



A STUDY OF ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS AND AQUEOUS EXTRACTS OF *Leucas longifolia* (Doron) LEAVES AGAINST *Eschreiashia coli*

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ABSTRACT

Leucas longifolia Benth also known as Doron (Assam) is an important herb frequently used in various folk remedies of Assam. The present study was designed to evaluate the antibacterial activity of aqueous extract and ethanol leaf extract of *Leucas longifolia* against disease causing *Eschreiashia coli*. Aqueous extract showed strong and superior antibacterial activity against *Eschreiashia coli* as compared to ethanol extract. These results were compared with standard antibiotic Norfloxacin. But the aqueous extract showed higher activity than the given standard antibiotic. These findings support the traditional use of the plant in the treatment of different infections in the area of medical science.

Key words: Assam, *Leucas longifolia*, Doron, Antibacterial, Norfloxacin

INTRODUCTION

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is due to the use of antibiotics as growth promoters in foods of animals¹. However, the widespread and indiscriminate use of antibacterial agents resulted in development of drug resistance among many virulently pathogenic bacteria species². A study published in the journal in Aug 2007 found that the rate of adaptive mutation in *E.coli* “on the order of 10^{-5} per genome per generation which is 1,000 times as high as previous estimates” a finding which may have significance for the study of bacterial antibiotic resistance³. Many of the currently used antibacterials are associated with adverse effects such as toxicity, hypersensitivity, immune suppression, and tissue residues posing public health hazard. So, further new alternative remedies for treatment of bacterial diseases are usually required. As the global scenario is now changing towards the use of non-toxic and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases.

Leucas longifolia Benth (Lamiaceae) is commonly called ‘Doron’ in Assam (India). In folk remedies of Assam its extract is used as analgesic and antidiarrhoeal. It is perineal herb found in wasted lands and road sides⁴. But Rapid fragmentation of natural habitats is greatly narrowing the distribution of this plant and increasing the risk of losing genetic diversity.

In the present study, the selection of this plant for evaluation was based on its traditional usages. Although very few works have been done on the antimicrobial activity of this medicinal plant, it needs further study for verification of its activity against disease-causing microorganisms. This paper describes the evaluation of the antibacterial potency of *Leucas longifolia* from Assam, India.

MATERIALS AND METHODS

Collection and Sterilization of Plant Materials

Plants were collected from Nameri National park; Sontipur district (Assam), India. The disease free and fresh plants were selected. About 5 g of fresh and healthy leaves were taken for each solvent extraction. They were washed with distilled water for three times. Then surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water for three times. After then plant leaves were air-dried the laboratory for four weeks and ground into powdered form, using a mortar and pestle.

Preparation of Extracts

The powdered plant material (5g) of leaves were percolated in 100mL redistilled ethanol in 1L capacity conical flasks, stopper and kept for two weeks with intermittent shaking. The percolates were filtered with Whatman’s No 1 filter paper. The extracts were Concentrated at 40°C under reduced pressure using rotary evaporator. The same quantity of plant material was again percolated with distilled water for one week and after filtration, the aqueous extract was concentrated in hot oven at 40°C. The concentrated extracts were labeled EEL (ethanol extract of leaf), AEL (aqueous extract of leaf).

Test Organism

E.coli was isolated from urine sample of clinical case at Doon Hospital; Dehradun by spread plate method. This was followed by Cultural and morphological identification as well as biochemical characterization of *E.coli*. Pure cultures of the *E.coli* were maintained in appropriate media for future use.

| Colony Morphology of <i>E.coli</i> on Agar Plate | | | | | |
|--|----------|-----------|--------|-------|-----------------|
| Size (mm) | Shape | Elevation | Edge | Color | Cell morphology |
| 2 | circular | flat | entire | cream | bacillus |

| Physical Test Of <i>E.coli</i> | | | | | |
|--------------------------------|---------------|-------------|-------------|-----------|----------|
| Gram Reaction | Cell Size(µm) | Oxygen use | Glucose Use | Endospore | Motility |
| Negative | 2 | Facultative | Yes, gas | no | yes |

| Biochemical Test Of <i>E.coli</i> | | | | | | | |
|-----------------------------------|--------------|-----------|-----------------------|-----------------|-----|----------------------|--------------------------|
| Starch hydrolysis Test | Nitrate Test | Reduction | Indol Production Test | Methyl Red Test | Red | Voges proskauer Test | Citrate Utilization Test |
| positive | positive | | positive | positive | | negative | negative |

Antimicrobial Disc preparation

The blank discs of about 6mm diameter were made from Whatman's No.1 filter paper using a paper puncher. Batches of 100 discs were transferred into Bijou bottles and sterilized in the oven at 121°C for 15minutes. After sterilization blank discs were separately impregnated with each of extract.

Positive and Negative Control

Blank paper discs impregnated with sterile solvents (Distilled water) served as negative control and where as blank discs impregnated with Norfloxacin (10mg/ disc) was used as positive control for comparison of the antibacterial activity.

Antimicrobial Susceptibility Testing:

The antimicrobial activities were found by using a modified agar well diffusion method⁵.

Antimicrobial bioassay

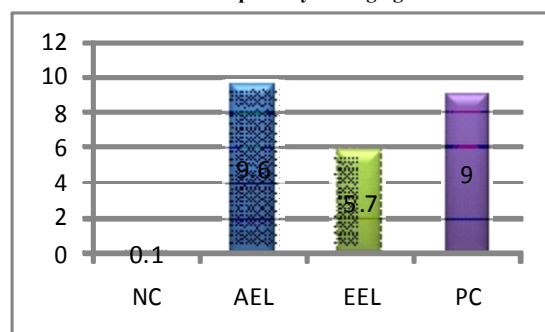
The bacteria were grown in nutrient broth, incubated at 37°C overnight. One ml of the broth culture of bacterium was spread over the nutrient agar taken in glass Petri dishes aseptically. The extract impregnated discs, blank paper discs impregnated with sterile solvents and the reference antibiotic Norfloxacin disc were placed on the inoculated nutrient agar in the Petri dishes and incubated at 37°C. After 24 hr incubation the zones of inhibition of bacterial growth around the discs were measured. The experiments were repeated thrice. All values are expressed as means \pm standard deviation.

| Zone of Inhibition (Diameter in mm) | | | |
|---|-------------------------------|-------------------------------|-------------------------------------|
| Negative control (Distilled Water) (NC) | Ethanol extract of leaf (EEL) | Aqueous extract of leaf (AEL) | Positive Control (Norfloxacin) (PC) |
| 0 \pm 0.1 | 5.5 \pm 5.7 | 9.2 \pm 9.6 | 9 |

RESULTS AND DISCUSSION

The present investigation showed that Aqueous extract zone of inhibition against *E.coli* was 9.2 \pm 9.6 mm, and positive control (Norfloxacin) zone of inhibition was 9 mm. Ethanol extract zone of inhibition against *E.coli* was 5.5 \pm 5.7mm, and positive control was (Norfloxacin) 9 mm.

Antimicrobial Susceptibility testing against *E.coli*



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Aqueous extract of leaves showed good activity against *E.coli* in comparison to EEL and PC. Ethanol extract shows less activity against tested *E.coli* in comparison to AEL and PC. According to the experimental result AEL shows good inhibition against *Eschreiashia coli*.

CONCLUSION

India has about 45000 plant species where several thousands have been claimed to possess medicinal properties⁷. Various parts of plants such as leaves, roots, barks, tubers and seeds are employed in ethanomedicine⁸. A considerable part of this indigenous knowledge was documented from the past into the organize systems of medicines such as Ayurveda, Yunani, Sidha or other systems. Some of these are having activity against microbial species, which are causing dreadful diseases. One such plant is *Leucas longifolia Benth*, an angiosperm (belongs to family Lamiaceae) found to be having antimicrobial activity by present investigation. *Leucas longifolia* is a vulnerable medicinal plant source rarely available. This plant has medicinally important compounds. The medicinal activities and secondary metabolite products have not been extensively documented. Hence further studies are required to identify and characterize chemical compounds present in leaf so that *Leucas longifolia* might be used as better alternative for synthetic antimicrobials.

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