Phytochemical Profiling and Antioxidant Potential of Enhydra fluctuans: An In-Vitro Study

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Abstract-

Enhydra fluctuans is a semi-aquatic herbaceous vegetable commonly consumed in Southeast Asia and traditionally acclaimed for its medicinal properties. Ethnobotanical literature associates this plant with hepatoprotective, anti-inflammatory, and detoxifying effects; however, its phytochemical and antioxidant attributes require robust scientific corroboration. In light of the growing preference for botanical antioxidants in the nutraceutical and pharmaceutical sectors, this study investigates the antioxidant capacity of a selected plant extract, utilizing microwave-assisted techniques for compound extraction and assessing activity through the DPPH free radical method. The leaves were shade-dried, ground into powder, and subjected to cold maceration using 80% methanol.

The phytochemical profile of the extract was analyzed through both qualitative and quantitative approaches. For the estimation of total phenolic content (TPC) and total flavonoid content (TFC), the Folin–Ciocalteu reagent and aluminum chloride colorimetric techniques were utilized, respectively. The screening revealed the presence of key bioactive compounds such as phenolics, flavonoids, tannins, and saponins, suggesting a potentially beneficial biological effect. The quantified TPC and TFC were found to be 80.2 ± 2.5 mg of gallic acid equivalent (GAE) per gram and 54.6 ± 1.8 mg of quercetin equivalent (QE) per gram, correspondingly. In terms of antioxidant activity, the DPPH free radical scavenging method demonstrated a concentration-dependent inhibition, with an IC_{50} value calculated at 42.3 µg/mL.

These outcomes validate the traditional medicinal use of Enhydra fluctuans and suggest its potential for development in therapeutic or functional food applications. Future research

focusing on bioactive compound isolation, structural characterization, and in vivo validation is warranted to advance its pharmacological utilization.

Keywords:

Enhydra fluctuans, Phytochemicals, Antioxidant Activity, Phenolics, Flavonoids, DPPH Assay, Methanolic Extract, Natural Antioxidants

Introduction-

For centuries, medicinal plants have served as essential components in the maintenance of human health and continue to be integral to traditional medical practices globally. These plant-based resources are recognized for their abundance of biologically active secondary metabolites, including compounds such as alkaloids, flavonoids, phenolic substances, terpenoids, tannins, and glycosides. A wide array of these phytoconstituents exhibit therapeutic potentials, offering effects like antimicrobial, anti-inflammatory, antioxidant, and anticancer properties. In contemporary research, there is growing interest in naturally occurring antioxidants because of their effectiveness in neutralizing free radicals and mitigating the damaging effects of reactive oxygen species (ROS). These reactive molecules are closely linked to the onset and progression of chronic health issues, including heart disease, cancer, neurodegenerative conditions, and diabetes. Due to their minimal toxicity, natural abundance, and compatibility with human physiology, antioxidants derived from plants are being increasingly utilized in nutraceuticals, health foods, and drug development.

Enhydra fluctuans Lour., a semi-aquatic plant of the Asteraceae family, is a traditionally valued species that has received limited scientific attention. Commonly referred to as “Helencha” or “Marsh Herb,” it thrives in marshy environments, rice fields, and riverbanks throughout tropical and subtropical areas, particularly in India, Bangladesh, and Southeast Asia. The plant is widely consumed as a leafy vegetable due to its mild taste and cooling nature. In traditional medicine systems such as Ayurveda and folk remedies, it is used to manage liver disorders, skin diseases, epilepsy, inflammatory conditions, and general detoxification. It is also believed to possess blood-purifying properties and is often administered as part of postnatal care. Despite its extensive ethnomedicinal usage, scientific research into the plant's phytochemical makeup and antioxidant potential remains insufficient.

Bridging this gap between traditional knowledge and scientific evidence is essential, particularly for underutilized edible plants like Enhydra fluctuans, which may possess valuable therapeutic properties. Preliminary studies have indicated the presence of bioactive phytoconstituents, yet comprehensive experimental evaluations—especially those involving

validated in-vitro techniques—are sparse. Given the global shift towards natural remedies and the growing emphasis on sustainable health solutions, a detailed analysis of this plant's phytochemistry and antioxidant properties is both timely and necessary.

In light of the growing interest in plant-based therapeutics, the present investigation aimed to analyze both qualitative and quantitative phytochemical characteristics of the methanolic extract derived from *Enhydra fluctuans* leaves. Established spectrophotometric methods were utilized to determine the levels of total phenolics and flavonoids, while antioxidant efficacy was assessed through the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method, known for its reliability in such evaluations. This research primarily sought to substantiate the traditional medicinal claims associated with the plant and to examine its potential as a natural antioxidant candidate for further utilization in the fields of pharmaceuticals and functional nutrition.

2. Materials and Methods

2.1 Plant Material Collection

Fresh, disease-free leaves of Enhydra fluctuans were collected from a nearby vegetable market. Plant identity was confirmed through floristic taxonomic keys and validated by a certified botanist. The harvested leaves were washed initially under running tap water and then with distilled water to ensure removal of any particulate matter or surface residues. After cleaning, the leaves were shade-dried under ambient conditions (25–30°C) for approximately 7 to 10 days until they became brittle. The air-dried plant sample was mechanically pulverized into a coarse powder and subsequently stored in a sealed, moisture-resistant container until further analysis.

2.2 Preparation of Plant Extract

A total of 100 grams of finely powdered leaf material was extracted using 80% methanol as the solvent medium. The extraction process employed a cold maceration technique, wherein the plant material was immersed in a solvent at a 1:10 weight-to-volume ratio and maintained at ambient temperature for a duration of 72 hours, with intermittent shaking by hand to enhance solute diffusion. After maceration, the extract was initially sieved through muslin cloth and then further clarified using Whatman No. 1 filter paper. The resulting filtrate was concentrated under reduced pressure using a rotary vacuum evaporator operated at a controlled temperature range of 40 to 45°C, yielding a viscous, semisolid mass. This final extract was stored at 4°C to ensure its stability for subsequent experimental procedures.

2.3 Qualitative Phytochemical Screening

The methanolic extract was screened for various classes of phytoconstituents using standard chemical assays:

- Alkaloids: Identified using Dragendorff's reagent, which forms an orange-red precipitate.
- Flavonoids: Detected via the Shinoda reaction, which shows pink to red coloration upon treatment with magnesium turnings and hydrochloric acid.
- Phenolics: Confirmed by the Ferric chloride test, producing a blue-green color.
- Saponins: Indicated by persistent foam formation during the froth test.
- Tannins: Detected using Lead acetate, which yields a pale yellow or whitish precipitate.

2.4 Quantitative Estimation of Phytochemicals

2.4.1 Total Phenolic Content (TPC)-

The quantification of total phenolic content (TPC) was carried out using the Folin–Ciocalteu reagent-based colorimetric technique. In this protocol, 1 mL of the methanolic plant extract was combined with 5 mL of the Folin–Ciocalteu reagent that had been pre-diluted to one-tenth of its original strength using distilled water. Subsequently, 4 mL of a 7.5% sodium carbonate solution was added to the mixture. The reaction system was then incubated at room temperature in a dark environment for 30 minutes to allow optimal color development. The absorbance of the resulting solution was measured at 765 nm with the help of a UV-Visible spectrophotometer. Gallic acid served as the calibration standard, and the outcomes were expressed in terms of milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

2.4.2 Total Flavonoid Content (TFC)-

The total flavonoid content was estimated utilizing the aluminum chloride-based colorimetric method. In this assay, 1 mL of the plant extract was mixed with 4 mL of distilled water and 0.3 mL of a 5% sodium nitrite solution. Following a 5-minute incubation period, 0.3 mL of 10% aluminum chloride was introduced into the mixture, which was then supplemented with 2 mL of 1M sodium hydroxide. The total volume was adjusted to 10 mL by adding distilled water. The optical density of the final solution was recorded at 510 nm using a UV-Vis spectrophotometer. Quercetin served as the standard compound for calibration, and the flavonoid content was calculated and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

2.5 Antioxidant Activity: DPPH Free Radical Scavenging Assay

The antioxidant capacity of the plant extract was investigated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. For this purpose, a freshly prepared 0.1 mM solution of DPPH in methanol served as the working reagent. To assess dose-dependent activity, various concentrations of the extract (25, 50, 100, 150, and 200 µg/mL) were individually combined with 1 mL of the DPPH solution. These mixtures were then incubated in a dark environment at ambient temperature for 30 minutes to allow the reaction to proceed. Following incubation, absorbance values were measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was employed as the standard antioxidant for comparative evaluation.

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

$$\%\text{Inhibition} = \frac{(A0 - A1) \times 100}{A0}$$

Where:

- A0A_0A0: Absorbance of the control (DPPH + methanol)
- A1A_1A1: Absorbance of the test sample (DPPH + extract)

The IC_{50} value (i.e., the concentration of extract required to inhibit 50% of DPPH radicals) was determined using a plotted dose-response curve.

3. Results

3.1 Qualitative Phytochemical Profile

The initial phytochemical analysis of the methanolic leaf extract of Enhydra fluctuans confirmed the presence of various bioactive constituents. A strong intensity of flavonoids and phenolic compounds (+++) was observed, while tannins were found in moderate quantities (++). Alkaloids and saponins were present in trace amounts (+), whereas terpenoids were entirely absent in the extract.

These findings indicate that E. fluctuans is notably rich in polyphenolic compounds, which are widely recognized for their antioxidant activities. The summarized results of the qualitative screening are provided below:

Phytochemical	Result
Alkaloids	+
Flavonoids	+++
Phenolics	+++
Tannins	++
Saponins	+
Terpenoids	–

(Note: + = weak; ++ = moderate; +++ = strong; – = absent)

3.2 Total Phenolic Content (TPC)

The phenolic content in the methanolic extract was quantitatively analyzed employing the Folin–Ciocalteu reagent-based assay. The results revealed a significantly elevated level of phenolic constituents, measured as 80.2 ± 2.5 mg gallic acid equivalents (GAE) per gram of dry extract. Such a high concentration reflects considerable antioxidant potential, as phenolic compounds are recognized for their capacity to donate hydrogen atoms and effectively neutralize free radicals.

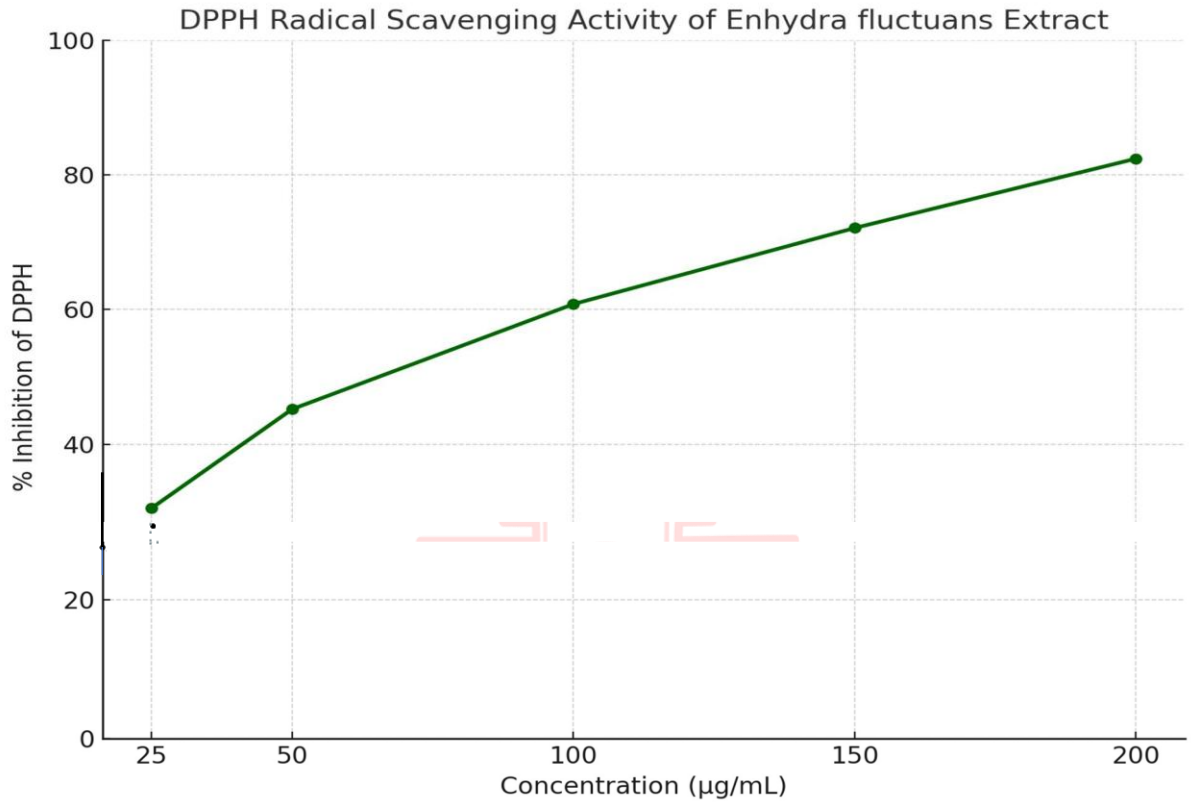
3.3 Total Flavonoid Content (TFC)

Flavonoid quantification was conducted using the aluminum chloride-based colorimetric assay. The extract showed a considerable amount of flavonoids, amounting to 54.6 ± 1.8 mg of quercetin equivalents (QE) per gram. Flavonoids, which are a key subclass of polyphenols, are associated with significant antioxidant, anti-inflammatory, and cytoprotective effects, particularly in conditions related to oxidative stress.

3.4 DPPH Radical Scavenging Antioxidant Activity

The antioxidant potential of the methanolic extract was assessed through the DPPH free radical scavenging assay. The extract exhibited a clear concentration-dependent inhibitory pattern, wherein higher extract doses resulted in greater neutralization of DPPH radicals.

The half-maximal inhibitory concentration (IC_{50}), indicating the extract amount required to scavenge 50% of free radicals, was determined to be 42.3 µg/mL, reflecting strong antioxidative capacity. These findings collectively affirm the robust antioxidant behavior of *Enhydra fluctuans*, which is plausibly attributed to its abundant phenolic and flavonoid constituents.



4. Discussion

The outcomes of this study demonstrate a substantial presence of phenolic and flavonoid compounds in the methanolic extract of Enhydra fluctuans, which are likely responsible for the notable antioxidant activity observed. Phenolic constituents, particularly flavonoids, are well-established for their role in neutralizing free radicals by donating electrons or hydrogen atoms. This mechanism effectively disrupts radical chain reactions and safeguards cellular structures from oxidative harm. The extract's pronounced free radical scavenging effect, as evidenced by an IC_{50} value of 42.3 µg/mL in the DPPH assay, highlights its potential to mitigate oxidative stress. These biochemical properties lend scientific credibility to the traditional use of E. fluctuans in managing liver disorders and inflammatory conditions, both of which are commonly associated with oxidative damage and redox imbalance.

In comparison with other reputed medicinal plants, the antioxidant performance of Enhydra fluctuans appears to be on par with many well-known phytotherapeutic agents. The elevated levels of total phenolics (80.2 ± 2.5 mg GAE/g) and flavonoids (54.6 ± 1.8 mg QE/g) point to a rich phytochemical composition that may serve as a strong basis for future pharmacological investigations. Additionally, the qualitative analysis revealed the presence of saponins and tannins, compounds that are also recognized for their antioxidative and anti-inflammatory properties, potentially enhancing the therapeutic value of the extract. Taken

together, these findings underscore the promise of Enhydra fluctuans as a valuable natural antioxidant source, with possible applications in the development of nutraceuticals and dietary supplements. Nonetheless, comprehensive research involving the isolation and characterization of individual bioactive compounds, along with in vivo biological assays, is essential to validate their mechanisms of action and confirm their therapeutic efficacy.

5: Conclusion

The antioxidant activity of the methanolic extract was assessed through the DPPH free radical scavenging method. Results exhibited a distinct concentration-dependent trend, where higher extract levels corresponded to increased radical inhibition. The IC_{50} value—indicating the extract concentration necessary to neutralize 50% of DPPH radicals—was found to be 42.3 µg/mL, suggesting notable antioxidant strength. These findings collectively support the robust free radical-quenching ability of *Enhydra fluctuans*, which can be attributed to its abundance of phenolic and flavonoid constituents.

Overall, these results lay a strong foundation for the development of plant-derived antioxidants, nutraceuticals, and functional foods that could support preventive healthcare strategies aimed at managing oxidative stress-related diseases.

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