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# Anti-Microbial Activity of *Barleria longiflora* L.F Against Human Pathogenic Microbes

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***Abstract:*** **The present study was designated to evaluate the antimicrobial activities of ethanolic extract of *Barleria longiflora* L.f. The antimicrobial activities of the extract against 6 strains belong to bacterial and fungi species were tested by using Disc diffusion method. The results showed that ethanolic extract of *Barleria longiflora* L.f had moderately significant antibacterial and significant antifungal activity. It inhibited the growth of both bacterial and fungal species dose dependently. The inhibition of growth was highest at 100mg/ml as compared to the controls. Ethanolic extract showed stronger antimicrobial activity against the fungi than that of the bacteria's. Thus we can conclude that *Barleria longiflora* L.f was a potent antimicrobial agent which can be tried as a novel anti-fungal agent.**

***Keywords:*** **Antimicrobial, *Barleria longiflora* L.f and Disc diffusion.**

## 1. INTRODUCTION

Medicinal plants have been identified as a part of the evolution of human healthcare for thousands of years. Medicinal components from plants play an important role in traditional as well as modern medicine[1]. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or synthetic derivatives [2]. The side effects associated with the extensive use of the synthetic medicines may lead to serious damages to many of human organs. Therefore, to overcome this limitation of synthetic drugs, researchers have shifted their focus towards medicinal plants which are recognized as rich sources of antimicrobial agents and are widely used by different countries for medicinal purposes. Traditionally used medicinal plants are known to produce a variety of compounds with therapeutic properties, such as antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic, gastroprotective effects [3].

Plants have diverse concentrations of bioactive constituents such as alkaloids, saponins, tannins, terpenoids, steroids, carbohydrates, proteins and lipids. These phytochemicals are used against an extensive range of bacteria (*Escherichia coli, Mycobacterium, Corynebacterium pervum, Bordetella pertusis, Klebsiella pneumoniae, Salmonella typhi*), viruses (simian-virus, retrovirus) and fungi (*Pseudomonas aeruginosa, Aspergillus fumigatus, Aspergillus flavus, Fusarium solani*) [4].

Plant extracts and their components have been known to exhibit biological activities, especially antimicrobial [5], antifungal [6], antibacterial [7] and antioxidant activities [8]. These compounds find in various medicinal plant organs such as stems, roots, leaves, barks, flowers, fruits and seeds [9]. The most important of these medicinally compounds are alkaloids, tannins, flavonoids and phenolic compounds [8]. Antimicrobial resistance is an increasingly serious threat to global public health. According to World Health Organization (WHO) report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major issue to the WHO for the next millennium. Screening of plants for antimicrobial agents has gained much importance because WHO is encouraging and promoting in the development and utilization of medicinal plant resources in the traditional system of medicine. Accordingly, the last decade witnessed an increase in the investigation of plants as a source of human infectious disease management [1].

There is a continuous and 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 [10]. Antimicrobial susceptibility testing against pathogenic microorganisms is the most significant task of clinical microbiology laboratory. Plants have a broad variety of antimicrobial agents which are extensively used as herbal drugs against different microbes. A variety of antibiotics (tetracycline, terramycin, ampicillin) has also been isolated from different medicinal plants [4].

To the best of our knowledge, similar studies have not been carried out on the *Barleria longiflora*.

## 2. PLANT COLLECTION

The plant materials (Leaf, Stem, and Root) were freshly collected in Rettamalai, Tiruchirappalli District, Tamilnadu, India. The plant was identified and authenticated (BSI/SRC/5/23/2014-15/Tech/538) by Dr.G.V.S.Murthy, Scientist 'F' & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India.

## 3. PREPARATION AND SELECTION OF PLANT EXTRACT

The collected plant material were shade dried, coarsely powdered and extracted by using hot continuous extraction technique in a soxhlet extractor using solvent ethanol until the extracts were colorless in the siphon tube. The extracts were concentrated and dried under vacuum.

## 4. TEST MICROORGANISMS

The following bacteria and fungi were used for screening the antimicrobial activity. Gram Positive bacteria such as *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063). Gram negative bacteria such as *Proteus vulgaris* (NCIM 2027), *Klebsiella aerogenes* (NCIM 2098) and Fungi such as *Candida albicans* (NCIM 3102), *Aspergillus niger* (NCIM 1054) were utilized for the study. The test microorganisms were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and fungi respectively.

## 5. ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The antibacterial and antifungal activity analyses were carried out by disc diffusion technique. The bacterial strains were inoculated into nutrient broth and incubated at 37 $^0$c for 18 hours. The Standardized inoculum about 0.1 ml was inoculated on Muller Hinton Agar (Hi media) uniformly. The sterile disc (watt man No. 2 of 6 mm diameter) was placed at equal interval on uniformly inoculated plate and a standard disc Ciprofloxacin 5 µg/disc was also placed by aseptic technique. The test sample about 100 µl was loaded to the sterile disc by using aseptic precautions. The plates were incubated at 37 $^0$ C for 24 hours.

The fungal strains were inoculated were brought to the active phase by sub culturing in Sabouraud Dextrose broth and incubated at room temperature for 4 days. The Standardized inoculum about 0.1 ml was inoculated on Sabouraud Dextrose Agar uniformly. The sterile disc (watt man No. 2 of 6 mm diameter) was placed at equal interval on uniformly inoculated plate and a standard disc Nystatin 100 units/disc was also placed by aseptic technique. The test sample about 100 µl was loaded to the sterile disc by using aseptic precautions. The plates were incubated at room temperature for 2 to 4 days. At the end of incubation, inhibition zones formed around the disc. The diameter of the inhibition zones observed and its value noted (in mm).

## 6. RESULTS AND DISCUSSTION

Herbal plants are nature's gift used to prevent and control the diseases in all over the world [11]. So that the antimicrobial activity of *Barleria longiflora* has been evaluated in vitro against four bacteria and two fungus species. They are frequently incriminated in human infection. Most of the tested plant extracts showed some level of antimicrobial activity. The result of the antimicrobial activity of the ethanolic extract of *Barleria longiflora* stem on showed maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (30mm) and Root on minimum against Gram negative bacteria *Bacillus subtilis* (8mm). *Barleria longiflora* root showed maximum antifungal activity towards Candida albicans (28mm) and leaf and stem showed minimum antifungal activity (26mm). The stem and leaf showed maximum antifungal activity towards *Aspergillus niger* (28mm) and minimum activity 25mm showed in root (Table 1 & 2).

The previous results showed that medicinal plants which were used in traditional medicine against infections may have some antimicrobial activity. This is true for *Barleria longiflora* L.f ethanolic extract. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [12]. In the present work, the extracts obtained from *Barleria longiflora* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. In this screening work, extracts of *Barleria longiflora* were found to be not inactive against any organism, such as Gram-positive, Gram-negative, and fungal strains were resistant to all the extracts of *Barleria longiflora* L.f.

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The antibacterial activities can be enhanced if the active components are purified and adequate dosage determined for proper administration. At last, the need of the hour is the development of an effective phytocompound into an exploitable herbal product, which is devoid of side effects and drug resistance problem [13]. The results showed significant activity of *Barleria longiflora* and suggesting its use as natural antimicrobial agent. The result of present study indicated that ethanolic extract of *Barleria longiflora* shows potent antimicrobial and antifungal activity.

## 7. CONCLUSION

From the recent study it is concluded that, as dose of the *Barleria longiflora* L.f increases the antimicrobial activity as well as antifungal activity increases. From the observations it clearly indicate that *Barleria longiflora* L.f has potent antimicrobial activity as well as antifungal activity but it act by dose dependent manner.

**Table 1: Effect Of *Barleria longiflora* L.f Extract On Growth Of Bacteria *In Vitro* Zone Of Inhibition (Mm).**

| S.No. | Name of the Microorganism | Zone of inhibition in mm | | | | |
|---|---|---|---|---|---|---|
| | | B.l Leaf | B.l Stem | B.l Root | Solvent control | Standard |
| 1. | *Staphylococcus aureus* ( NCIM 2079) | 28 | 30 | 26 | NIL | 35 |
| 2. | *Bacillus subtilis* (NCIM 2063) | 14 | 12 | 08 | NIL | 40 |
| 3. | *Proteus vulgaris* (NCIM 2027) | 26 | 20 | 10 | NIL | 30 |
| 4. | *Klebsiella aerogenes* (NCIM 2098) | 29 | 14 | 12 | NIL | 30 |

**Standard:** Ciprofloxacin 5µg /disc for bacteria.

**Solvent:** DMSO

**Table 2: Effect Of *Barleria longiflora* L.f Extract On Pathogenic Fungi Zone Of Inhibition (Mm).**

| S.No. | Name of the Microorganism | Zone of inhibition in mm | | | | |
|---|---|---|---|---|---|---|
| | | B.l Leaf | B.l Stem | B.l Root | Solvent control | Standard |
| 1. | *Candida albicans* (NCIM 3102) | 26 | 26 | 28 | NIL | 32 |
| 2. | *Aspergillus niger* (NCIM 105) | 28 | 28 | 25 | NIL | 35 |

**Standard:** Nystatin 100 µg / disc for fungi.

**Solvent:** DMSO.

**Figure 1: Effect of *Barleria longiflora* L.f extract on Pathogenic bacteria Zone of inhibition (mm).**

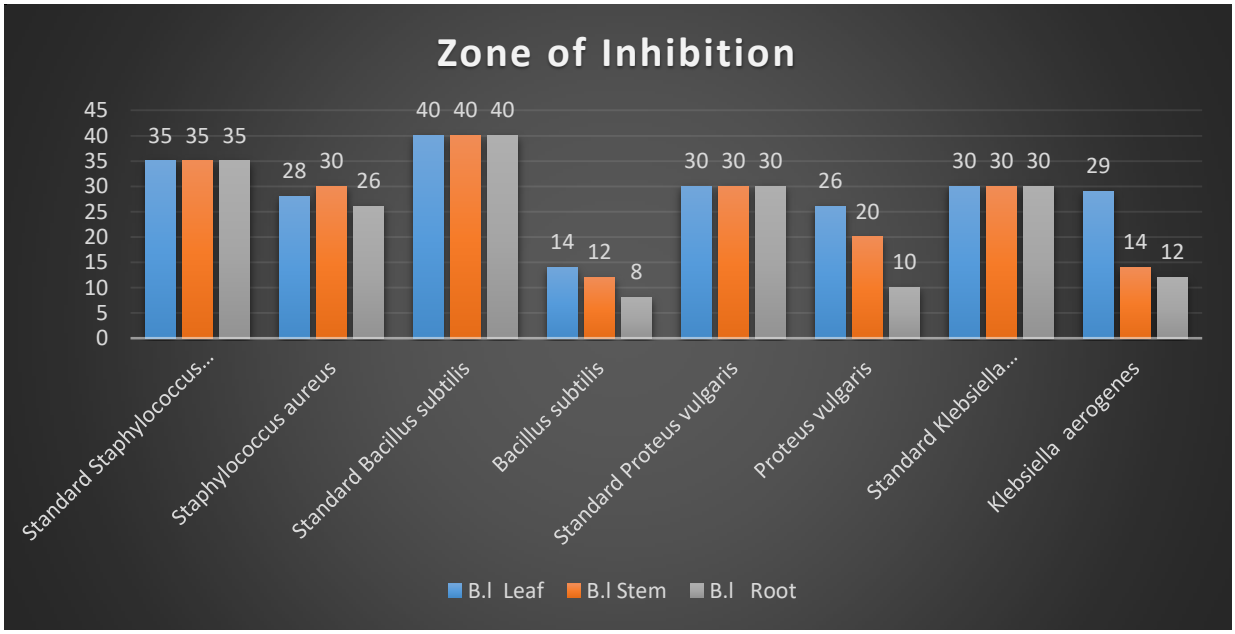

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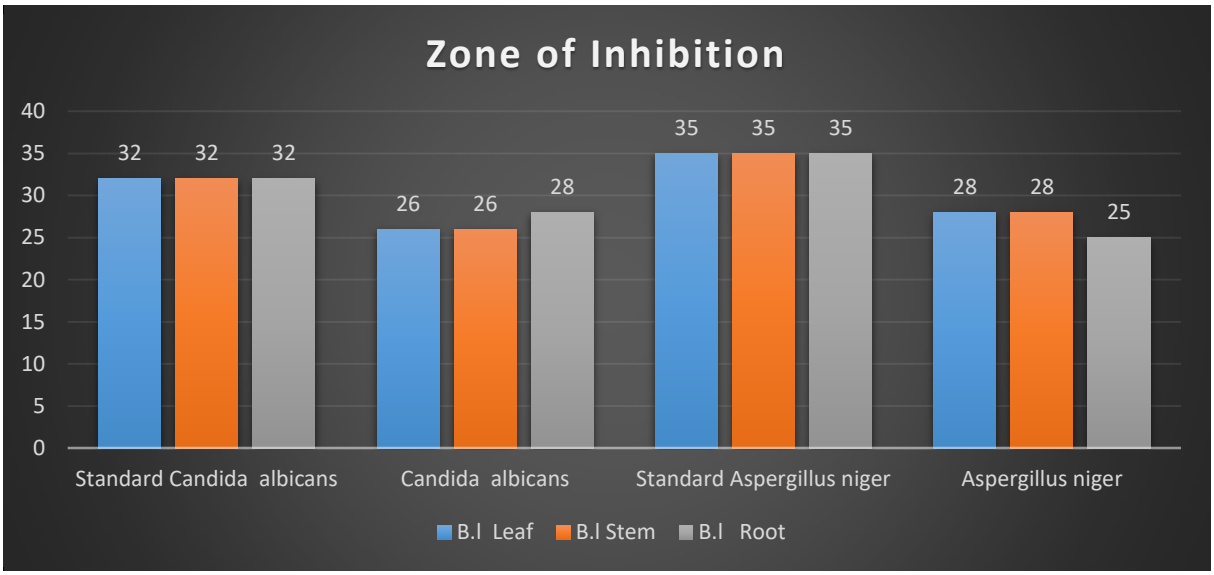
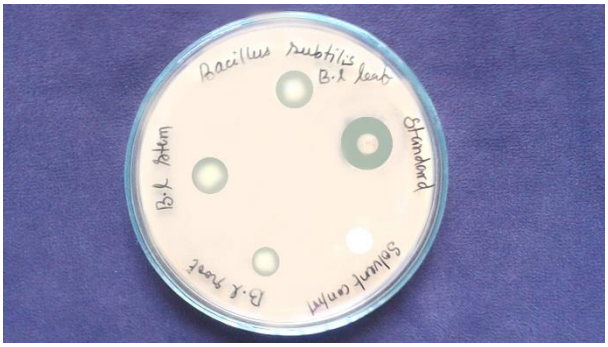
**Figure 2: Effect of *Barleria longiflora* L.f extract on Pathogenic fungi Zone of inhibition (mm).**

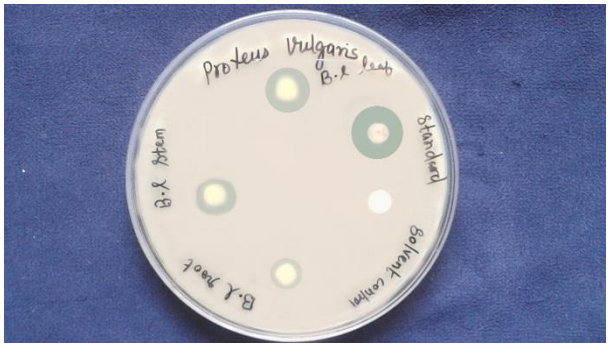



**PLAT 1**

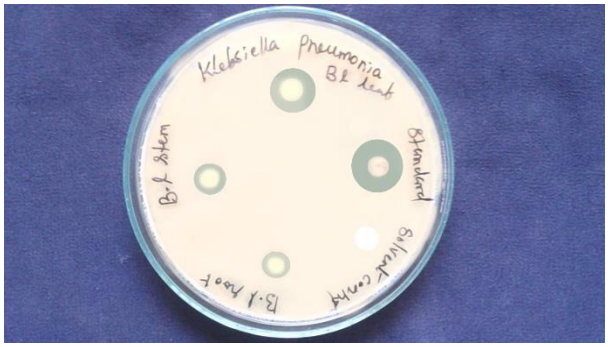

Staphylococcus aureus



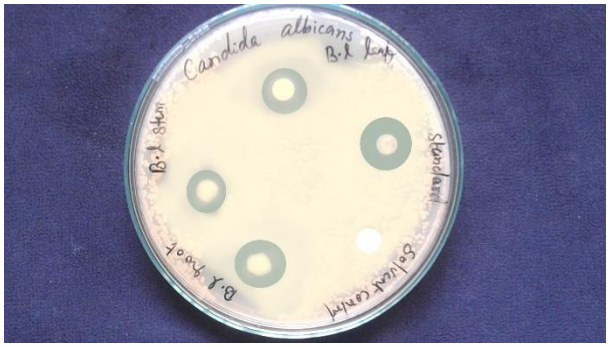

Bacillus subtilis



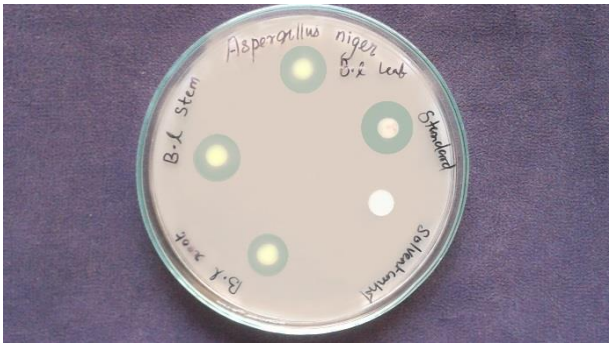

Proteus vulgaris




Klebsiella aerogenes




Candida albicans




Aspergillus niger

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