

Screening of Antibacterial Potential and Phytochemical Analysis of Medicinal Plant *Barleria Prionitis*.

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Abstract – Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, phenols, terpenoids, steroids, saponins, tannins etc. and getting these chemicals out into the herbal remedy depends upon the solubility of these compounds in various solvents. In the present study aqueous ethanol diethyl ether acetone and methanol extracts of *Barleria prionitis* leaves were investigated for phytochemical and anti-microbial activity. The micro-organisms employed were *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and *Bacillus subtilis*. The susceptibility of bacterial strains against the all extracts was determined using the disk diffusion method. The findings showed that potential antibacterial properties of the extracts against the organisms tested. The most susceptible microorganisms were *S. aureus*, while the least susceptible was *E. coli*. Aqueous extracts had no activity against the test bacteria the leaves of plant were found abundant with biologically active phytochemicals.

Keywords: *Barleria prionitis*, Phytochemical, Antimicrobial.

Introduction

Nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbs are safe, less toxic,

economical and a reliable key natural resource of drugs all over the world. (Al-Essa et al., 1998). Antibiotics is one of the most serious public health problems, especially in developing countries where infectious diseases still represent a major cause of human mortality (World Health Organization, 2014). *Barleria prionitis* is a shrub in the family Acanthaceae, native to Island and Mainland Southeast Asia, China, the Indian Subcontinent, the Arabian Peninsula and northeastern Africa. It used not only as an ornamental but also as a hedge and extensively as a component of folk medicines to treat whooping cough and tuberculosis. (Malik, 2021). One of the oldest forms of medical practice is the use of plants for therapeutic purposes; teas, syrups, tinctures, among others have been used as medicines and in many cases come to be the sole therapeutic resource of certain communities and ethnic groups (Amorozo, 2002; Oliveira et al., 2012). Thus, knowledge about the therapeutic potential of plants is of great scientific and medical interest, as an effective alternative to the battle against resistant microorganisms (dos Santos et al., 2015). Herbal treatment is still used for many health problems. The current investigation was carried out to screen the antibacterial activity and phytochemical analysis of medicinal plant *Barleria prionitis* leaves used for herbal treatment by local communities against some pathogenic bacterial strains.

Material and Methods

Collection of plant material: The leaves of *B. prionitis* were collected from the Botanical Garden, Department of Botany, D.A.V(PG) College, Muzaffarnagar, UP, India and identified by the HOD of the department, Dr. Sanjeev Kumar. The plant leaves were washed with running tap water. The leaves were shade dried at room temperature for 14 days and blender to get fine powders. The powders were stored in airtight container at room temperature for further studies.

Preparation of plant leaves extracts: The powdered leaves of the plant *Barleria Prionitis* were homogenised, and about 30g of the sample from each variety was extracted separately with 300mL (10%) of aqueous and four different solvents: methanol, acetone, di ethyl ether and ethanol for 48 h in an orbital shaker at 100 rpm and room temperature. The extracts were filtered and filtrates were concentrated in a rotary evaporator at 45°C. Extracts were kept at 4 °C until the further analyses.

Bacterial cultures: Four standard human enteric pathogenic bacteria viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were procured from the Laboratory of the Department. All the pathogens were sub cultured on nutrient agar slants and preserved at 4°C for further study.

Evaluation of antibacterial activity of extracts of *Barleria prionitis*: Antibacterial assay was carried out by agar disk diffusion method. (Bauer A, 1959). One ml of standardized suspensions of the microorganisms was deposited in Petri dishes (diameter 90 mm) and 20 ml of nutrient Agar at 50°C was added. After solidification, aliquots of 10% of the ethanol extract of filtered sample were applied to paper disks (6mm in diameter, Whatman No.1), which resulted in disks containing 180-200µg of the extract. After evaporation of the loading solvent, each disk was placed at

the centre of the Petridishes containing previously inoculated Nutrient agar medium plates and incubated at 37°C for 24 h. At the end of the incubation time, the diameter of microbial growth inhibition zone was measured in millimeter (mm).

Phytochemical screening of leaves of *Barleria Prionitis*: Chemical tests were carried out on the aqueous extract of *Barleria prionitis* using standard to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984), (Omoya and Akharaiyi, 2012), (Jyothi-prabha and Venkatachalam, 2016). (Harborne and Williams, 2000)

Screening for alkaloids: To 5 ml each of the spice extracts, 5 ml of aqueous hydrochloric acid was added on a steam bath at 60°C for 5 min. The spice extract was filtered with a 3 layered muslin cloth. In one ml of the filtrate, few drops of Dragendorff's reagent were added. Appearance of Blue black turbidity was positive for alkaloids.

Screening for steroids: 1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening for tannins: 5 ml each of the extracts was stirred separately with 100 ml distilled water and filtered. One millilitre ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins.

Screening for terpenoids: 5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for flavonoids: 5 ml of diluted ammonia solution was added to aqueous

extract followed by the addition of 1 ml concentrated H_2SO_4 . Appearance of yellow colour indicated the presence of flavonoids.

Screening for saponins: 5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence of the presence of the saponins.

Test for coumarins: Two ml of every extract is treated with 3 ml of 10% NaOH. A yellow colouration determined in every extract indicated the presence of coumarins.

Screening for anthraquinones: 5ml of every extract resolution is hydrolysed with diluted targeted H_2SO_4 extracted with benzene. 1ml of dilute ammonia is additional thereto. Pink coloration steered the positive response for anthraquinones.

Screening of Phenols: 2ml of plant extract, 2ml of distilled water followed by 10 % $FeCl_3$ solution was added. Bluish black colour indicates the presence of phenol.

Results and Discussion

From the ancient time, analysis of biologically active natural yields from plants has attracted several natural product researchers. Natural products play a very important role within the field of recent medication analysis and development due to its low toxicity, simple handiness, and low price. The latest analysis investigation has ascertained that

the bioactive and inhibitor potentials of those plants are attributed to the presence of phenols, flavonoids, alkaloids, terpenoids, saponin, and tannins (Agboret *et al.*, 2011). Hence, this work principally focuses on phytochemical screening in the leaves of *B. prionitis*. The results are summarized and mentioned below. The current study was carried out on the *B. prionitis* unconcealed the presence of medicinally active constituents. The results investigated and summarized in Table-1. The phytochemical screening in the leaves of *B. prionitis* showed that the abundant presence of tannin, saponins, steroids, flavonoids, terpenoids, anthraquinones, phenol, and coumarins in aqueous extracts. Terpenoids are concerned with medication and antineoplastic functions thus employment of the plant to treat burns, skin diseases, and bug stings (Bown Deniet *et al.*, 1995). Flavonoids are also present in all the extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Salah N, 1995; Rio DA, 1997]. Tannin rich medicinal plants are used as healing agents in a number of diseases Doughari JH (2012). Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemo-therapeutic agents, anaesthetics and Central Nervous Stimulants (Noble RL 1990; Madziga HA, 2010).

Table – 1 Phytochemical screening of different extracts of leaves of *Barleria prionitis*.

S.No.	Phytochemical	Aqueous	Ethanol	Acetone	Di ethyl ether	Methanol
1	Alkaloids	+	+++	++	+	+++
2	Steroids	-	+	+	+	++
3	Terpenoids	++	+++	+++	+	+++
4	Tannins	+	+++	+++	+	+++
5	Flavonoids	+	++	++	+	+++
6	Saponin	++	++	++	+	+
7	Anthraquinone	++	++	++	+	+
8	Coumarin	+	++	++	+	++
9	Phenols	++	++	++	+	++

(- = absence, + = presence)

Evaluation of antibacterial activity of extracts:

This study was conducted to evaluate the efficacy of ethanol, methanol, aqueous di-

ether and acetone extracts of *Barleria prionitis* against selected human bacteria pathogens namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The ethanolic extract of *Barleria prionitis* significantly inhibited the growth of all the bacterial pathogens, whereas the aqueous extract did not inhibit the growth of any bacteria. However, ethanolic extract at 100% concentration posed more lethal effect followed 50% concentration. Table-2 showed antibacterial activity of ethanol, methanol, di ethyl ether, acetone and aqueous extracts of *Barleria prionitis* (leaves). Aqueous extracts had no activity against the test bacteria. The result of antibacterial activity of ethanol, methanol and aqueous extracts of *Barleria prionitis* leaves showed that ethanol extracts had good activity against *Staphylococcus aureus* and *Bacillus subtilis*, this study is in agreement with a study by Omokhua et

al., (2008) who also reported that crude extract of neem plant was very effective against *Staphylococcus aureus* and *E. coli* (Saba et al., 2011). These antibiotic principles are actually the defensive mechanism of the plants against different pathogens (Hafiza, 2000). *E. coli* was less susceptible to all the plant extracts. This study is in conformity with the study of Saradhajyothi (Sofowara, 1993) which showed that neem plant leaf posse good antibacterial activity, The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals (Talaly and Talaly, 2001).

Table – 2 Showing the antibacterial potential of different extracts of leaves of *Barleria prionitis* against various bacterial strain: (Inhibition zone in mm)

S.No.	Bacterial Culture Name	Aqueous	Ethanol	Acetone	Di ethyl ether	Methanol
1	<i>E.Coli</i>	-	2.2±0.22	2.6±0.19	4.0±0.27	2.9±0.28
2	<i>Pseudomonas aruginosa</i>	-	4.6±0.27	5.5±0.28	4.3±0.23	5.4±0.33
3	<i>Staphylococcus aureus</i>	-	8.1±0.17	8.3±0.56	6.8±0.18	7.9±0.37
4	<i>Bacillus subtilis</i>	-	7.7±0.24	7.8±0.36	7.6±0.28	8.2±0.33

Conclusion

Determination of the natural phytochemicals and antimicrobial compounds can facilitate to develop of new drug candidates for antimicrobial medical aid. *Barleria prionitis* is used regionally for herbal drugs however, nonetheless to be totally explored. Our results suggest that *Barleria prionitis* can serve as potential source of bioactive healthy compounds and their consumption could be useful in the prevention of diseases. It is also suggested that aqueous extracts were autoclave-sterilized before use as autoclaving is reported to cause less damage to the antibacterial activities of the aqueous extract. However, further studies

ought to be administered on this plant so as to isolate, identify, characterize and elucidate the structure of the bioactive compounds and verify their mechanism of action.

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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