management of liver and jaundice.

Material and Methods

Collection and Processing of plant

Matured roots of *Boerhavia diffusa* were collected from the Himalaya Wellness company. There plant roots were washed with tap water to remove soil and unwanted dust particles. Then the roots were shaded, dried, and then powdered by using mechanical blender and stored in air tight bottles.

Extract Preparation

The powdered plant roots were soaked (10g/100ml) in different solvents aqueous, methanol and chloroform, for over night in rotator shaker.

HPTLC Profile

Mobile Phase

Chloroform: Methanol: Glacial acetic acid (35:6:1)

Application

Applied the sample and standard solution as 10-12mm band, in a distance of 12mm from the bottom of a pre coated thin layer silica plate of uniform thickness, made a mark up to a distance of 8.5cm from the application point as a development mark using pencil.

Preparation of development tank

Used Camag twin trough development tank (10×10cm). Chamber was covered from one side with required size of whatman no-1 filter paper, measured 20ml of mobile phase and transferred into the chamber along the side of the filter paper and allowed for the saturation process.

Visualization and Documentation

Visualized the dried plate under UV 254 nm and 366 nm using Camag Reprostar 3.

Identification Test

The individual sample was subjected to the qualitative phytochemical screening. Phyto-chemical tests were carried out adopting standard procedures (Trease et.al 1983, Kokate et.al 2003 and Harbone, 1998).

All of the reagents were made by adopting standard procedures (Indian Pharmacopoeia 2014).

Test for Alkaloids

To 1 mL of extract was added 1 mL of Mayers reagent and few drop of Iodine solution. Formation of yellow colour precipitate indicates the presence of Alkaliods.

Test for Terpenoids

To 1 mL of crude extract was added 1 mL of concentrated H2SO4 and heated for 2 minutes. A grayish colour indicates the presence of terpenoids.

Test for Tannins

To 1 mL of crude extract added 1 mL of $FeCl_3$. A blue green indicated presence of tannins.

Test for Saponins

To 1 mL of extract added 2 mL of distilled water and was shaken well and formation of 1 cm layer of foamindicates presence of saponins.

Test for Flavonoids

To 1 mL of extract added few fragments of magnesium ribbon and added few drops of concentrated HCl drop wise. Appearance of pink scarlet colour confirmed the presence of flavonoids.

Test for Steroids

1 mL of extract mixed with 1 mL of chloroform and concentrated H_2SO_4 sidewise. A red colour presence at the lower chloroform layer indicated presence of steroids.

Antibacterial activity

Antibacterial activity of *Boerhavia diffusa* was studied against Gram-positive, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli and Pseudomonas aeruginosa* compared to Ciprofloxacin.

Method Used

The zone of inhibition was measured by the Agar well diffusion method (Stoke, 1975) used for antibacterial activity .The sterilized nutrient agar was melted and allowed to set in a sterile petri dish and then inoculated with the nutrient broth containing the test inoculation. Holes were punched in agar of 6 mm diameter to a depth of about 2mm. Each of the holes in a Petri-dish was filled with the extract and incubated for 24 hrs at 37^0C. The active extracts had zone of inhibition which were measured to indicate the degree of sensitivity.

The antibacterial activity of the dried leaves extract of *Boerhavia diffusa* on the test bacteria are shown in **table-1**.

Results and Discussion

The bioactive substances in plant are produced as secondary metabolites. Secondary metabolites are substances manufactured by plants that make them competitive in their own environment.

These small molecules exert a wide range of effect on the plant itself and on other living organisms. The search for new secondary metabolites in plant with the hope of discovery of new products, new approaches for the treatment of disease is an on-going process. Aqueous, methanol and chloroform extracts are rich with secondary metabolites. **Table-1** shows the detection of secondary metabolites in *Boerhavia diffusa* root extract.

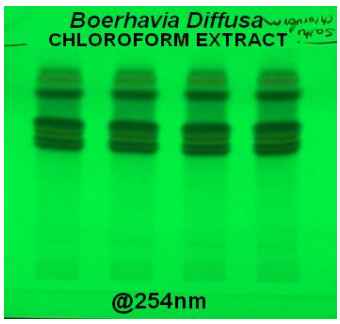
Table-1 Detection of secondary metabolites in *Boerhavia diffusa* root extract.

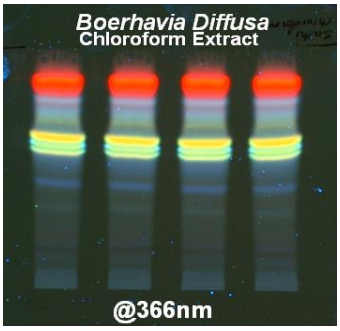
Class of compound	Aqueous	Methanol	Chloroform
Tannin	+	++	++
Flavanoid	++	+++	++
Saponin	+	++	+
Alkaloids	-	+++	++
Steroids	-	+	+
Terpenoids	-	++	+

Where +++ shows strong presence, ++ shows partially strong, + shows week and – shows absence of phytochemical activities.

The antibacterial activity of the roots extracts of *Boerhavia diffusa* on the test bacteria are shown in table-2. The antibacterial activity of *Boerhavia diffusa* roots were carried out. Most of the extract shows an antibacterial activity against the pathogens such as *S.aureus, E.coli* and *Pseudomonas aeruginosa*. The results of this work on chloroform, aqueous and methanol extracts of *Boerhavia diffusa roots had activity on E.coli, S.aureus and Pseudomonas aeruginosa*. The activity of plant extract showed the good antibacterial activity. The plant extracts contained active principle with broad antibacterial spectrum *(Bankole, 1992*). Methanol and chloroform had highest susceptibility. E.coli exhibited the least susceptibility.

HPTLC fingerprinting is proved to be a linear, precise, and accurate method for herbal identification (Cortes et.al 2014). Such finger printing is useful in quality control of herbal products and checking for the adulterants (Teo.P, et.al, 2013). The significant antibacterial effect of *Boerhavia diffusa* against all the three pathogens confirmed that the compounds present in the crude extract are responsible for the effective antibacterial activity. Thin layer chromatography studies of different extracts of roots (Methanol, Chloroform and Aqueous) indicated the presence of more than ten different compounds (Fig. 1), further confirming the synergistic action.





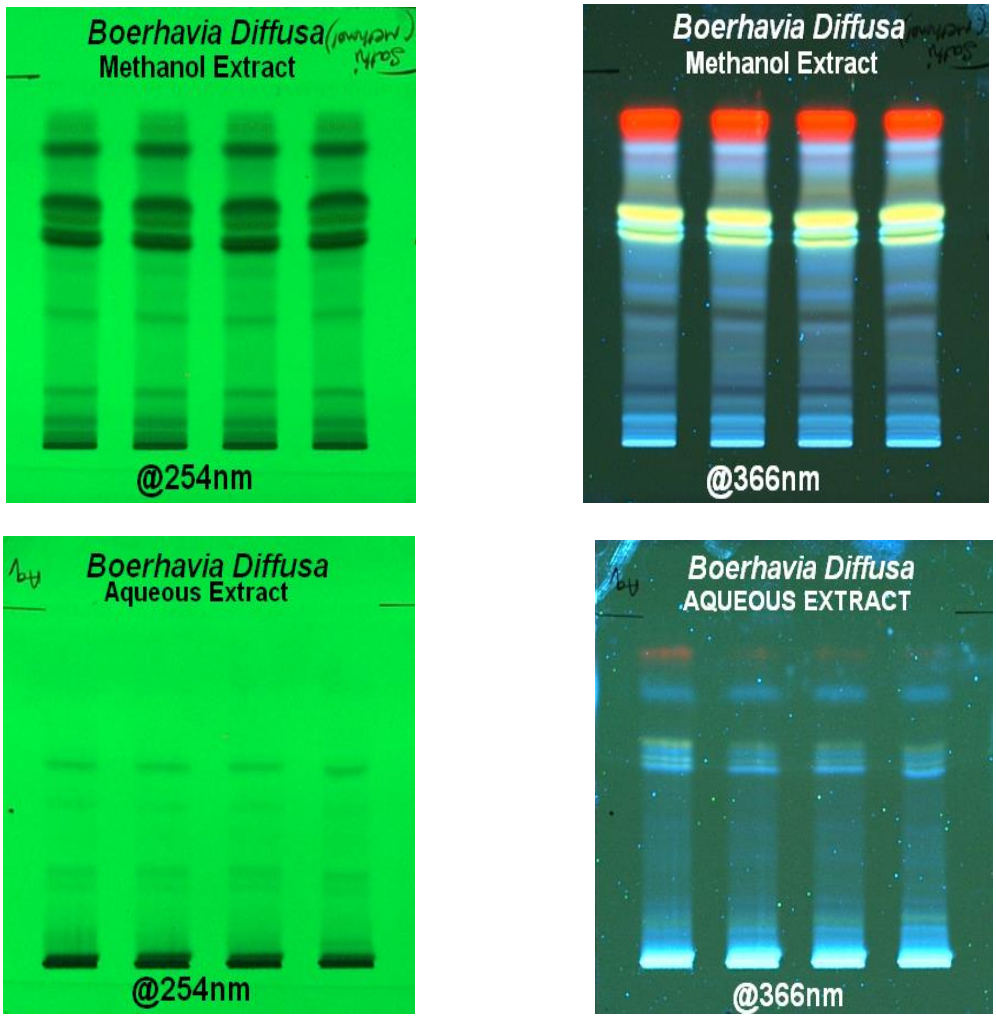


Figure-1 HPTLC profile of root extract of *Boerhavia diffusa in different solvent*

Table-2 Antibacterial activity of *Boerhavia diffusa* root and standard (Ciprofloxacin)

Tested Bacteria	**Diameter of ZI (in mm)**	**Diameter of ZI (in mm)**	**Diameter of ZI (in mm)**	**Diameter of Zone of Inhibition (in mm)**
	Methanolic Extract	**Chloroform Extract**	**Aqueous Extract**	**Standard (Ciprofloxacin-50 mcg/ml)**
Escherichia coli	20mm	19mm	14 mm	25 mm
Staphylococcus aureus	18mm	19mm	16 mm	28 mm
Pseudomonas aeruginosa	18mm	17mm	15 mm	22 mm

ZI→ Zone of Inhibition

Conclusion

From the above research, it is seen that the results of this investigation support the ethanomedicinal use of this plant by local practitioners. India has a rich flora used in traditional medical treatment. These plants have medicinal properties because of their phytochemical components. These phytochemicals show therapeutic effect on mankind. From this current study, we concluded that the root of *B.diffusa* showed high therapeutic activity because the presence of potential phytochemicals. Extracts of this plant is abundant in flavonoids, diterpenoids, alkaloids, tannins, saponins. In this study, we concluded that biologically active phytochemicals present in methanol and chloroform extracts of *Boerhavia diffusa* roots. *B.diffusa* root extracts showed good antibacterial activity on *E.coli, S.aureus and Pseudomonas aeruginosa.* However more research has to be carried out so as to characterize the bioactive ingredients of the plants.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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