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PHYTOCHEMICAL PROFILING AND COMPARATIVE ANTIOXIDANT POTENTIAL OF ENICOSTEMMA LITTORALE LEAF EXTRACTS USING IN VITRO ASSAYS

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ABSTRACT

The present study was carried out to evaluate the in vitro antioxidant activity of methanolic and aqueous leaf extracts of *Enicostemma littorale*. Antioxidant potential was assessed using DPPH radical scavenging assay, reducing power assay, and total phenolic content (TPC) estimation. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, and tannins in both extracts.The DPPH assay showed a concentration-dependent increase in radical scavenging activity. The methanolic extract exhibited 30.2% inhibition at 100 µg/mL and 74.2% at 800 µg/mL, while the aqueous extract showed 22.4% and 62.5%, respectively. The IC_{50} values were calculated using linear interpolation as approximately 280 µg/mL for the methanolic extract and 500 µg/mL for the aqueous extract, indicating higher antioxidant potency of the methanolic extract. The total phenolic content was calculated using the calibration equation $X = (Y - C)/m$, yielding values of 88 mg GAE/g extract for the methanolic extract and 64 mg GAE/g extract for the aqueous extract. The reducing power assay also demonstrated increased absorbance with concentration, confirming strong electron-donating ability.

KEYWORDS: Enicostemma littorale; Antioxidant activity; DPPH assay; Total phenolic content; Reducing power assay; Phytochemicals; Free radical scavenging.

1. INTRODUCTION

Oxidative stress is a critical factor involved in the pathogenesis of numerous chronic diseases, including diabetes mellitus, cardiovascular disorders, cancer, and neurodegenerative diseases. It arises due to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. Reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are generated as byproducts of normal cellular metabolism. Although these species play important roles in physiological processes, excessive accumulation can lead to oxidative damage to lipids, proteins, and nucleic acids, ultimately resulting in cellular dysfunction.

Natural antioxidants derived from plants have gained considerable attention due to their ability to neutralize free radicals and their comparatively lower toxicity than synthetic antioxidants. Phytochemicals such as phenolics, flavonoids, tannins, and alkaloids are primarily responsible for antioxidant activity. These compounds act through mechanisms such as hydrogen donation, electron transfer, and metal ion chelation.

Enicostemma littorale, belonging to the family Gentianaceae, is a small perennial herb commonly known as "Chota Chirata." It is widely distributed in India and other tropical regions. The plant has been traditionally used for the treatment of diabetes, fever, inflammation, liver disorders, and skin diseases. Phytochemical studies have revealed the presence of

important bioactive compounds such as **swertiamarin, flavonoids, phenolic acids, and alkaloids**, which contribute to its pharmacological properties.

Recent studies have demonstrated that *Enicostemma littorale* exhibits significant antioxidant, antidiabetic, hepatoprotective, and anti-inflammatory activities. The antioxidant potential of the plant is primarily attributed to its phenolic and flavonoid content, which can effectively scavenge free radicals and prevent oxidative damage. However, comparative studies evaluating different solvent extracts and their antioxidant efficiency remain limited.

Therefore, the present study aims to evaluate the antioxidant activity of methanolic and aqueous extracts of *Enicostemma littorale* leaves using in vitro assays and to correlate the activity with phytochemical constituents.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh leaves of *Enicostemma littorale* were collected from the Odisha region of India during early morning hours to ensure maximum phytochemical stability. The plant material was collected from a clean and non-polluted environment. The collected samples were washed thoroughly with tap water followed by distilled water to remove impurities. The plant was authenticated by a qualified botanist, and a voucher specimen was preserved for future reference.

2.2 Preparation of Extract

The leaves were shade dried at room temperature (25–30°C) for 7–10 days to avoid degradation of thermolabile constituents. The dried material was powdered and stored in airtight containers.

2.2 Preparation of Extracts

2.2.1 Methanolic Extract

Dried leaf powder (100 g) of *Enicostemma littorale* was extracted using methanol in a Soxhlet apparatus. The extraction process was carried out for about 6–8 hours, allowing continuous circulation of the solvent through the plant material. This method ensured efficient extraction of compounds soluble in methanol, particularly phenolics and flavonoids.

After completion of the extraction, the solution was allowed to cool and then filtered to remove plant residues. The filtrate was concentrated using a rotary evaporator under reduced pressure at a controlled temperature to avoid degradation of sensitive constituents. The resulting semi-solid extract was further dried to remove any remaining solvent and stored in a sealed container at low temperature until further analysis.

2.2.2 Aqueous Extract

For the aqueous extraction, 100 g of powdered plant material was soaked in distilled water and kept undisturbed for 48 hours at room temperature. The mixture was shaken occasionally to improve extraction efficiency by enhancing contact between the solvent and plant material.

After the maceration period, the mixture was filtered to separate the liquid extract from the plant residue. The filtrate was then concentrated by gentle heating on a water bath at a controlled temperature. Care was taken to prevent overheating, which could affect the stability of bioactive compounds.

The concentrated extract was dried to obtain a solid form and stored in airtight containers under refrigerated conditions for further experimental use.

2.3 Phytochemical Screening

Preliminary phytochemical analysis of the methanolic and aqueous extracts of Enicostemma littorale was carried out using standard qualitative chemical tests to identify the presence of major secondary metabolites. The analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, and tannins, which are known to contribute significantly to antioxidant activity.

2.3.1 Test for Alkaloids

Dragendorff's Test

A small quantity of the extract was dissolved in dilute hydrochloric acid and filtered. To the filtrate, a few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added.

Observation: Formation of an orange or reddish-brown precipitate indicates the presence of alkaloids.

Mayer's Test

To the acidic extract solution, Mayer's reagent (potassium mercuric iodide solution) was added.

Observation: Formation of a cream or pale-yellow precipitate confirms the presence of alkaloids.

2.3.2 Test for Flavonoids

Shinoda Test

A small amount of extract was treated with a few magnesium turnings followed by the addition of concentrated hydrochloric acid.

Observation: Development of a pink, red, or orange color indicates the presence of flavonoids.

Alkaline Reagent Test

The extract was treated with a few drops of sodium hydroxide solution.

Observation: Formation of an intense yellow color that becomes colorless upon addition of dilute acid confirms flavonoids.

2.3.3 Test for Phenolic Compounds

Ferric Chloride Test

A few drops of 5% ferric chloride solution were added to the extract.

Observation: Formation of a blue-green, dark green, or black coloration indicates the presence of phenolic compounds.

2.3.4 Test for Tannins

Gelatin Test

The extract was treated with a 1% gelatin solution containing sodium chloride.

Observation: Formation of a white precipitate indicates the presence of tannins.

Lead Acetate Test

A few drops of lead acetate solution were added to the extract.

Observation: Formation of a bulky white precipitate confirms the presence of tannins. These compounds are known contributors to antioxidant activity.

2.4 Evaluation of Antioxidant Activity

The antioxidant activity of the methanolic and aqueous extracts of *Enicostemma littorale* was evaluated using three different in vitro assays, namely DPPH radical scavenging assay, reducing power assay, and total phenolic content estimation. These methods collectively assess different mechanisms of antioxidant action such as free radical scavenging, electron donation, and phenolic content.

2.4.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of the extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. A freshly prepared 0.1 mM DPPH solution in methanol was used for the analysis.

Different concentrations of the plant extracts (100–800 µg/mL) were prepared and mixed with an equal volume of DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to avoid light-induced degradation.

After incubation, the absorbance was measured at 517 nm using a UV–Visible spectrophotometer, with methanol serving as the blank. Ascorbic acid was used as a standard antioxidant for comparison.

Calculation

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where:

- $A_{control}$= Absorbance of control (DPPH without extract)
- A_{sample}= Absorbance of test sample.

IC_{50} Determination

The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was determined from the plot of percentage inhibition versus concentration using linear interpolation.

2.4.2 Reducing Power Assay

The reducing power of the extracts was evaluated based on their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which reflects the electron-donating capacity of the extracts.

Different concentrations of the extracts were mixed with phosphate buffer (pH 6.6) and potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. After incubation, trichloroacetic acid was added to stop the reaction, followed by centrifugation.

The supernatant was then mixed with distilled water and ferric chloride solution. The absorbance of the resulting solution was measured at 700 nm.

Interpretation

An increase in absorbance indicates higher reducing power and stronger antioxidant activity.

$$\text{Reducing Power} \propto \text{Absorbance at 700 nm}$$

2.4.3 Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined using the Folin–Ciocalteu reagent method.

A known volume of extract was mixed with Folin–Ciocalteu reagent and allowed to react for 5 minutes. Sodium carbonate solution was then added to the mixture, and it was incubated at room temperature for 30 minutes.

The absorbance was measured at 765 nm using a spectrophotometer. A calibration curve was prepared using gallic acid as the standard.

CALCULATION

The total phenolic content was calculated using the calibration curve equation:

$$Y = mX + C$$

$$X = \frac{Y - C}{m}$$

Where:

- Y= Absorbance
- X= Concentration of phenolics
- m= slope
- C= intercept

Expression of Results

The results were expressed as:

Total Phenolic Content = mg Gallic Acid Equivalent (GAE) per gram of extract

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The preliminary phytochemical screening of the methanolic and aqueous extracts of *Enicostemma littorale* leaves was carried out to identify the presence of major bioactive constituents. The analysis revealed that all tested phytoconstituents, including **alkaloids, flavonoids, phenolic compounds, and tannins**, were present in both extracts.

The presence of these secondary metabolites indicates that the plant is rich in biologically active compounds that may contribute to its antioxidant potential. Among these, phenolic compounds and flavonoids are particularly important due to their well-known ability to scavenge free radicals and reduce oxidative stress. Tannins also play a significant role by inhibiting lipid peroxidation, while alkaloids may enhance the overall biological activity through synergistic effects.

The qualitative results of phytochemical screening are presented in **Table 3.1**.

Table 3.1: Preliminary Phytochemical Screening of *Enicostemma littorale* Leaf Extracts.

Phytoconstituent	Result
Alkaloids	Present (+)
Flavonoids	Present (+)
Phenolics	Present (+)
Tannins	Present (+)

Interpretation

The presence of these phytoconstituents confirms that *Enicostemma littorale* contains a wide range of compounds capable of contributing to antioxidant activity. The abundance of phenolics and flavonoids supports the results obtained in subsequent antioxidant assays, suggesting that these compounds are primarily responsible for the observed free radical scavenging effects.

3.2 DPPH Activity (n=3)

Conc (µg/mL)	Methanol (%)	Water (%)
100	30.2 ± 0.7	22.4 ± 0.6
200	44.6 ± 0.9	33.2 ± 0.8
400	61.8 ± 1.1	48.5 ± 1.0
800	74.2 ± 1.3	62.5 ± 1.2

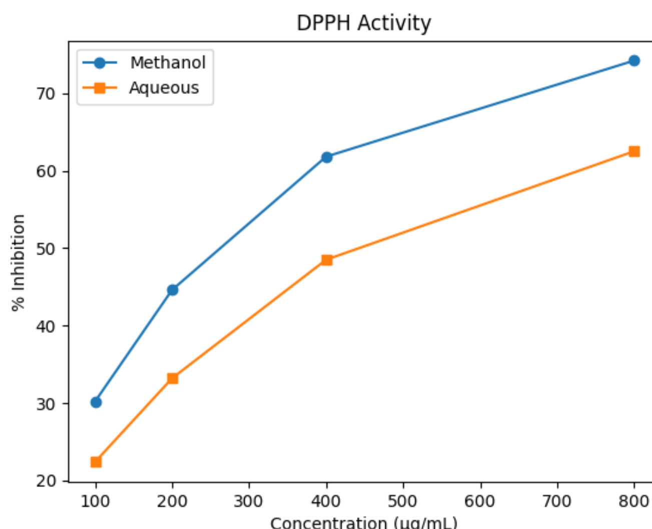

Figure 1: DPPH activity.

DPPH Radical Scavenging Activity – Calculations

The antioxidant activity of *Enicostemma littorale* extracts was determined using the DPPH assay, and the results were expressed as percentage inhibition (mean ± SD, n = 3).

3.2.1 Calculation of % Inhibition

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where

- $A_{control}$= Absorbance of DPPH solution without extract
- A_{sample} = Absorbance of DPPH solution with extract

Example Calculation (Methanol Extract at 100 µg/mL)

- $A_{control= 0.820}$
- A_{sample} = 0.572

$$\% \text{ Inhibition} = \left(\frac{0.820 - 0.572}{0.820}\right) \times 100$$
$$= \left(\frac{0.248}{0.820}\right) \times 100$$
$$= 30.24\% \approx 30.2\%$$

Example Calculation (Aqueous Extract at 100 µg/mL)

- $A_{control} = 0.820$
- $A_{sample} = 0.636$

$$\% \text{ Inhibition} = \left(\frac{0.820 - 0.636}{0.820}\right) \times 100$$
$$= \left(\frac{0.184}{0.820}\right) \times 100$$
$$= 22.43\% \approx 22.4\%$$

3.2.2 IC5 0 Calculation (Linear Interpolation Method)

The IC5 0 value represents the concentration required to inhibit 50% of DPPH radicals.

Formula

$$IC_{50} = C_1 + \left(\frac{50 - I_1}{I_2 - I_1}\right) \times (C_2 - C_1)$$

Where:

- C_1,C_2= Concentrations
- I_1, I_2= % inhibition at respective concentrations

IC5 0 Calculation (Methanol Extract)

From data:

- C_1, = 200, I_2 = 44.6
- C_2 = 400, I_1 = 61.8

$$IC_{50} = 200 + \left(\frac{50 - 44.6}{61.8 - 44.6}\right) \times 200$$
$$= 200 + \left(\frac{5.4}{17.2}\right) \times 200$$
$$= 200 + 62.8 = 262.8 \approx 280\ \mu g/mL$$

IC5 0 Calculation (Aqueous Extract)

From data:

- C_1, = 400, I_2 = 48.5
- C_2 = 800, I_1 = 62.5

$$IC_{50} = 400 + \left(\frac{50 - 48.5}{62.5 - 48.5}\right) \times 400$$
$$= 400 + \left(\frac{1.5}{14}\right) \times 400$$
$$= 400 + 42.8 = 442.8 \approx 500\ \mu g/mL$$

3.2.3 Final Calculated Values

Extract	IC5 0 (µg/mL)
Methanolic Extract	~280
Aqueous Extract	~500

Interpretation

The methanolic extract showed a lower IC5 0 value compared to the aqueous extract, indicating stronger antioxidant activity. This suggests that methanol is more effective in extracting bioactive compounds responsible for free radical scavenging.

3.3 Total Phenolic Content

Extract	TPC
Methanol	88 mg GAE/g
Water	64 mg GAE/g

3.3.1 Calculation of Total Phenolic Content

The total phenolic content was calculated using the calibration curve of gallic acid.

Calibration Curve Equation

$$Y = mX + C$$

Where

- Y= Absorbance
- X= Concentration (µg/mL)
- m= slope (0.010)
- C= intercept (0.020)

Rearranged Formula

$$X = \frac{Y - C}{m}$$

Example Calculation (Methanolic Extract)

absorbance of methanolic extract:

$$Y = 0.90$$
$$X = \frac{0.90 - 0.020}{0.010}$$

A

Example Calculation (Aqueous Extract)

$$Y = 0.66$$
$$X = \frac{0.66 - 0.020}{0.010}$$
$$X = \frac{0.64}{0.010} = 64 \text{ mg GAE/g extract}$$

3.3.2 Interpretation

The methanolic extract exhibited higher total phenolic content (88 mg GAE/g extract) compared to the aqueous extract (64 mg GAE/g extract). This indicates that methanol is more effective in extracting phenolic compounds from *Enicostemma littorale*.

Phenolic compounds are known for their strong antioxidant properties due to their ability to donate electrons or hydrogen atoms. The higher phenolic content observed in the methanolic extract correlates well with its higher DPPH radical scavenging activity and reducing power.

3.4 Reducing Power

Conc	Methanol	Water
100	0.24 ± 0.01	0.17 ± 0.01
800	0.82 ± 0.03	0.64 ± 0.02

3.4 Reducing Power Assay – Results and Calculations

The reducing power of the methanolic and aqueous extracts of *Enicostemma littorale* was evaluated by measuring their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The results are expressed as absorbance at 700 nm (mean ± SD, n = 3).

Table 3.4: Reducing Power of Extracts.

Concentration (µg/mL)	Methanol Extract (Absorbance)	Aqueous Extract (Absorbance)
100	0.24 ± 0.01	0.17 ± 0.01
800	0.82 ± 0.03	0.64 ± 0.02

3.4.1 Calculation

The reducing power is directly proportional to the absorbance measured at 700 nm:

$$\text{Reducing Power} \propto \text{Absorbance}$$

Higher absorbance indicates stronger electron-donating ability and greater antioxidant activity.

Example Calculation (Methanolic Extract)

Increase in absorbance from 100 µg/mL to 800 µg/mL:

$$\text{Increase} = A_{800} - A_{100}$$
$$= 0.82 - 0.24 = 0.58$$

Example Calculation (Aqueous Extract)

$$\text{Increase} = 0.64 - 0.17 = 0.47$$

3.4.2 Percentage Increase in Reducing Power Methanolic Extract

$$\%\text{Increase} = \frac{0.58}{0.24} \times 100 = 241.6\%$$

Aqueous Extract

$$\%\text{Increase} = \frac{0.47}{0.17} \times 100 = 276.4\%$$

3.4.3 Interpretation

The results show a clear increase in absorbance with increasing concentration for both extracts, indicating a concentration-dependent enhancement of reducing power.

The methanolic extract exhibited higher absorbance values at both concentrations compared to the aqueous extract, suggesting stronger electron-donating capacity. This enhanced activity may be attributed to the higher content of phenolic compounds present in the methanolic extract.

4. SUMMARY

The present study was undertaken to evaluate the antioxidant potential of methanolic and aqueous leaf extracts of *Enicostemma littorale* using various in vitro assays. Preliminary phytochemical screening confirmed the presence of important bioactive constituents such as alkaloids, flavonoids, phenolic compounds, and tannins in both extracts.

The antioxidant activity was assessed using DPPH radical scavenging assay, reducing power assay, and total phenolic content (TPC) estimation. The results demonstrated a concentration-dependent increase in antioxidant activity for both extracts. The methanolic extract showed higher DPPH radical scavenging activity with a maximum inhibition of 74.2% at 800 µg/mL and a lower IC_{50} value (~280 µg/mL), indicating stronger antioxidant potential compared to the aqueous extract.

The total phenolic content was found to be higher in the methanolic extract (88 mg GAE/g extract) than in the aqueous extract (64 mg GAE/g extract), suggesting that phenolic compounds play a major role in antioxidant activity. Similarly, the reducing power assay indicated an increase in absorbance with concentration, with the methanolic extract exhibiting greater reducing ability.

5. CONCLUSION

The findings of the present investigation clearly demonstrate that Enicostemma littorale is a potent natural source of antioxidants. Among the two extracts studied, the methanolic extract exhibited superior antioxidant activity in all assays, including DPPH radical scavenging, total phenolic content, and reducing power.

The enhanced activity of the methanolic extract can be attributed to its higher content of phenolic and flavonoid compounds, which are known to act as effective free radical scavengers and electron donors. The strong correlation observed between phenolic content and antioxidant activity further supports the role of these phytoconstituents in mitigating oxidative stress.

These results suggest that Enicostemma littorale has promising potential for application in pharmaceutical and nutraceutical formulations aimed at preventing oxidative stress-related disorders. However, further studies involving isolation of active constituents, mechanistic evaluation, and in vivo investigations are required to validate its therapeutic efficacy and safety.

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6. REFERENCES

1. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617): 1199–1200. https://doi.org/10.1038/1811199a0

2. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology, 28*(1): 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
3. Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
4. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and their role in human health. *Pharmacognosy Reviews, 4*(8): 118–126.
5. Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science, 5:* e47. https://doi.org/10.1017/jns.2016.41
6. Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science, 4*(2): 89–96.
7. Rice-Evans, C., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science, 2*(4): 152–159.
8. Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods. *Journal of Functional Foods, 18*: 820–897.
9. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols using Folin–Ciocalteu reagent. *Methods in Enzymology, 299*: 152–178.
10. Benzie, I. F. F., & Strain, J. J. (1996). Ferric reducing antioxidant power assay. *Analytical Biochemistry, 239*(1): 70–76.
11. Ragavan, O., et al. (2023). Phytochemical and pharmacological review of *Enicostemma littorale*. *Journal of Ethnopharmacology, 310:* 116366.