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### *In-Vitro* Assessment of Anti-Microbialactivity of Extracts of *Enicostemma Littorale* Belonging to Family *Gentianaceae*

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#### Abstract

Plants have been used for thousands of years to treat health disorders to prevent diseases including epidemics, flavor, cosmetics and food. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment. Numerous studies presented earlier have proved their chemical constituents and its therapeutic benefits.

**Aim and objective:** Here an attempt is performed to establish antimicrobials activity of extracts of *Enicostemma Littorale* common name *Chhota chirayta*. belonging to Family *Gentianaceae*.

**Materials and Method:** The shade dried leaves of plant

were powdered and using Soxhlet extractor Aqueous and Ethanolic extract was collected. Antimicrobial activity was evaluated for these extracts by cup plate method using nutrient agar medium and gentamicin as reference.

**Results:** The results revealed that of *Enicostemma littorale* MIC at 1000 µg/ml to 32.5 µg/ml while the reference drug gentamicin showed MIC at 1.8625 µg/ml to 3.625 µg/ml.

**Conclusion:** Thus, present study revealed the antibacterial activity of the herb *Enicostemma littorale* has been proved against various species of microbes, giving evidence to utilize this plant extract against the particular microbes satisfactorily.

**Keywords:** Plants, Antimicrobial, Extract, Therapeutics, Activity, Microbes

#### Introduction

Infection is an important cause of morbidity and mortality in mass public especially children's and elderly leading to hospitalization and treated with loads of synthetic drugs including antibiotics. <sup>[1,2]</sup> In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. <sup>[3,4]</sup> Antimicrobials agents are categorized according to their application and spectrum of activity as germicides, bactericide, fungicide etc. where agents inhibit the growth of infection causative pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. <sup>[5]</sup> Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. <sup>[6]</sup> Currently antimicrobial activity of numerous plants and plant produce is established so far empirical with scientifically evident methodology and increasing number of reports are published in reputed journals. <sup>[7,8]</sup>

In the present work, leaves extract of medicinal plants *Enicostemma Littorale* belonging to Family *Gentianaceae* commonly known as *Chhota chirayta* is evaluated for its antimicrobial properties was evaluated using aqueous and alcoholic extract and results are presented for future findings for researchers.

#### Plant profile

##### Taxonomy of *Enicostemma littorale*

Kingdom: Plantae

Subdivision: Angiospermae

Class: Dicotyledonae

Subclass: Gamapetalae

Series: Bicarpellatae

Order: Gentianales

Family: Gentianaceae  
 Genus: *Enicostemma*  
 Species: *Littorale*



Fig 1: Plant *Enicostemma littorale*

The Indian Chhota Chirayta (*Enicostemma littorale*) belonging to the family Gentianaceae, occurs as a weed in diverse natural habitats such as savannas, grasslands, forests to beaches, from wet to very dry habitats, and also survives well in a very saline environment. [9] The plant is also recognized by folk name in different languages and diversity of region like in Sanskrit *Nagajihva*, *Mamajjaka*, *Nahi*, *Tiksnapatra*, *Vitikshnika*, *Krimihrit*, in English *Indian Gentian* and Common Name *Chhotaa Chirayataa*, *Naai*, *Vellargu*, *Mamejavi*, *Nelaguli*. Additional name includes common name in English as Indian Gentian generally refers to *Swertia* spp. Whitehead is a common name that applies to *E. verticillatum*, in the New World. [10]

### Plant Morphology

*Enicostemma littorale* Blume (*E. littorale*) belonging to Gentianaceae family is a highly nutritious perennial medicinal plant and used as a medicine in Siddha Medical System to treat several disease conditions such as diabetes mellitus, rheumatism (Vata diseases), skin diseases (Pitta diseases), constipation, abdominal ulcers, swelling, obesity and insect poisoning. [11] The plant is erect perennial herb, 5–30 cm. tall, simple or branched at the base. Stem cylindrical, glabrous with a decurrent ridge below each leaf. Leaves narrowed into a petiolelike base, longer than the internodes; lamina (1)5–8 x 0.3–1 cm., linear to lanceolate or narrowly oblong, entire, obtuse and mucronate at the apex, somewhat narrowing towards the base, glabrous. Flowers are white with green lines, drying yellowish, sessile or subsessile. Calyx tube 1–2 mm. long; lobes usually unequal, 0.7–1.5(2) x 0.4–0.7 mm., triangular to lanceolate, acute at the apex and narrowly scarious at the margin, or obovate to subcircular, obtuse and mucronate at the apex, with wide scarious margin. Corolla tube 3.5–6 mm. long; lobes 1.5–2 x 0.7–1 mm., ovate and abruptly narrowing to an acute or mucronate apex. Stamens inserted below the sinuses, just above the middle of the tube; filaments 1.5–2.3 mm. long, with a double hood at the insertion point; anthers. 1 mm. long, erect, shortly apiculate. Ovary 5–6 x 1 mm., ovoid; style 2–2.5 mm. long, subulate; stigma subcapitate. Capsule 3–4.5 x 2–2.5 mm., obovoid. Seeds 0.4–0.5 mm. in diam., subglobose, reticulate faveolate. [12, 13]

### Earlier findings: A brief Review

Thayalini T. *et al.* (2012) described about *Enicostemma littorale* as a glabrous perennial herb belonging to the family

Gentianaceae. The whole plant is used in traditional medicine for the treatment of various diseases. Phytochemical screening revealed the presence of alkaloids, saponins, steroids, flavonoids, glycosides and triterpenoids as active functional components. They also demonstrated antimicrobial activity of aqueous extract of the whole plant of *E. littorale* on eight bacterial isolates, *viz.* *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and five wild strains of Methicillin resistant *Staphylococcus aureus* by cut well diffusion and agar dilution methods. They found aqueous extract of *E. littorale* showed growth inhibitory action against *S. aureus* and 5 MRSA strains. Thus, they suggested the activity of the aqueous extract against a wider range of skin pathogens such as Kiranthi, Itching and Scabies. [14] Kowsalya R *et al.* (2019) [15] in their study synthesized silver and Zinc nanoparticles using aqueous extract of *E. littorale* and found in experimental work that there was reduction of Ag<sup>+</sup> ions to metallic Ag which could attribute the antibacterial effect of *E. Littoreale* nanoparticles against gram positive and gram-negative bacteria. [15]

Padul MV *et al* (2008) [16] carried out an experimental work which demonstrated the antibacterial activity of aqueous, ethanolic, methanolic, ether, acetone, chloroform and hexane extracts of plant *E. littorale* in *in vitro* model against *Serratia* sp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Screening of bacterial activity was carried out by agar well method on *E. coli*, *Serratia* sp., *P. aeruginosa* and *S. aureus*. The result stated in their literature emphasize antibacterial activity against *Serratia* sp and *P. aeruginosa* but very less activity against *E. coli* and *S. aureus*. [16] Patel D B *et al.* (2015) [17] evaluated the antimicrobial activity of by *Enicostemma littorale* using ethyl acetate extract of root and stem by Disk diffusion method. The Extract of root and stem show the zone of inhibition against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* may be due to the presence of flavonoid and flavones component. [17]

Deepake U S *et al.* (2012) [18] conducted a study to determine the colonization frequency of endophytic microbes and to evaluate the antimicrobial activity of crude extracts of fungal endophytes from plant *Enicostemma axillare*. They isolated endophytic fungi from leaves, stem and roots of the plant and pure culture were raised. The found highest colonization frequency of bacteria and actinomycetes in leaves while colonization frequency of endophytic fungi was slight less in stem. The endophytic fungi that displayed broad spectrum antimicrobial activity include *Aspergillus flavus*, *Penicillium* sp., *Eurotium* sp., *Sartorya* sp. and *Phomopsis* sp. indicating crude extracts of endophytic fungi of *E. axillare* may possess some antimicrobial compounds. [18] Abirami P *et al.* (2012) [19] studied antimicrobial activity of *Enicostemma littorale* using different solvents extract like Chloroform, methanol and acetone of leaf, stem and root using disc diffusion method against some gram-negative species such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and gram-positive species *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis* and two fungal species *viz.*, *Aspergillus fumigates* and *Aspergillus flavus*. In their findings, chloroform extracts of stem showed the highest antibacterial activity than leaf or root extracts. None of the extracts had shown any significant antifungal activity

against *Aspergillus fumigates* and *Aspergillus flavus*. Thus, this literature suggests the antimicrobial use of *E. littorale* plant. [19]

Indumathi C *et al.* (2014) [20] presented in their experimental study which states presence of terpenoids content of plant *Encostemma littorale* which attributes the antimicrobial activity of said plant. The presence of terpenoids was determined both qualitatively and quantitatively, while different solvent extract was tested for antimicrobial property against *E. coli* and *B. Subtilis* strains of bacteria. There was sufficient zone of inhibition for bacterial culture plate was observed. [20] Pillai JR, *et al* (2020) [21] In view to explore the antimicrobial property of *Encostemma littorale* and to identifying the molecular entities using different solvent extract lead them to satisfactory result. The Antimicrobial activity was ascertained using strains *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The ethyl acetate extract was found to possess significant antimicrobial activity when compared to other extracts. This paved way to explore further molecular entity which were responsible for such activity. [21]

The *in vitro* antimicrobial activity of plant extract of *Encostemma littorale* was studied and documented by Praveena P *et al* (2011) [22]. In their study they evaluated the Minimum Inhibitory Concentration by Micro-titre plate method of Chloroform, Ethyl acetate, Methanol, Petroleum ether extract of whole plant on four bacterial species *Staph aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei* and two fungal strains *Aeromonas hydrophila* and *Candida albicans*. It was observed by the scholars that Ethyl acetate, Methanolic extract showed prominent antimicrobial activity against all microorganisms. [22] Mathur R *et al* (2013) [23] performed an experiment work using *Encostemma littorale* aimed to investigate antimicrobial property. The extracted petroleum ether, chloroform, n-Butanol, ethanol and aqueous extracts of the whole plant and subjected to *in vitro* antimicrobial activity against common human pathogenic microorganisms using agar gel diffusion susceptibility test by standard technique. They compared their results of various plants extracts with commercially available antibiotics. The Minimum inhibitory concentration, Minimum Bactericidal Concentration and Minimum Fungicidal Concentration of the plant extracts was determined according to the Micro broth dilution technique. Among all solvents used ethanol extract gave the highest zone of inhibition. With *Escherichia coli*, zone of inhibition was 20.0mm, with *Staphylococci* it was 27.3 mm., with *Pseudomonas aeruginosa* 25.0 mm., with *Enterococcus sp.* 23.6 mm., with *Candida albicans* and *Candida parapsilosis* it was 22.0 mm and 20.5 mm respectively. [23]

## Material and methods

### Preparation of the plant extract

The leaves were collected in October 2022 and were subjected to shade drying to a constant weight in an atmospheric controlled research laboratory. The dried leaves pulverized for size reduction by the help of blender to increase the surface area for extraction. Dried powder was stored in air tight container while some part of the leaves powder was used for soxhlet extraction procedures using water and ethanol as solvent. Both extracts were concentrated to recover solvent and used further for investigation. [24, 25]

## Phytochemical analysis

Different chemical tests were performed in laboratory in order of identification of phytochemical constituents in the plants leave powder and extract were carried using the standard procedures. The standard test procedure was followed for detection of alkaloids, steroids, terpenoids, flavonoids, tannins, glycosides etc. and the results are mentioned in following table 1.

**Table 1:** Table showing presence of phytoconstituents

S No.	Phytochemicals	Results
1	Alkaloids	+
2	Steroids	+
3	Terpenoids	-
4	Flavonoids	+
5	Tannins	+
6	Saponin	+
7	Glycosides	+

“+” Presence of the compound, “-” Absence of compound

## *In vitro* Antimicrobial Analysis

Synthesized herbal drug or extract substances that either suppress or inhibit the growth of microorganisms are generally analyzed for their antimicrobial potential by microbial method. The procedure employed for this testing is Cup plate method or Agar well diffusion method.

## Selection of Bacterial species

The four bacterial species were used in this *in vitro* evaluation among the gram-negative bacterial species which used were *Escherichia coli* and *salmonella typhi*, while the gram-positive species used are *Staphylococcus aureus*, *Bacillus subtilis*.

## Preparation of nutrient agar medium

Readymade dehydrated medium was used for testing the antimicrobial activity of plant extracts. The dehydrated medium was dissolved in 100ml of distilled water and heated to boiling to dissolve the medium completely. The medium was distributed on clean glass tubes and plugged with cotton and sterilized by autoclaving at 15lb/sq. inch pressure at 121°C for 20 minutes. [26]

## Cup plate method

Antibacterial activity of each extract of plant (1000mg/ml) was evaluated by the cup plate method. Cups were made in Petri plates using sterile cork borer (0.85 cm) and 50µl of each extract was added into each well. Then bacterial plates were incubated at 37° C 24 hrs. [27] Active culture of test bacteria was grown in nutrient broth medium at 37°C for 24 hours. A lawn culture then prepared on Muller Hinton agar. Sterile filter paper discs (5mm in diameter) impregnated with each extract was placed on the culture plates and incubated at 37°C. Take DMSO solvent as negative control and streptomycin as positive control. After 24 hours of incubation, the antibacterial activity was assessed by measuring the inhibition zone. [28, 29] Measured inhibition zones were recorded as mean diameter in mm. [30] Gentamycin antibiotic was used as control.

## Results

The result of antimicrobial activity of plant extract both aqueous and alcoholic extract is given in Table 2. The both Aqueous extract of *E. littorale* and Alcoholic extract *E.*



littorale show highest activity against *Salmonella typhi* as observed in dimensions of inhibition zones while least with staph aureus. The results were compared with control drug gentamycin and found to be satisfactory.

**Table 2:** Antimicrobial activity of extract of *E. littorale*

Extract	<i>Escherichia Coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Aq. <i>E. E. l.</i>	14	20	11	10
Al. <i>E. E. l.</i>	12	10	6	10
Gentamycin	27	29	22	26

Aq. *E. E. l.*- Aqueous Extract of *E. littorale*, Al. *E. E. l.*- Alcoholic Extract of *E. littorale*.

### Conclusion and discussion

Plants are the basic source of remedies for human ailments in proper category and alternative to modern medicine accompanying side effect. When herbal source medicine preparations with relatively lower incidence of side effect to plant, coupled with their reduced cost compared to modern pharmaceuticals, encourages health providers and consumers to consider plant medicines as alternative to synthetic drugs. As of now a vast range of antibiotics which were used as antibacterial earlier has lost their efficiency due to the development of resistant strains of bacteria. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. In this present study, the antibacterial activity of the herb *Encostemma littorale* using its Aqueous and Alcoholic extract has been performed to prove its activity against various species of microbes by spread cup plate method. The results will motivate researchers further as the it provides evidence of utilizing the phytocomponents of plant *Encostemma littorale* against the particular microbes satisfactorily.

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### Conflict of Interest

The authors declare no conflict of interest.

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