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Preliminary Phytochemical analysis and Antimicrobial activity of Leaves of *Barleria longiflora* Linn.

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Abstract

Present investigation deals with the preliminary phytochemical analysis and antibacterial activity of *Barleria longiflora*. The leaves of this plant was subjected to successive Soxhlet extraction using petroleum ether, chloroform and ethanol. The various leaf extracts, were subjected to preliminary phytochemical screening for different classes of phytoconstituents. Phytochemical analysis, ethanol extracts showed presence of all compounds. The three different concentrations range from 30 , 60 and 90 µl /ml of the extracts were tested for antibacterial activity using agar disc diffusion assay method against *Salmonella typhi*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Streptomycin was used as a reference drug. The ethanolic extract exhibit highest zone of inhibition against *Staphylococcus aureus* is 36 mm with 90 µl /ml concentration and followed by *Streptococcus pyogenes.*

Keywords: Phytochemical, Antibacterial, *Barleria longiflora*, *Escherichia coli*, *Klebsiella pneumoniae.*

Introduction

The search for antimicrobial agents from plants has been a growing interest in the last few decades. The threat of the disease in day to day life, increases the invention of new drugs for various diseases. Even today the world is in crisis of medicine for new illeness. There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. Higher plants produce hundreds of thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991). It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms (Harborne, 1990). These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of them to yield active principles (Zgoda-Pols *et al*., 2002). From an estimated 250,000 higher plants in the world, only 5–15% has been studied for a potential therapeutic value (Kinghorn, 2003). A large number remains to be investigated. Medicinal plants have long been the subject of human curiosity and need. Plant-derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations

that are currently recommended by medical practitioners and they form an important part of the health-care system in the western world. It is estimated that there are about 2,500,000 species of higher plants and the majority of these have not been examined in detail for their pharmacological activities. The antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Perumalsamy *et al*., 2000), and a few attempts were made on inhibitory activity against certain pathogenic bacteria and fungi (Taylor *et al*., 1995).

Barleria longiflora (Acanthaceae), It common name is long flowered barleria and kattu mullai in Tamil. It is small shrub grow up to 1-2 m. Steams are covered with glandular hairs and oppositely arranged ovate long pointed leaves with densely hairy on both sides. Leaves are 4-7 cm long and leaf base is rounded and the stalk is about 1 cm long. Flowers have a very long and narrow tube 8-9cm long and white with bluish purple tinge colour. Flowers are borne singly or groups of 2-3 on top of stalk in leaf axils on October-December moths. Root decoction in dropsy. Powder from the dried whole plant taken with water to treat poisonous bites (Chennaiyan *et al*., 2016). In the present study, preliminary phytochemical screening and antibacterial activity of selected bacteria tested against *B. longiflora.*

Materials and Methods

Collection of plant materials

The leaves of *Barleria longiflora* Linn. was collected from Pullian Solai (Longitude 78º 17'05" to 78º 27'45" and North Latitude 11º 55' 05" to 11º 21'10"), Namakkal District, Tamil Nadu, India. Plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India. The voucher specimen of *B. longiflora* (IPH No. 3) was prepared and deposited in PG and Research Department of Zoology, Government Arts College, Musiri, Tamil Nadu, India.

Test microorganisms

The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* and *Salmonella typhi*. The bacterial cultures were maintained on nutrient agar medium.

Preparation of Inoculum

The gram positive bacteria *Streptococcus pyogenes, Staphylococcus aureus,* and gram negative bacteria *E. voli, Klebsiella pneumoniae, Salmonella typhi,* were pre-cultured in nutrient broth over night in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm).

Anti-bacterial Activity

The solvent crude extracts of petroleum ether, chloroform and ethanol with three different concentrations were tested for antibacterial activity using agar disc diffusion assay method (Bauer *et al*., 1996). The plant leaves extract of *Barleria longiflora* was tested by the well diffusion method. Different concentration of the extracts (100 µg/ml) was prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptomycin (10µg/disc) was used as standards. All data on antibacterial activity were average of triplicate. The solvent loaded disc without extracts in it served as control. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained.

Preliminary studies on phytochemical screening

The leaf, extracts of *B.longiflora* were tested for the presence of major phytochemicals such as alkaloids, flavonoids, tannins, phytosterols, saponins, glycosides, flavonol glycosides, fixed oils, phenolic compounds, carbohydrates and proteins according to standard methods (Raman, 2006).

Alkaloids

Hager's test (Wagner *et al*., 1996)

Solvent free extract, 50 mg was stirred with 5 mL of dilute hydrochloric acid and filtered. To the filtrate, 2 mL of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Flavonoids

Alkaline reagent test (Raman, 2006)

An aqueous solution of the extract was treated with 10 % ammonium hydroxide solution. A bulky white precipitate indicated the presence of flavonoids.

Tannins

Potassium hydroxide test (Williamson *et al*., 1996)

The extract (0.5 g) was added into 10 mL of freshly prepared 10 % potassium hydroxide (KOH) in a beaker and shaken to dissolve. A dirty precipitate indicated the presence of tannin.

Phytosterols

Libermann and Burchard's test (Finar, 1986)

About 50 mg of extract was dissolved in 2 mL of acetic anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the sides of the test tube. An array of colour changes showed the presence of phytosterols.

Saponins

Frothing test (Kokate, 1999)

The extract (50 mg) was diluted with distilled water and made up to 20 mL.
The suspension was shaken in a graduated cylinder for 15 minutes 2 cm layer of foam indicated the presence of saponins.

Glycosides

Borntrager's test (Evans, 1997)

50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 h on water bath and filtered. To 2 mL of filtered hydrolysate, 3 mL of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added to it. Pink colour indicated the presence of glycosides.

Flavonol glycosides

Magnesium and hydrochloric acid reduction (Harborne, 1990)

The extract (50 mg) was dissolved in 5 mL alcohol and few fragments of magnesium ribbon were added. Concentrated hydrochloric acid was added drop wise into the test tube. Development of pink or crimson colour indicated the presence of flavonol glycosides.

Fixed oils and fats

Saponification test (Kokate, 1999)

A few drops of 0.5 N alcoholic potassium hydroxide solutions were added to a small quantity of extract along with a drop of phenolphthalein. Then the mixture was heated on boiling water bath for 2 h. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils

Phenolic compounds

Ferric chloride test

About 50 mg of the extract was dissolved in 5 mL of distilled water. To this, few drops of 5% neutral ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green colour.

Results and Discussion

Preliminary phytochemical was studied in petroleum ether, chloroform and ethanol extracts of *B. longiflora*. Among the crude extracts tested, ethanol extract showed the presence of alkaloids, flavonoids, tannins, phytosterols, anthraquinones and phenolic compounds and presented in table 1. Antibacterial activity of petroleum ether, chloroform and ethanol extract of *B.longiflora* tested against five important human pathogenic bacteria *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* and *Salmonella typhi* at 30, 60 and 90µl concentrations and the data pertaining to the experiments are shown in table 2. Among the tested bacteria the results showed the highest zone of inhibition against *Staphylococcus aureus* is 36 mm in 90 µl /ml ethanolic extract. Whereas, chloroform extracts showed the maximum zone of inhibition is 32mm in 90 µl /ml and followed by 26mm in pertoleum ether extract of same concentration.

Table 1. Preliminary phytochemical analysis of different crude extracts of *B. longiflora*

S.No	Compounds	Tests/Methods	Results		
			Petroleum Ether	Chloroform	Ethanol
1	Alkaloids	Hager's test	-	-	+
2	Flavonoids	Alkaline reagent test	-	+	+
3	Tannins	Potassium hydroxide test	+	-	+
4	Phytosterols	Libermann - Burchard's test	-	-	+
5	Saponins	Frothing test	-	-	-
6	Glycosides	Borntrager's test	-	-	-
7	Anthraquinones	Dragendroff's test	+	-	+
8	Fixed oils	Saponification test	-	_	-
9	Phenolic compounds	Ferric chloride test	+	+	+

+ Denotes Presence of compound - Denotes Absence of compound

Table 2. Effect of petroleum ether extract of *B. longiflora* on different group of bacteria

Bacteria	Zone of inhibition (mm)									Standard (Streptomycin)
	Petroleum ether			Chloroform			Ethanol			
	30 µl	60 µl	90 µl	30 µl	60 µl	90 µl	30 µl	60 µl	90 µl	90 µl
Salmonella typhi	8	10	21	-	10	25	12	18	22	30
Streptococcus pyogenes	9	15	24	7	9	20	10	25	34	35
Staphylococcus aureus	7	14	26	7	18	32	12	20	35	35
Escherichia coli	7	10	21	7	17	31	24	29	31	33
Klebsiella pneumoniae	-	8	14	8	14	21	18	27	30	32

Values showing in the table are zone of inhibition obtained through disc diffusion method; Control = commercially available chemical drug: Streptomycin.

Phytochemical analysis conducted on the plant extracts revealed that the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993). Phytochemical constituents of the plant *B. longiflora* leaf extracts showed the presence of phenols, alkaloids, tannins and flavonoids, it may influence the bioactivity . The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Crown, 1999). Preliminary phytochemical analysis showed the presence of tannins and phenolic compounds. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). In the present study ethanol extracts of *B. longiflora* showed highest inhibition activity on

Staphylococcus aureus. The present findings agreed with recent works. Mohamed Mahmoud Jouda, (2013) assessed the antibacterial effect of some medicinal plant extracts and their synergistic antibiotic and non-antibiotic drugs against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Their results showed that ethanolic extracts used against *E. coli*, *S. aureus* and *P. aeruginosa* were showed antimicrobial and synergistic effect with most antibiotics better than methanolic and aquatic extracts. Water extracts were showed synergistic effect with the Paracetamol and Loperamide Hcl better than methanolic and ethanolic extracts against *E. coli* and *S. aureus*. Ethanolic extracts were showed synergistic effect with the Paracetamol and Loperamide Hcl better than methanolic and aquatic extracts against *P. aeruginosa*. Previous studies showed that some researchers like (Singh *et al*., 2002; Naziri *et al*., 2012; Vasconcelous *et al*., 2003) stated that *Punica granatum* peel extracts in different concentrations were effective against *Streptococci* strains (*Streptococci mutans*; *Streptococci aureus*; *Streptococci salivarius*; *Streptococci sanguinis* and *Streptococci epidermidis*). So, it is concluded that *B. longiflora* possesses good antibacterial activity. The antimicrobial activity of *B.longiflora* may be due to the presence of phenolic compounds, alkaloids.

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