WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 8, 2235-2243 Research Article ISSN 2278 – 4357

# PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *BOERHAVIA ERECTA*

**Ayushi Gupta, Ismail Shareef M.*, Gopinath S. M. and Sonia Gupta**

Department of Biotechnology, Acharya Institute of Technology, Bangalore, India.

Article Received on
15 June 2017,
Revised on 05 July 2017,
Accepted on 26 July 2017
DOI: 10.20959/wjpps20178-9901

***Corresponding Author**
**Dr. Ismail Shareef M.**
Department of Biotechnology, Acharya Institute of Technology, Bangalore, India.

## ABSTRACT

The objective of the present study was to evaluate the phytochemical constituents and antimicrobial activity of methanolic extract of dried whole plant of medicinal important herbs of *Boerhavia erecta* utilized in our daily routine in the form of vegetables. Qualitative analysis of phytochemical constituents are carbohydrates, glycosides, flavonoids, tannins, saponins, alkaloids, phenolics, ferric chloride, etc. was performed by well-known tests protocol available in the literature. The phytochemical screening was revealed the extract richness in carbohydrates, glycosides and ferric chloride. The antimicrobial activity was determined in the extract by using ZOI and MIC. The antibacterial and antifungal activities of extract on different concentration (2000, 1000, 500, 250, 125, 62.5 µg/ml) of *Boerhavia erecta* were tested against 2 Gram-positive bacteria- *Staphylococcus aureus* and *Streptococcus mutans*; 2 Gram-negative bacteria- *Salmonella typhimurium* and *Pseudomonas aeruginosa*; and two Fungal strains- *Candida albicans* and *Aspergillus niger*. ZOI and MIC were compared with an antibiotic Ciprofloxacin as a standard. The results showed that the remarkable inhibition was shown against only Gram-negative bacteria in both ZOI and MIC. Hence, this plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research.

**KEYWORD:** *Boerhavia erecta*, Phytochemical constitution, *In-vitro* antibacterial and antifungal activity.

## INTRODUCTION

In India, the different types of several medicinal plants are used to cure the specific ailments have been in vague from ancient times. The different system of medicine namely Ayurvedic,

Siddha and Unani have been the existence in several centuries (Khan, S. S., and Chaghtai, S. A. 1982). The plants supply us the large number of 'chemicals' which form the sources of drugs. The present study of modern medicine is towards a change from the use of cellulose coated medicinal pills to extract from medicinal plants and supplied either in pure form or in a synthetic form to cure many human diseases. Natural products performed various functions and many of them has very useful biological functions (Galal, M., Bashir, A. K., et. al., 1991).

*Boerhavia erecta*, commonly known as the erect spiderling, erect boerhavia, in Hindi Shweta and in Marathi pandhari punarnava. *Boerhavia erecta* is an herbaceous member of the family Nyctaginaceae and mainly used as a traditional medicinal plant in Africa (Stintzing, F. C., Kammerer, D., et. al., 2004). It has a similar property to *Boerhavia diffusa*, which possesses a diuretic (Gaitonde, B. B., Kulkarni, H. J., et. al., 1974), anti-inflammatory (Bhalla, T. N., Gupta, M. B., et. al., 1968), antifibrinolytic (Jain, G., and Khanna, N. 1989), anticonvulsant (Adesina, S. K. 1979) and hepatoprotective activities (Chandan, B. K., Sharma, A. K., et. al., 1991 and Rawat, A. K. S., Mehrotra, S., et. al., 1997) which make it a very useful medicinal plant. In moderate doses, it is useful in the treatment of asthma (Schmelzer, G. H. 2006). In West and East Africa, the leaves are eaten as a vegetable or use for the preparation of sauces (Grubben, G. J. 2004).

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology, and prediction of therapeutic outcome. Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthesized molecules as potential microbial agents (Runyoro, D. K., Matee, M. I., et. al., 2006; Mabona, U., Viljoen, A., et. al., 2013 and Nazzaro, F., Fratianni, F., et. al., 2013). Dilution methods are the most appropriate method for the determination of MIC values by agar dilution or broth medium (microdilution or macrodilution). MIC values are recorded for the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of microorganisms tested which expressed in µg/ml or mg/L (Pfaller, M. A., Sheehan, D. J., et. al., 2004).

## MATERIALS AND METHODS

**Materials:** Molish reagent, conc. $H_2SO_4$, HCL (0.1% N), 1% $FeCl_3$, 5% $FeCl_3$, glacial acetic acid, dragendroff's reagent, water bath, NaOH, chloroform, antibiotic, resazurin dye, incubator, side arm flask, petri plates, laminar air flow, methanol, weighing balance, etc.

**Sample collection:** *Boerhavia erecta* plant was collected from the district of Tamil Naidu.

**Extraction of bioactive compounds:** *Boerhavia erecta* plant was dried and weighed approximately 270 gm along with 2.5 L methanol solvent. The extraction process was done by hot continuous extraction method using soxhlet apparatus. The process of transferring the partially soluble components of a solid to the liquid phase using a soxhlet extractor. The solid was placed in a filter paper thimble which was then placed into the main chamber of the soxhlet extractor. The solvent (heated to reflux) travels into the main chamber and the partially soluble components were slowly transferred to the solvent.

**Phytochemical analysis:** The qualitative phytochemical assay was done in order to detect the presence of several compounds like alkaloids, saponins, flavonoids, carbohydrates, glycosides and many more.

**Table 1: Preliminary phytochemical tests for plant extract.**

| Phytoconstituents | Test | Observation |
|---|---|---|
| Carbohydrates (Molisch's test) | 2ml extract + 2 drops Molish reagent + few drops conc. $H_2SO_4$ | purple-violet ring |
| Glycosides (Keller-Killiani test) | 2ml extract + Few drops glacial acetic acid + 1 drop 5% $FeCl_3$ + conc. $H_2SO_4$ | reddish brown color at the junction of two layers and upper layer turned bluish green |
| Saponins (Foam test) | 1ml extract + 20ml distilled water + slowly shakes in a graduated cylinder for 15 mins | 1 cm layer of foam |
| Alkaloids (Dragendrodroff's test) | 2ml extract + few drops dragendroff's reagent | red colored precipitate |
| Flavonoids (Alkaline reagent test) | 2ml extract + few drops NaOH | intense yellow color that becomes colorless on addition of few drops of dil. HCl |
| Phenolic nucleus (Ferric chloride test) | 2ml extract + 1% ferric chloride | bluish black color |
| Tannins | 2ml extract + 0.5ml NaOH | precipitate |
| Triterpenes (Leiberman-Bucharat test) | 2ml extract + few drops acetic acid + conc. $H_2SO_4$ | Deep red ring at the junction of 2 layers |
| Sterols (Salkowaski test) | 2ml extract + few drops conc. $H_2SO_4$ + shaken + allowed to stand | Lower layer turns red |
| Oily spot test | 1 drop of extract placed on filter paper and solvent allowed to evaporate | An oily stain on filter paper |
| Resins test | 1ml of solvent extract + few drops acetic anhydride + 1ml conc. $H_2SO_4$ | coloration ranging from orange to yellow |

**Antimicrobial screening**

**Antibacterial studies:** The antimicrobial activity of extract was studies systematically against 2 Gram-positive and 2 Gram-negative bacteria by ZOI and MIC.

*Staphylococcus aureus* is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin.

*Streptococcus mutans* is a facultatively anaerobic, Gram-positive coccus commonly found in the human oral cavity and is a significant contributor to tooth decay.

*Pseudomonas aeruginosa* is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans.

*Salmonella typhimurium* is a pathogenic Gram-negative bacterium predominately found in the intestinal lumen. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharides (LPS) which protect the bacteria from the environment.

**Antifungal studies:** The antimicrobial activity of extract was study systematically against 2 fungal strains.

*Candida albicans* is a type of yeast that is commonly used as a model organism for biology. It is generally referred to as a dimorphic fungus since it grows both as yeast and filamentous cells.

*Aspergillus niger* is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food.

Zone of inhibition (ZOI), the medium was used SCDA for bacteria and SDA for fungi. The medium plates were coated with the inoculums and wells were formed on a different concentration (2000, 1000, 500 µg/ml) and standard as an antibiotic Ciprofloxacin. Plates were incubated overnight at 37°C. Minimal Inhibitory Concentration (MIC), MIC plates consists of test sample (extract), standard as an antibiotic Ciprofloxacin, growth control and negative control. The medium was used SCDM broth for bacteria and SDB for fungi. Serially dilute it and kept it for overnight incubation at 37°C. After incubation, add resazurin dye 10 µl and incubate it for 3-5 hrs.

## RESULTS

**Preliminary phytochemical screening:** It was found that *Boerhavia erecta* whole plant extract contained carbohydrates, glycosides and ferric chloride.

**Table 2: Qualitative analysis of phytochemical constituents.**

| Phyto-constituents | Test name | Presence/Absence |
|---|---|---|
| Carbohydrates | Molisch's Test | + |
| Glycosides | Keller-Killiani Test | + |
| Saponins | Foam Test | - |
| Alkaloids | Dragendrodroff's Test | - |
| Flavonoids | Alkaline reagent test | - |
| Phenolics<br>Tannins | Ferric chloride test<br>Test for Tannins | +<br>- |
| Phyto sterols<br>Triterpenes | Salkowaski Test<br>Leiberman-Bucharat Test | -<br>- |
| Fixed oil & fats | Oily spot Test | - |
| Resins | | - |

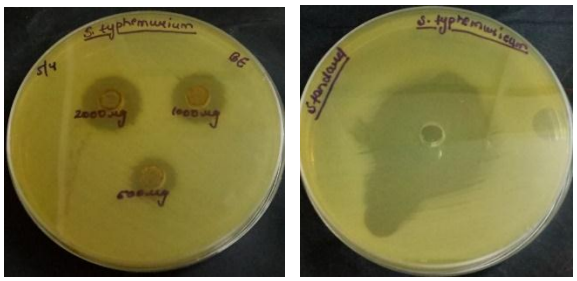
Here, + means presence and – means absence

**Antimicrobial studies:** The antimicrobial activity of the extract *Boerhavia erecta* medicinal plant was studies in different concentration (2000, 1000, 500, 250, 125, 62.5 µg/ml) against six microorganisms, 2 Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans)* and 2 Gram-negative (*Salmonella typhimurium* and *Pseudomonas aeruginosa)*, and two fungal strain (*Candida albicans* and *Aspergillus niger*). These strains were selected on the basis of their application purpose for further formulation study.

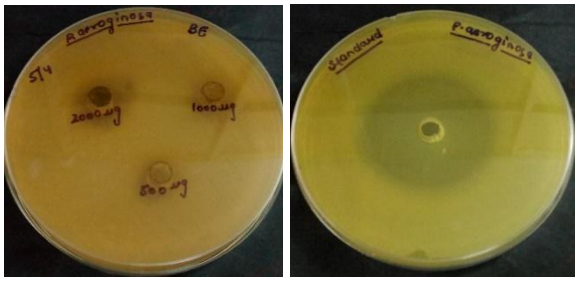
The extract *Boerhavia erecta* was showing the activity only against gram-negative bacteria in MIC and ZOI.

**Table 3: Zone of inhibition of *Boerhavia erecta.***

| Concentration | *Salmonella typhimurium* | *Pseudomonas aeruginosa* |
|---|---|---|
| 2000 µg/ml | 18 mm | 15 mm |
| 1000 µg/ml | 16 mm | 9 mm |
| 500 µg/ml | 12 mm | 6 mm |
| Standard | 38 mm | 41 mm |



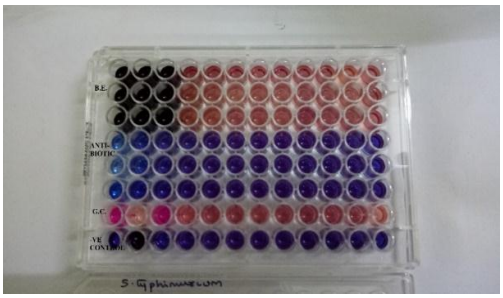
**Zone of inhibition showing *S. typhimurium* on different concentrations with the standard Ciprofloxacin**



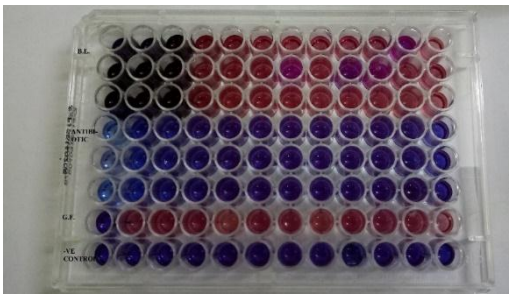
**Zone of inhibition showing in *P. aeruginosa* on different concentrations with the standard Ciprofloxacin**

**Table 4: Minimal inhibitory concentration of *Boerhavia erecta.***

| | ***Salmonella typhimurium*** | ***Pseudomonas aeruginosa*** |
|---|---|---|
| Concentration | 500 µg/ml | 500 µg/ml |
| Standard | 0.024 µg/ml | 0.024 µg/ml |



**MIC assay of *S. typhimurium***



**MIC assay of *P. aeruginosa***

The particular technique was performed by using standard as an antibiotic (Ciprofloxacin) with varying concentration of the plant extract namely 2000, 1000, 500, 250, 125, 62.5 µg/ml. The zone of inhibition and minimal inhibitory concentration are developed against the bacterial species only which seems to be interesting and notable. The organisms responded to the plant extract but the inhibitory zone varied according to the concentration. Nearly, the concentration (2000 µg/ml) seems to show the maximum inhibitory zone against the bacterial strains.

The Gram-positive bacterial and fungal strain were not showing any activity in MIC and ZOI because mainly it depends on the plant part and Gram-positive bacteria didn't have cell membrane so the organism was not affecting the drug. The fungal strain is unicellular or multicellular/filamentous, so *C. albicans* is unicellular and *A. niger* is multicellular so they were not affecting the drug or antibiotic, it means they were resistant to it.

The increasing prevalence of multidrug resistance strain of bacteria and the recent appearance of strain with reduced susceptibility to antibiotic raises the specter of untreatable bacterial infections and add urgency to the search of new infection fighting strategies (SIERADZKI, K., WU, S. W., et. al., 1999). The result obtain nearly signifies that the plant selected for the screening of bacteria and the result further control confirms that the plant extract possesses antibacterial effect.

## DISCUSSION

Antimicrobial studies of medicinal plant is being increasingly reported from different parts of the world. The WHO estimates that plant extract or their bioactive constituents are used as folk medicine in traditional therapies of 80% of the world's population.

In the present study, the extract obtained from *Boerhavia erecta* show strong activity only in Gram-negative bacteria. The results were compared with standard as an antibiotic (Ciprofloxacin). The above results showed that the activity of methanolic extract of *Boerhavia erecta* showed significant antibacterial activity. This study also showed the presence of different phytochemicals with biological activity that can be valuable of therapeutic agents. The results of phytochemicals in the present investigation showed that the plant contains more or less same components like carbohydrates, glycosides and phenolic ion. The plant is rich in these components that possess an antimicrobial activity against the different microorganisms.

The result obtain nearly signifies that the plant selected for the screening of bacteria and the result further control confirms that the plant extract possesses antibacterial effect.

## CONCLUSION

Phytochemicals derived from plant has a great prospect and promise in providing good and effective antimicrobial agents to treat against intractable life-threatening diseases, with this view, the present study has explored the efficiency of the *Boerhavia erecta* as a valuable natural source.

## ACKNOWLEDGMENT

The authors were grateful to Acharya Institute of Technology and Radiant Research Services Pvt. Ltd. for providing necessary chemicals and equipment's for the completion of following work.

## REFERENCES


1. Adesina, S. K. Anticonvulsant properties of the roots of Boerhaavia diffusa Linnaeus. *Quarterly Journal of Crude Drug Research*, 1979; *17*(2): 84-86.
2. Bhalla, T. N., Gupta, M. B., Seth, P. K., & Bhargava, K. P. Anti-inflammatory activity of *Boerhaavia diffusa*, 1968.
3. Chandan, B. K., Sharma, A. K., & Anand, K. K. Boerhaavia diffusa: a study of its hepatoprotective activity. *Journal of Ethnopharmacology*, 1991; *31*(3): 299-307.
4. Gaitonde, B. B., Kulkarni, H. J., & Nabar, S. D. Diuretic activity of Punarnava (*Boerhavia diffusa*). *Bull Haffkine Inst*, 1974; *2*.
5. Galal, M., Bashir, A. K., Salih, A. M., & Adam, S. E. I. Activity of water extracts of Albizzia anthelmintic and A. lebbek barks against experimental Hymenolepis diminuta infection in rats. *Journal of ethnopharmacology*, 1991; *31*(3): 333-337.
6. Grubben, G. J. *Plant Resources of Tropical Africa (PROTA)* (Vol. 1). Prota, 2004.
7. Jain, G., & Khanna, N. Punarnavoside: a new fibrinolytic agent from *Boerhaavia diffusa* Linn. *Indian J. Chem. B*, 1989; *28*: 163-166.
8. Khan, S. S., & Chaghtai, S. A. Ethnobotanical study of some plants used for curing skin afflictions. *Ancient science of life*, 1982; *1*(4): 236.
9. Mabona, U., Viljoen, A., Shikanga, E., Marston, A., & Van Vuuren, S. Antimicrobial activity of southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. Journal of ethnopharmacology, 2013; 148(1): 45-55.
10. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 2013; 6(12): 1451-1474.
11. Pfaller, M. A., Sheehan, D. J., & Rex, J. H. Determination of fungicidal activities against yeasts and molds: lessons learned from bactericidal testing and the need for standardization. *Clinical Microbiology Reviews*, 2004; *17*(2): 268-280.
12. Rawat, A. K. S., Mehrotra, S., Tripathi, S. C., & Shome, U. Hepatoprotective activity of Boerhaavia diffusa L. roots-a popular Indian ethnomedicine. Journal of Ethnopharmacology, 1997; 56(1): 61-66.
13. Runyoro, D. K., Matee, M. I., Ngassapa, O. D., Joseph, C. C., & Mbwambo, Z. H. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC complementary and alternative medicine, 2006; 6(1): 11.

14. Schmelzer, G. H. Boerhavia erecta L. *PROTA (Plant Resources of Tropical Africa/Ressources végétales de l'Afrique tropicale), Wageningen (http://database. prota. org/search. htm, Date of access: 1 October 2008)*, 2006.
15. SIERADZKI, K., WU, S. W., & TOMASZ, A. Inactivation of the methicillin resistance gene mecA in vancomycin-resistant Staphylococcus aureus. *Microbial Drug Resistance*, 1999; *5*(4): 253-257.
16. Stintzing, F. C., Kammerer, D., Schieber, A., Adama, H., Nacoulma, O. G., & Carle, R. Betacyanins and phenolic compounds from Amaranthus spinosus L. and *Boerhaviaerecta* L. *Zeitschrift für Naturforschung C*, 2004; *59*(1-2): 1-8.