**In vitro antimicrobial and antioxidant studies on Enicostemma axillare (Lam.) Raynal. leaves**

Sharada L Deore*, S S Khadabadi, Lalita Bhagure and D S Ghorpade  
Govt. College of Pharmacy, Kathorna naka, Amravati-444 604, Maharashtra, India  
*Correspondent author, E-mail: khadabadi@yahoo.com, sharudeore_2@yahoo.com  
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**Abstract**  
Enicostemma axillare (Lam.) Raynal. syn. *E. littorale* Blume (Family—Gentianaceae) is a perennial herb found throughout the greater part of India. Locally it is known as *chota chirayita* and used in indigenous medicines in the treatment of fevers and as bitter tonic and forms one of ingredients of many hypoglycemic marketed formulations. In the present study *in vitro* antimicrobial activity (Well diffusion method) of aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves of this plant has been evaluated. Six bacterial species and two fungal strains used for study are: *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella sonni*, *Aspergillus niger* and *Candida albicans*. It was observed that chloroform, ethyl acetate and hydroalcoholic extract showed prominent antimicrobial activity against all microorganisms. *In vitro* antioxidant activity of each extract except ethyl acetate and the possible mechanism involved was investigated by using two different model covering nitric oxide and DPPH method. The result indicated efficacy of extracts for antioxidant activity in following sequence: methanol>hydro alcoholic>aqueous>chloroform.

**Keywords:** Enicostemma axillare, Enicostemma littorale, Chota chirayita, Antimicrobial activity, *In vitro* antioxidant activity, DPPH method, Nitric oxide method.

**IPC code; Int. cl.** A61K 36/00, A61P 31/00, A61P 31/04, A61P 31/10, A61P 17/18

**Introduction**  
Enicostemma axillare (Lam.) Raynal. syn. *E. littorale* Blume (Family — Gentianaceae), locally called as Chota chirayita in Marathi and Mamajaka in Sanskrit, has been used traditionally for many diseases1. According to Ayurvedic literature survey, the fresh juice of leaves has been used as a bitter tonic, to control arthritis, in typhoid fever and as cooling agent. It is used as stomachic and laxatve, blood purifier in dropsy, rheumatism, abdominal ulcers, hernia, swellings, itches and insect poisoning. The plant paste is applied on boils. The leaves are fed to cattle to increase appetite. Plant extracts were reported for the biological activities such as antidiabetic, anti-inflammatory, stimulant, astringent and diuretic, and useful in skin diseases. The plant possesses stimulant, astringent, diuretic and anthelmintic properties 2,3. It also acts as ethnomedicine for snakebite4. The plant is used to cure leucorrhoea. The root extract showed antimalarial activity both *in vitro* and *in vivo*5. Methanolic extract showed antidiabetic effect in alloxan induced diabetic rats6, 7. It inhibited carrageen-induced edema and its anti-inflammatory activity is comparable to that of hydrocortisone8. It also acts as potential hypolipidemic in *p*-DAB (*p*-dimethylaminoazobenzene) induced hepatotoxic animals. The hypolipidaemic and antioxidant effect of its aqueous extract in cholesteral fed rats has also been reported9. It is also evaluated for hepatoprotective activity10. Anticancer activity of plant has been evaluated for Dalton’s ascitic lymphoma11. It contains bitter principle and acts as substitute for Katvinayi (*Chirata*). Various parts of plant are extremely bitter due to presence of amrogentian (0.05%)12. It contains swertimarine, a secoiridoid glycoside, bitter glycoside ophelic acid, gentianin, phytosterol and tannins13-15. The *n*-Butanol extract afforded triterpenoids and unsaponifiable matter has yielded *n*-hexasanol, heptacosane, nanocosane; while nonsaponifiable matter of petroleum ether extract is enriched with myristic acid, stearic acid and oleic acid15. Since, there was no report on its antimicrobial activities and plant phenols, terpenoides are tannis are reported to have antimicrobial and antioxidant activities16, 17 hence present study was carried out to evaluate its *in vitro* antioxidant and antimicrobial activities. Six bacterial species and two fungal strains were used for antimicrobial study. Antioxidant activity was studied by using two different model covering nitric oxide and DPPH method.
Materials and Methods

Plant material and Preparation of extracts

The aerial plant parts of *Enicostemma axillare* at flowering stage were collected from the University area of Amravati in Vidarbh region in the month of August-September 2005. The plant species was identified and confirmed (by Botanist Dr. Prabha Bhogaonkar) at Vidarbh Institute of Science and Humanities (V.M.V), Amravati. The collected material was dried under the sunlight and powdered.

The powdered plant material was extracted using solvents of increasing polarity chloroform, ethyl acetate, methanol and 70% alcohol, in a soxhlet extraction apparatus. Aqueous extract was obtained by hot water decoction. The solvent was completely removed by using rotary flash evaporator to get dry mass. Percentage yield (w/w) of each extract was found to be 1.52, 3.4, 1.8, 2 and 1.8, respectively. Same extracts were prepared for antioxidant activity.

Microbial strains and Standard drugs

*Bacillus subtilis* NCIM-2063, *Staphylococcus aureus* NCIM -2608, *Escherichia coli* NCIM-2065, *Shigella sonni* NCIM-2957, *Pseudomonas aeruginosa* NCIM-2036, *Proteus vulgaris* NCIM-2813, *Aspergillus niger* NCIM-545 and *Candida albicans* NCIM-3100 were used as microbial and fungal strains for the study. Standard drugs used for study were Gentamycin and Amphotericin.

The bacterial strains employed in the study were obtained from National Collection of Industrial Microorganisms (NCIM), NCL, Pune. Standard drugs used for study Gentamycin and Amphotericin were obtained as a free sample from Sai Adventium, Hyderabad.

Well diffusion method (zone of inhibition)

Using micropipette, 0.2ml of each of the seeded broth containing $10^6 - 10^7$ cfu/ml test organisms were inoculated on the three plates of solidified agar and spreaded uniformly with a glass spreader. Then 3 wells were cut out in the agar layer of each plate with an aluminum bore of 5 mm diameter to contain equal volume of extract, standard drug and solvent DMSO. All the work was carried out under strict aseptic conditions. The plates were kept in fridge for 30 minutes after addition to allow diffusion of the solution into the medium and then incubated at 37°C ±1 for 18 hours (For antifungal activity 48 hours). After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured which has been shown in Table 1. Antifungal study was carried out by same procedure as used in antibacterial study but the media used

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test organism</th>
<th>AE</th>
<th>ME</th>
<th>HAE</th>
<th>CE</th>
<th>EAE</th>
<th>GENTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Bacillus subtilis</em> NCIM-2063</td>
<td>17.33± 2.08</td>
<td>17.00±2.08</td>
<td>15.00± 2.08</td>
<td>17.00±2.082</td>
<td>13.33±2.08</td>
<td>15.33</td>
</tr>
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<td>2.</td>
<td><em>Staphylococcus aureus</em> NCIM -2608</td>
<td>11±1.03</td>
<td>11.67±1</td>
<td>11.33±1</td>
<td>13.00±1</td>
<td>12.33±1</td>
<td>16.33</td>
</tr>
<tr>
<td>3.</td>
<td><em>Escherichia coli</em> NCIM-2065</td>
<td>13±1.01</td>
<td>13.3±1.53</td>
<td>13.33±3.06</td>
<td>13.3±1</td>
<td>13.33±3.22</td>
<td>17</td>
</tr>
<tr>
<td>4.</td>
<td><em>Shigella sonni</em> NCIM-2957</td>
<td>14.33±1.52</td>
<td>13.00±1.52</td>
<td>15.33±1.53</td>
<td>14.33±1.53</td>
<td>11.00±1.53</td>
<td>16</td>
</tr>
<tr>
<td>5.</td>
<td><em>Pseudomonas aeruginosa</em> NCIM-2036</td>
<td>12.33±2.31</td>
<td>15.33±2.31</td>
<td>14.67±2.31</td>
<td>19.67±2.31</td>
<td>15.67±2.31</td>
<td>15.67</td>
</tr>
<tr>
<td>6.</td>
<td><em>Proteus vulgaris</em> NCIM-2813</td>
<td>17±1.02</td>
<td>14.00±1</td>
<td>15.67±1</td>
<td>13.00±1</td>
<td>16.33±1</td>
<td>16.33</td>
</tr>
<tr>
<td>7.</td>
<td><em>Aspergillus niger</em> NCIM-545</td>
<td>14.67±0.58</td>
<td>17.67±2.52</td>
<td>17.33±2.08</td>
<td>15.33±2.08</td>
<td>15.00±1</td>
<td>18.78</td>
</tr>
<tr>
<td>8.</td>
<td><em>Candida albicans</em> NCIM-3100</td>
<td>11.00±1</td>
<td>13.00±1</td>
<td>11.00±1</td>
<td>14.00±1.07</td>
<td>12.00±1</td>
<td>16.33</td>
</tr>
</tbody>
</table>

AE: Aqueous extract, ME: Methanol extract, HAE: Hydro alcoholic extract, CE: Chloroform extract, EA: Ethyl Acetate extract, GENTA: Gentamycin, AMPHO: Amphotericin B
for antifungal study was Sarboud's dextrose agar media (SDA Medium) instead of Nutrient agar medium which was used for antibacterial study. Gentamycin was used as standard for antibacterial while Amphotericin B for antifungal activity. Results of antimicrobial activity are summarized in Table 1.

**Antioxidant activity**

Antioxidant properties were analyzed by two different methods:

*DPPH method*: The different extracts were measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH. one ml of different concentration of extract solutions (100, 250, 500, 1000 µg/ml) and standard were taken in different vials. 2.8 ml of DPPH solution (45 mg/ml) were rapidly mixed with each solution of plant extracts. The absorbance at 515 nm on spectrophotometer Shimandzu 1700 was measured after 5 minutes (Fig. 1).

*Nitric oxide radical inhibition assay*: Nitric oxide radical inhibition was estimated by the use of Griess Illosvoy reaction. Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and 1 ml of extracts were incubated at 25°C for 150 minutes. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 minutes at 25°C. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions (Fig. 2).

**Statistical analysis**: The experimental results were expressed as the mean ± SD.

**Results and Discussion**

Results of phytochemical investigation of *E. axillare* showed the presence of anthraquinone glycoside, saponin glycoside, steroids, terpenoids, phenolic compounds including tannins and flavonoids. Phenolic and triterpenoid constituents are prominent with bitter principles.

According to ethnomedicinal claim this plant is used in typhoid fever, dropsy, malaria and skin diseases. As plant contains phenolic and terpenoidal compounds hence present study has undertaken to evaluate antimicrobial and
antioxidant activity\textsuperscript{16, 17}. Antimicrobial activity on Gram positive, Gram negative bacteria and some fungal strains have been performed for the first time. The results of antimicrobial activity are shown in Table 1. All five extracts exhibited prominent antimicrobial activity against all microorganisms used in the study. It is observed that chloroform, ethyl acetate and hydro alcoholic extracts showing prominent antimicrobial activity against all microorganisms as compare to aqueous and methanol extracts.

The decline in radical concentration indicated the radical scavenging activity of the sample\textsuperscript{18}. Results shown in Fig. 1 illustrate decrease in the concentration of DPPH radical due to the scavenging of different extracts. Results of nitric oxide radical inhibition assay are shown in Fig. 2 which illustrates scavenging effect of different extracts. The results of antioxidant activity (Figs 1 & 2) indicate efficacy of extracts in following sequence: methanol>hydro alcoholic>aqueous>chloroform.

Conclusion

The prominent antioxidant and antimicrobial activity may be due to presence of higher content of tannins, phenolic and saponins. Future scope involves isolation and identification of different constituents responsible for these activities.

References