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Assessment of Antibacterial, Antioxidant & Anti-inflammatory Activities and Phytochemical Screening of *Typha elephantina* Roxb. (Hogla) Leaves

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Abstract

Typha elephantina Roxb, locally known as 'Hogla' in Bangladesh, is widely found in wet areas. In this work, *T. elephantina* leaves were used for developing the methanolic crude extract, which afterwards went through antibacterial, antioxidant and anti-inflammatory assays in addition to phytochemical investigations. Alkaloids, carbohydrates, tannins, phenolics, carbohydrates and flavonoids were identified in the extract. The methanolic extract of the leaves of *T. elephantina* revealed the highest level of antibacterial activity against *E. coli*. It exhibited an IC_{50} of 42.02 µg/ml in the DPPH free radical scavenging assay. The total phenolic content (TPC) of it was 29.96 milligrams of gallic acid equivalents (GAE) per gram of test extract. In the anti-inflammatory assay, the extract reduced paw edema 28.8% ($p < 0.05$) and 38.83% ($p < 0.01$) in the third and fourth hours of carrageenan administration. Taken together, the leaves of *T. elephantina* might be considered biologically active.

Key words: *Typha elephantina,* antibacterial, antioxidant, anti-inflammatory, phytochemical screening.

Introduction

The discovery of drugs from natural sources is crucial for the development of today's therapeutic systems, as demonstrated by ethnopharmacological research (Pirintsos *et al.*, 2022). For millennia, substances derived from plants, animals and minerals have served as the foundation for the management for numerous illnesses. Plants with their secondary metabolites are extremely significant regarding the natural resource usage (Chaachouay and Zidane, 2024).

Typha elephantina Roxb., belonging to the Typhaceae family, is a tiny bush-like plant. There are numerous significant medicinal plant species in the family Typhaceae (Ahmad *et al.*, 2021). The plant, usually known as Hogla in Bangladesh, is found close to the country's lakes, rivers, canals and wetlands. Long fibrous roots are common characteristics of *T. elephantina* (Al-Sodany *et al.*, 2021). Aerenchymatous spongy tissue makes up the flattened leaf blades of the plant. The flowering stem that forms the rachis of the male and female spikes is

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encased at the base by the leaf sheaths. By the local people, the plant is commonly used to make useful handicrafts (Haq *et al.*, 2022). Many clinical disorders are seen to be managed with various parts of *T. elephantina*. The plant has been used to treat leprosy, splenic enlargement, strangury and other clinical conditions. It also possesses cooling and aphrodisiac properties. The rootstock is used to treat measles, gonorrhea and diarrhea. It also acts as a diuretic and astringent. In order to treat wounds and ulcers, both male and female spikes are found to be utilized as medicated absorbents (Sen *et al.*, 2018). As the plant is used in managing different clinical conditions, the leaves of the plant were collected and its phytochemical screening was executed as well as the antibacterial, antioxidant and anti-inflammatory properties were evaluated in the study.

Materials and Methods

Sample preparation from plant materials: The leaves of *T. elephantina* were collected from Nasirabad, Dhaka, Bangladesh, in 2023. After collection, the sample of the collected materials was sent to an expert in the botanical field (affiliated with Bangladesh National Herbarium), and the person confirmed the identity of the plant and provided the identification number (DACB accession number 104601). After being chopped into smaller pieces, the leaves were allowed to dry in a hygienic shed. With the aid of a grinder, powdered materials were extracted from the dried materials. In a tightly sealed container, 850 g of powder and 1.5 liters of methanol solvent were poured. Gentle stirring was applied sometimes to that mixture while soaking the materials. After 14 days, the entire contents were filtered and the crude extract (27 g) of *T. elephantia* was obtained by rotary evaporation technique.

Qualitative phytochemical screening: The presence of phytochemicals in the test materials was confirmed by a number of qualitative tests. Dragendrof's test, Molish's test, Benedict's test, Fehling's test, Borntrager's test, xanthoproteic test, lead acetate test, ferric chloride test, potassium dichromate test, sulfuric acid test and Froth test were executed to confirm the presence of alkaloids, carbohydrates, sugars, glycosides, proteins, flavonoids, tannins, steroids, saponins and acidic substances (Shaikh and Patil, 2020).

In vitro assays

Antibacterial properties evaluation: Antibacterial activity was determined by disk diffusion method (Bauer *et al.*, 1966). Several gram-positive and gram-negative bacterial species were selected for this assay. Subcultures were prepared from the species including *Shigella boydii*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aureus*, *Bacillus cereus*, *Staphylococcus aureus* and *Bacillus subtilis*; those were taken in the petridishes. On the prepared bacterial media, methanolic leaf extracts of *T. elephantia* at three different concentrations (250 µg/disc, 500 µg/disc, and 1000 µg/disc) were placed, along with standard antibiotic discs of ciprofloxacin (5 µg/µl). The dispersed plates are then incubated for 18 to 24 hours at 37°C. The antibacterial effect was then checked out through determining the zone of inhibition's area on a millimeter scale.

Antioxidant properties evaluation

DPPH free radical scavenging activity assay: To assess the antioxidant capacity of *T. elephantina* leaves methanolic extract, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity experiment was used (Baliyan *et al.*, 2022). To perform the assay, an initial solution of 500 µg/ml concentrations of ascorbic acid in methanol was prepared. Further dilution of the prepared solution produced a group of diverse concentrated solutions (serially from 250 µg/ml to 0.98 µg/ml). Solutions with the same concentrations were prepared from the *T. elephantia*'s leaves extract. In an amber reagent vial, a 0.1 mM DPPH solution was made ready for use. 2 ml of that solution was added to all the previously prepared solutions of the standard and the sample. They were incubated for half an hour at room temperature in a dark environment. A UV-Vis spectrophotometer was used to measure each solution's absorbance at 517 nm following

decolorization. To evaluate the antioxidant potency, the given formula was used:

Percent (%) inhibition={($A_{control}$-A_{sample})/$A_{control}$} ×100

In order to create a calibration curve, the computed percent of inhibition (I%) was plotted against the test sample strength. Finally, the fifty percent inhibitory concentration (IC_{50}) value—a qualitative assessment of the DPPH radical scavenging affinity—was determined for every test sample.

Total phenolic content: Total phenolic content (TPC) had been determined using the Folin-Ciocalteu (FC) colorimetric technique, where gallic acid was the standard (Molole *et al.*, 2022). The FC reagent was 10-fold diluted in distilled water while executing the assay. A stock solution of 1000 µg/ml concentration was prepared from dried leaf extract of *T. elephantina*, diluted FC reagent, and 7% Na_2CO_3 solution. A standard solution of gallic acid was prepared at 500 µg/ml concentrations by the FC reagent and Na_2CO_3 solution, then serially diluted to obtain a number of solutions of 250, 125, 62.5, 31.25 and 15.62 µg/ml. The absorbance was measured with a UV spectrophotometer at a wavelength of 750 nm to construct a calibration curve by plotting the absorbance values against the respective concentrations. The TPC of the dried plant extract was computed using the standard prepared curve.

In vivo anti-inflammatory properties evaluation

Experimental animal: The *in vivo* experiment was conducted using Swiss Albino mice of either sex that were five to six weeks old. The mice weighed between 30 and 40 g on average. In the animal housing conditions, twelve hours of alternating light and dark was employed with the temperature at 24 ± 1°C. Animal experimentation guidelines were followed rigorously and before commencing the experiment, the animal using the assay's protocol was submitted to the ethical review committee to obtain the relevant permission (Ref No: CPP/DIU/EC/14). The mice were given recommended food and water during their time in housing, but they were kept unfed for twelve hours before starting the assay.

Carrageenan induced hind paw edema method: The pathological alterations brought on by inflammation-producing chemicals can be reduced by an anti-inflammatory substance. Carrageenan is an inflammation-producing agent and carrageenan-induced hind paw edema was employed in the work (Mansouri *et al.*, 2015). The *T. elephantina* methanolic leaves extract at 250 and 500 mg/kg body weight dose and diclofenac sodium as a positive standard at 50 mg/kg dose were administered to the test animals orally. Besides, mice of the negative control group were treated with only saline solution. One hour later, the sub-plantar region underwent an injection of 0.1 ml of 1% carrageenan. Plethysmometer was used to measure the hind paw volume at each of the four hours after the administration. The percent inhibitory values of the hind paw volume expressed the anti-inflammatory potentiality of the *T. elephantina* leaves.

Statistical analysis: To justify the statistical significance of the data obtained from *in vivo* assays as well as to construct the curve in the case of in vitro assays, statistical and mathematical calculations were executed. MS Excel (version 2010) was used in these regards. When the *in vivo* assay's p value was less than 0.05, the data was regarded as statistically significant.

Results and Discussion

Phytochemical screening: Phytochemical constituent screening tests revealed that the leaves of *T. elephantina* contain diverse types of secondary metabolites, including glycosides, phenolic compounds, alkaloids, flavonoids, tannins and others (Table 1). The existence of several different types of chemicals may be the key element behind the plant's traditional application.

Antibacterial activity: The methanolic leaf extract of *T. elephantina* at three different concentrations was tested against a number of gram-positive and gram-negative bacterial species. With a zone of inhibition value of 22 mm, *T. elephantina*

leaves extract at 1000 µg/disc had the highest level of antibacterial action against *E. coli*, while standard ciprofloxacin at 5 µg/disc demonstrated a 36 mm zone of inhibition against that bacterium (Table 2). Regarding the gram-positive species, the maximum zone of inhibition against *B. cereus* and *S. aureus* was 19 mm at 1000 µg/disc.

Table 1. Qualitative phytochemical screening of the methanolic leaves extract of *T. elephantina.*

Test name	Phytochemicals	Test outcome
Dragendrof 's	Alkaloids	+
Molish's test	Carbohydrates	+
Benedict's test	Sugars	–
Fehling's test	Sugars	–
Borntrager's test	Glycosides	+
Xanthoproteic test	Proteins	–
Lead acetate test	Flavonoids	+
Potassium dichromate test	Tannins	+
Ferric chloride test	Phenolic compounds	+
	Tannins	+
Sulphuric acid test	Steroids	–
Froth test	Saponin	–

[The '+' sign indicates presence and the '–' sign indicates absence of the phytochemicals]

Table 2. Antibacterial potency determination of the *T. elephantina* leaves extract using zone of inhibition values.

Type of bacterial strains	Bacterial species	Diameter of zone of inhibition (mm)			
		Extract 250 µg/disc	Extract 500 µg/disc	Extract 1000 µg/disc	Standard (Ciprofloxacin) 5 µg/disc
Gram negative	*Pseudomonas aureus*	9	12	16	34
	Shigella bodydii	12	16	18	32
	Shigella dysenteriae	-	-	-	33
	Escherichia coli	12	14	22	36
Gram positive	*Staphylococcus aureus*	10	16	19	38
	Bacillus cereus	14	18	19	33
	Bacillus subtilis	13	15	18	37

A number of diseases, including some critical ones, are caused by pathogenic bacterial species. These species are associated with common clinical conditions, like pneumonia, gonorrhea, whooping cough, urinary tract infection (UTI), other organ infections and so on (Doron and Gorbach, 2008). The point to be noted is that already existing antibiotics are becoming resistant (Ventola, 2015). In addition, there are huge gaps and difficulties in discovering new antibiotics. In this work, the methanolic leaves extract of *T. elephantina* exhibited moderate to good antibacterial properties against different bacterial species. The phenolics, flavonoids, and tannins might exert antibacterial activities (Medini *et al*., 2014).

Antioxidant activity: To evaluate the antioxidant potency of the methanolic leaves extract of *T. elephantina*, the IC_{50} value was determined in the DPPH free radical scavenging activity assay and the total phenolic content (TPC) value was calculated.

The calculated IC_{50} value of methanolic leaves extract of *T. elephantina* was 42.02 µg/ml. where that value of standard ascorbic acid was 7.24 µg/ml (Table 3).

In the total phenolic content (TPC) assay, the obtained equation from the standard curve constructed by using FC reagent and Na_2CO_3 solution was y = 0.0018x+0.2224 (Figure 1). Using the formula, the TPC was 29.96, which was represented as mg of GAE/g of the test sample extract (Table 3). The result indicated the existence of notable amount of the phenolic compounds in the leaves of *T. elephantina*.

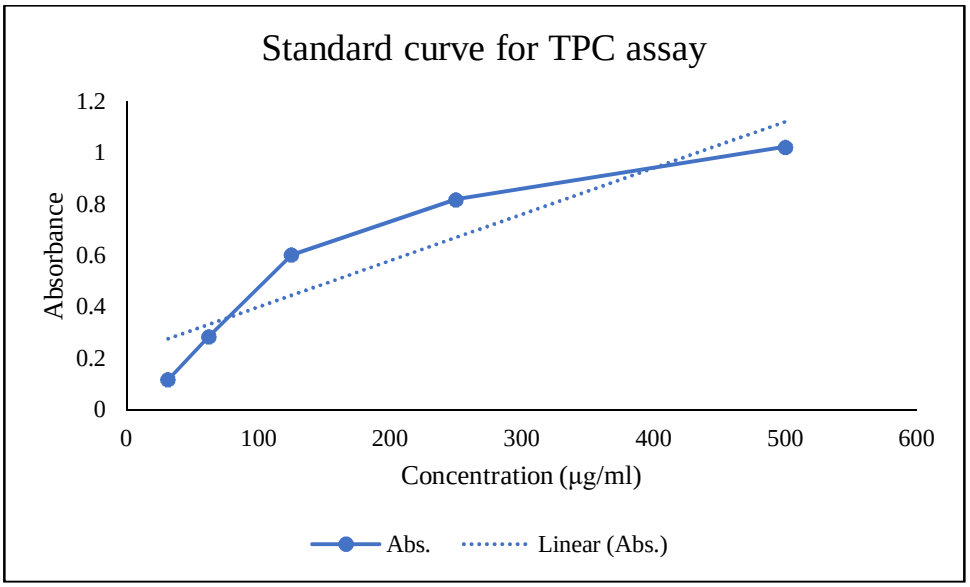

Figure 1. Standard curve for determining total phenolic content (TPC).

Table 3. IC_{50} values from DPPH assay and determination of TPC using the equation obtained from standard curve.

Test sample	Antioxidant potential assays	
	IC_{50} value (µg/ml)	TPC (mg of GAE/g) of the test sample extract
Methanol extract	42.02	29.96
Standard (Ascorbic acid)	7.24	

According to the determined IC_{50} values and the total phenolic content (TPC) analysis, *T. elephantina* leaves extract possesses antioxidant qualities. Besides phenolic substances, other agents with antioxidant properties (flavonoids, tannin) were identified in the phytochemical screening (Table 1). These assays suggested that the leaves of *T. elephantina* might be a potential reservoir of secondary metabolites with antioxidant properties.

Anti-inflammatory activity: Two dosages of the *T. elephantina* leaves extract were used in the anti-inflammatory test and the test sample exhibited 15.84%, 24.84%, 28.8% ($p < 0.05$), and 38.83% ($p < 0.01$) paw edema inhibition after one, two, three and four hours of carrageenan administration, respectively, at the higher dose (500 mg/kg). Whereas the standard (diclofenac sodium) showed 34.65% ($p < 0.05$), 55.28% ($p < 0.01$), 57.07% ($p < 0.01$) and 64.36% ($p < 0.001$) mice paw edema inhibitory

effects at the same time duration (Figure 2). Besides, 8.91%, 13.67%, 19.57% and 20.74% paw edema suppression was recorded by the test sample at 250 mg/kg dose at the same time interval.

It was evident from the phytochemical screening experiments (Table 1), and the previous report that the methanolic extract of *T. elephantina* leaves contained anti-inflammatory substances, such as flavonoids and tannins (Medini *et al.*, 2014). The results of the assay specified the significant anti-inflammatory properties of the *T. elephantia* leaves at the higher dose (500 mg/kg).

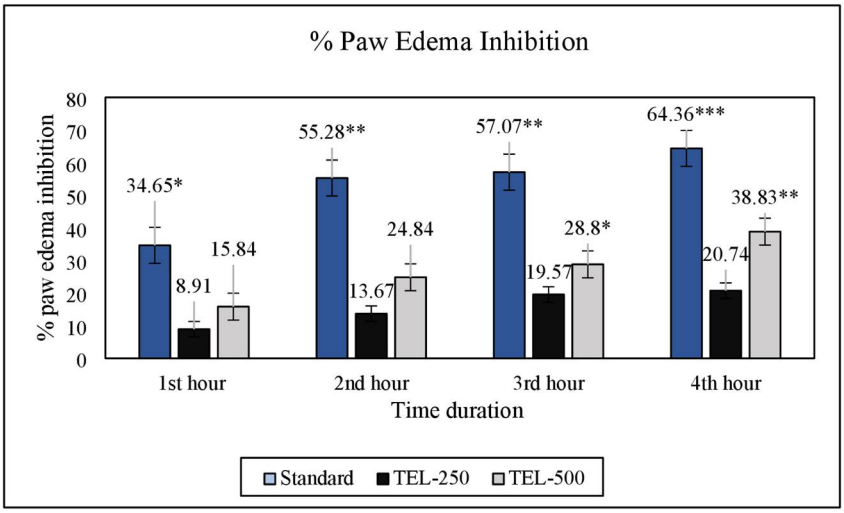

Figure 2. Anti-inflammatory properties the methanolic leaves extract of *T. elephantina* [***: p<0.001, **: p<0.01, *: p<0.05]

In the initial phase of carrageenan-induced inflammation, mediators like histamine, serotonin, and so on are usually associated. Prostaglandins in inflammation are linked to the vascular permeability. The later phase is associated with the release of TNF-a, IL-1, and IL-6. Kinins, once released, provoke releasing other inflammatory mediators, causing cell influx and plasma extravasations (Patil *et al.*, 2019). The anti-inflammatory substances are reported to exert their pharmacological effects mainly by interfering in the later phase activities (Meirer *et al.*, 2014). So, it might be predicted that the antioxidant substances present in the leaves of *T. elephantina* are able to limit the actions of the released inflammatory mediators. The currently used anti-inflammatory medications have negative physiological effects (Harirforoosh *et al.*, 2014). Natural sources, including medicinal plants, may be a potential option in finding newer anti-inflammatory agents. So, more works need to be executed to identify the potential bioactive substances from *T. elephantina* leaves.

Conclusions

The methanolic crude extract of *T. elephantina* leaves was obtained in the work and subjected to phytochemical tests. Phytochemicals like flavonoids, tannins, carbohydrates, alkaloids and phenolics were identified. The test sample also exhibited antibacterial potentiality against several bacterial species. *In vitro* total phenolic content (TPC) assay and DPPH free radical scavenging assay revealed antioxidant properties of the *T. elephantina* leaves. Mild to moderate anti-inflammatory activity was shown by the *in vivo* assay. The *T. elephantina* leaves thus revealed their medicinal potentials in the work.

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Conflict of interest

Regarding the publishing of the article, the authors declare that they have no conflict of interests.

Authors' contributions

Md. Kawser: Phytochemical screening tests, *In vitro* experimentation, Writing- Original draft; Sharmistha Dhar Shaily: Phytochemical screening tests, Supervision, *In vitro* experimentation; Sumiya Akter: *In vitro* experimentation; Md. Sabbir Hossain: Conception, *In vivo* experimentation, Supervision, Writing- Original draft, Writing- Review; Md Raihan Chowdhory: Writing- Original draft; Shahenul Islam: *In vivo* experimentation, Writing-Review; Md. Raihan Sarkar: Writing and proof reading.

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