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Antibacterial and phytochemical Analysis of Leaves Extract *Barleria cuspidata* against Common Human Pathogens*:* An *invitro* Study

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A B S T R A C T

Phytochemical components of *Barleria cuspidata* leaves extract were detected in aqueous, methanol, ethanol and acetone extracts, and their antibacterial activity was tested against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter aerogenes. Pseudomonas aeruginosa* and *Salmonella typhimurium.* There were anthraquinones, alkaloids, saponins, tannins, glycosides, and phenolic compounds detected in the acetone extract; however, the methanol and ethanol extracts had a slightly higher antibacterial activity than aqueous extract. Various extracts of leaves of *Barleria cuspidata* are showing antibacterial properties which will lead to the development of some compounds that could be used to develop new and more effective natural antimicrobials. The active constituents of these plants considered to be responsible for their antibacterial properties are still being isolated and identified.

Introduction

There are over 6,000 medicinal plants in India, which have provided the nation with natural health products for thousands of years. Plants are the richest source of drugs for traditional systems, modern medicine, nutraceuticals, and nutritional supplements. Folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Medicinal plants are widely used to treat human illnesses all over the world since they contain components with therapeutic value (Sharma *et al.,* 2009). Secondary metabolites produced by plants in response to bacterial infections have been used as medicine since the dawn of time. According to World Health Organization (WHO, 2000) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

In the pharmaceutical sector, natural products play an essential part in drug development. There are several stories on the traditional usage of medicinal herbs by tribal peoples or indigenous populations. (Rastogi and Mehrotra, 2002). Plants and plant

derivatives have long been used as medicines, dating back to the dawn of civilization. Over the last two centuries, research into the biological functions of plants has provided a plethora of chemicals for the development of contemporary medications (Yuan *et al.,* 2016). Plants have medical value because they contain a chemical compound that has a specific physiological function on the human body. Alkaloids, saponins, flavonoids, tannins, and phenolic compounds are the most important bioactive chemicals (Nadkarni, 2005; Chaudhari *et al.,* 2018)

Emergence of multidrug resistant pathogens has been reported to be one of the leading causes of death world (Reddy *et al.,* 2009) wide with infectious diseases responsible for 68% of all deaths globally in 2012 (WHO, 2000). Many infectious bacteria have developed resistance to synthetic medications, which has become a serious issue for health institutions, pharmaceutical corporations, and governments around the world; hence, an alternate therapy is required (Tambekar and Dahikar, 2011).

The *Barleria Cuspidata* (Family: Acanthaceae) which is commonly known as piwali koranti or kate koranti in Marathi. These plant shows ethnomedicinal properties due to presence of bioactive compound in it. The chemical constituent which present in plant known phytochemical, such phytochemicals have potential to work against the microbial pathogen (Nadkarni, 2005). These phytochemicals present in the different part of plants such as leaves, roots, fruits, etc. as a secondary metabolite (Alkaloids, Phenols, Terpenoids, Tannins, Quinones, cardiac glycosides, saponins, carbohydrates flavonoids and proteins, etc.)

A growing body of plant indicates that secondary plant metabolite plays vital role in human health and may nutritionally important. Extraction and characterization of phytochemical are an important part of research. There is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity (Mazumder *et al.,* 2009; Anonymous, 1998; Gunabakshi, 1999). Many researchers had studied antimicrobial activity of other parts of plant like bark, leaves and fruits of *Barleria cuspidata* which are used to cure many infectious diseases in traditional system of medicine but still very, less work has been done on antibacterial activities of leaves of *Barleria cuspidate.*

To prove the validity of traditional medicine the present work has been undertaken to evaluate the antimicrobial screening of leaves of *Barleria cuspidata* against the human bacterial pathogens.

Materials and Methods

Sample Collection

Barleria cuspidata

Barleria cuspidata leaves were collected from Badami Village of Karnataka State, India in the month of March and authenticated by Botanical Survey of India, Pune (M.S), India.

Preparation of plant material

Leaves were collected and dried at room temperature. The dried samples were powdered separately. 100gm each of the sample was extracted separately with different solvents starting with polar to non-polar solvents in the order of aqueous, ethanol, methanol and acetone. The crude residues were obtained by removing the solvents in rotary evaporator and each of the extracts were resuspended in the respective solvents for further study.

Preparation of extracts

Solvent extraction method Thirty grams of dried powder of *Barleria cuspidata* leaves were extracted with aqueous, ethanol, methanol and acetone using soxhlet apparatus for 48 hrs. The collected extracts were filtered with Whatman No.1 filter paper and used for estimation of phytochemicals and antibacterial activity.

Phytochemical screening

Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001).

Test for Tannins

To 0.5 ml of extract solution, 1 ml of distilled water and one to two drops of ferric chloride solution were added, observed for blue or green black coloration.

Test for Saponins

Two ml of distilled water was added to 2 ml of the test solution shaken well and observed for frothing.

Test for Flavonoids

A volume of 1.5 ml of 50 % methanol was added to 4 ml of the extracts. The solution and magnesium metal were added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid were added to the solution and observed for red coloration.

Test for Steroids (Salkwoski's test)

Five drops of concentrated sulphuric acid (H2SO4) were added to 2 ml of each extract and observed for red coloration.

Test for Glycosides

To 4 ml of extract solution and add few drops of glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid and observed for a reddish brown coloration at the junction of 2 layers and bluish green colour in upper layer.

Test for Alkaloids

To 4 ml of extract filtrate, a drop of Mayer‟s reagent was added along the sides of the test tube. Creamy yellow or white precipitate indicates that the test is positive.

Test for Anthraquinones

One gram of powdered plant material was taken and extracted with 10 ml of hot water for five minutes and filtered. Filtrate was extracted with 10 ml of CCl4 then CCl4 layer was taken off. Five ml water and 5 ml dilute ammonia solution was added. No free anthraquinones were revealed as absence of appearance of pink to cherry red colour. One gram of second sample of the same plant material was extracted with 10 ml of ferric chloride solution and 5 ml of hydrochloric acid then it was heated on the water bath for 10 minutes and filtered. Filtrate was cooled and treated as mentioned above.

Test for phenolic compounds

Two ml of extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5 % ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds

Bacterial cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India, and used in the present study (table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18h and then stocked at 4^0C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37^0C for 3h until the culture attained a turbidity of 0.5 McFarland unit. The final inoculum size was standardized to 10^5 CFU/mL with the help of SPC and Nephlo-turbidometer.

Preparation of disc for antibacterial activities

The aqueous, ethanol, methanol and acetone extracts were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted extract in such concentration that the

amount of solution absorbed by each disc was 1mg, 2mg, 3mg, 4mg, 5mg of each extract of *Barleria cuspidata* leaves. The prepared disc was dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial activity using disc diffusion method

The modified paper disc diffusion method was employed to determine the antibacterial activity of aqueous, ethanol, methanol and acetone extracts. Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002). Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc was placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^0C.The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive.

Results and Discussion

The antibacterial activity has been attributed to the presence of some active constituents in the extracts. Phytochemical screening of the stem bark extract of *Barleria cuspidata* in the present study also revealed presence of terpenes and glycosides. In the Indian frameworks of medication, the greater part of the expert's detail and apportion their own plans without legitimate proof; thus, this requires appropriate documentation and research (Stephen and Richard, 2004). India is the biggest maker of therapeutic spices and is appropriately called the botanical garden of the world (Ahmedulla and Nayar, 1987).

As shown in the antibacterial profile (table 3), the aqueous extract had a maximum inhibitory effect only on *Staphylococcus aureus*.

However, it showed a moderate antibacterial effect against *Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes*, as well as mild inhibitory effects against *Salmonella typhi*. Extracts derived from methanol and ethanol showed a strong antibacterial effect against *Staphylococcus aureus* and a moderately antibacterial effect against *Escherichia coli, Enterobacter aerogenes* and *Salmonella typhimurium* but had a mild effect against *Pseudomonas aeruginosa*.

An ethanol extract and methanol extract showed excellent antibacterial activity against *Staphylococcus aureus* and moderate activity against *Escherichia coli, Enterobacter aerogenes*, and *Salmonella typhimurium*, but only mild activity against *Pseudomonas aeruginosa*. Methanol and ethanol extracts showed significant antibacterial activity against *Staphylococcus aureus*, moderate antibacterial activity against Escherichia coli, *Enterobacter aerogenes*, and *Salmonella typhimurium*, but mild effect against *Pseudomonas aeruginosa*. Acetone extract showed maximum inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium*, but moderate inhibitory effect on *Escherichia coli, Enterobacter aerogenes.* The medicinal properties of compounds derived from plants have been reported by several researchers. It is not surprising that plant extracts containing these classes of compounds have curative properties against a number of bacteria and are often used by herbalists to combat illnesses associated with bacteria.

Accordingly, these results suggest that the phytochemical constituents identified in this study might be the bioactive constituents responsible for the efficacy of *Barleria cuspidata l*eaves extract. The methanolic extract of the leaves of this plant possess wound healing activity and thus justifies its use in folklore medicine.

Table.1 Bacterial cultures used in study (IMTECH, Chandigarh, India)

Bacterial Pathogens	MTCC Number
Staphylococcus aureus	**96**
Escherichia coli	**739**
Pseudomonas aeruginosa	**424**
Klebsiella pneumoniae	**109**
Enterobacter aerogenes	**111**
Salmonella typhimurium	**98**

Table.2 Phytochemical analysis of leaves extract of *Barleria cuspidata*

Sr.No	Phytochemical Constitutes	Aqueous extract	Ethanol extract	Methanol extract	Acetone Extract
1	Alkaloid	+	+	+	+
2	Flavonoids	+	++	++	+++
3	Glycosides	+	+	+	+
4	Saponins	-	++	++	+
5	Steroids	-	+	+	+
6	Tannins	+	++	+++	+++
7	Anthroquinones	-	+	+	+
8	**Phenolic compounds**	**-**	**+++**	**+++**	**+++**

Table.3 Antibacterial activity of *Barleria cuspidate* leaves extracts against bacterial pathogens (Zone of inhibition of growth in mm, average of 3 readings)

Solvent extract	*S. aureus*	*E. coli*	*P. aeruginosa*	*Klebsiella pneumonia*	*E. aerogenes*	*S. typhimurium*
Aqueous	27	-	21	-	-	-
Ethanol	25	21	21	19	17	23
Methanol	27	22	23	17	19	18
Acetone	27	21	21	17	19	17
Water (Control)	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-
Methanol	-	-	-	-	-	-
Acetone	-	-	-	-	-	-
Ampicillin (10mcg/disc)	24	11	16	18	30	19

The preliminary findings can be further substantiated through further investigation and biochemical evaluation. Ultimately, it is hoped that these studies will lead to the development of new and more effective natural antimicrobial drugs.

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