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Phytochemical Screening and *in vitro* Antibacterial Activity of *Typhonium trilobatum* Methanolic extract

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Abstract: *Typhonium trilobatum* (Family: Araceae) is a common vegetable and a useful perennial herb in traditional medicine found all over Bangladesh. The purpose of this study was to evaluate the phytochemical screening, total phenolic content, total flavonoid content and antibacterial activity of *Typhonium trilobatum* species. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds and reducing sugar. The antibacterial study result confirmed that the crude methanolic extract of *Typhonium trilobatum* has the ability to inhibit bacterial growth. The higher antibacterial activity was found against Gram-negative bacteria *Shigella bodydii* where the zone of inhibition was found 12 mm for 1000µg/disc extract. The lowest inhibitory activity was found against *Salmonella paratyphi* with 8 mm zone of inhibition. The presence of total phenolic and flavonoid contents was found 10.8 mg gallic acid equivalent (GAE/gm) and 6.5 mg quercetin equivalent (QE/gm), respectively in each gram of *Typhonium trilobatum* methanolic extract. The antibacterial effects of the *Typhonium trilobatum* leaves extract may be linked to the presence of phenolic and flavonoid components. However, further studies are required to obtain more reliable information.

Keywords: *Typhonium trilobatum*, phytochemical, antibacterial activity, phenolic content, total flavonoid content.

Introduction

The genus *Typhonium* (Family: Araceae) comprises of about 69 species of tuberous perennial herbs and is widely distributed in tropical region of India, China, Bangladesh, Burma, Siam, Ceylon, Malaysia and North Australia. *Typhonium trilobatum* is one of them and it is a well-liked green vegetable that is less expensive due to its availability and is thus eaten by the under privileged. This perennial spice is a significant source of vitamins like thiamine, niacin, carotene, and folic acid. In Bangladesh, it is typically referred to as Bengal arum, Ghat kanchu, or Ghat kol [1].

The herb has historically been used to treat a variety of illnesses. Both the Unani and Ayurvedic medicines have provided detailed descriptions of the medicinal properties of this plant. According to scientific studies, the plant contains antibacterial, antioxidant, anti-inflammatory, and nematocidal

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properties. In addition, it treats spleen enlargement, liver disease, dermatitis, piles, boils, body aches, rheumatoid arthritis, edema, and piles [2].

The vast majority of the species of T. *trilobatum* are utilized customarily to treat regurgitating, hack, asthma, pyogenic sore throat, gastric ulcer, migraine, abscess, snake nibble [3] and feminine inconveniences [4]. The plant is also utilized as an energizer. The leaves are cooked as vegetables and given to the patient suffering from heaps and ailment [5, 6]. Different studies showed that the leaf contains reducing sugars, alkaloids, glycosides, gums, tannins and saponins. Rhizome contains lectins and roots contain flavonoids, carbohydrates and phenols [1]. A summary of the pharmacological activities reported from various authors is given below (Table 1).

Summary of pharmacological activities of *Typhonium trilobatum*

Pharmacological activity	**Experimental models**	**Animals/microbes/ larvae used**	**Parts of plant used**	**References**
Anti-inflammatory activity	Xylene-induced ear Edema model	Wistar rats	Leaf	[7]
Analgesic activity	Acetic acid-induced writhing method	Swiss-albino mice	Leaf	[7]
Wound healing activity	Excision wound model and the incision wound model	Adult albino rats	Whole plant	[9]
Anti-bacterial activity	Disc diffusion methods	*Salmonella typhi* (18), *Proteus mirabilis* (15), *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*	Tuber and aerial parts	[10,11]
Anti-fungal activity	Disc diffusion methods	Candida albicans and Aspergillus niger	Aerial parts	[11]
Anti-diarrhoeal activity	Castoroil-induced Diarrhoea	Swiss-albino mice	Leaf	[7]
Larvicidal activity	Dose response larvicidal bioassay	Culex quinquefasciatus	Leaf	[12]
Anti-oxidant activity	DPPH free reducing scavenging assay, scavenging of hydrogen peroxide and nitric oxide radical scavenging assay	Root		[13]
Anti-diabetic activity	Alloxan induced Diabetic model	Albino rats	Leaf	[14]
Anti-depressant Activity	Forced swimming Test method	Swiss-albino mice	Root	[13]

The objective of the present study was to evaluate antibacterial activity of methanol extract of *Typhonium trilobaum* leaf and stem extracts using disc diffusion method against few Gram-positive bacteria and Gram-negative bacteria and also to investigate the presence of different phytochemical compounds.

Methods and Materials

Chemicals and reagents

Methanol (Merck), ethanol (Merck), standard antibiotic disc (ciprofloxacin) (Bioqual, Inc.), Folin-ciocalteu (FC) reagent (Loba), gallic acid (Loba), quercetin (SRL), nutrient agar medium (Hi media), nutrient broth medium (Hi media), bismuth carbonate (Loba), sodium iodide (Merck), 5% glacial acetic acid (Merck), ethyl acetate (Merck), distilled water, mercuric chloride (Merck), potassium iodide (Merck), sodium citrate (Merck), sodium carbonate (Merck), copper sulphate (Merck), potassium sodium tartrate (Merck), NaOH (Merck), iodine solution (Unique Scientific Mart), alcoholic α- nepthol (Merck), conc. H_2SO_4 (Merck) , 5% ferric chloride (Merck), KOH pellets (Merck), nitric acid (Merck), lead acetate solution (Loba), sodium nitrite (Research Lab), potassium dichromate solution, sodium bicarbonate (Merck), sodium acetate trihydrate (Merck).

Collection and identification of plant materials

The leaves and stem of *Typhonium trilobatum* used in this experiment were collected in September 2022 from the area of Satarkul village, Dhaka, Bangladesh. Authentication was done by expert teachers at the Department of Pharmacy, Faculty of science and Engineering, Dhaka International University.

Figure 1: *Typhonium trilobatum*

Extraction and isolation

Fresh plant were harvested and washed thoroughly with running tap water. The washed and cleaned stem and leaves of the plant were dried in air and then dried in oven at 60℃.

Figure 2: Air dried *Typhonium trilobatum*

Figure 3: *Typhonium trilobatum* in oven

The stem and leaves of dried plant were crushed, powdered and weighed separately, before cold extraction. Dried powdered leaves and stem 150g of *Typhonium trilobatum* were soaked with 600ml methanol by cold extraction method in closed bottle container for 19 days, accompanying occasional shaking and stirring. After 19 days, the combined extract underwent a coarse filtration by a piece of clean white cotton, and then it was filtered with Whatman filter paper.

The filtrate (methanol extract) was evaporated using rotary evaporator (DLAB, USA). After drying, dried sticky extract was obtained and weighed to calculate the yield of the extract. This extract was designated as crude extract. Throughout the experiment, the plant extract was preserved in beaker (100ml) covered with aluminum foil and kept in cool place at 4℃.

Phytochemical analysis

Phytochemical screening of this plant extract:

Preliminary qualitative analysis of the extracts were carried out to determine the presence of various phytochemicals which include tannins, phenolics, flavonoids, alkaloids, saponins, steroids and glycosides in accordance with the methods as described below.

Reagent preparation for phytochemical screening

Reagent	Stock solution	Working solution	Compounds for identification
Drangendroff ’s reagent	5.2gm bismuth carbonate + 4gm sodium iodide + 50mL glacial acetic acid, boiled for few min, after 12hr precipitated sodium acetate crystals are filtered by sintered glass funnel; 40mL filtrate + 160mL ethyl acetate + 1mL distilled water, (stored in amber-coloured glass bottle).	10mL stock solution +20mL acetic acid + distilled water to make final volume 100mL.	Alkaloids
Mayer’s reagent	Solution A; 1.358gm mercuric chloride + 60mL distilled water Solution B; 5gm potassium iodide +10mL distilled water	Working solution; solution A + solution B + distilled water to make final volume 100mL.	Alkaloids

Wagner's reagent	1.27gm iodine + 2gm potassium iodide + distilled water to make final volume 100mL.		Alkaloids
Seliwanoff's reagent:	0.05% resorcinol +100mL dilute HCl		Reducing sugar
Benedict's reagent	**Solution A;** 173gm sodium citrate + 100gm sodium carbonate+800mL water, dissolve and boil to make solution clear **Solution B;** 17.3gm of copper sulphate dissolved in100mL distilled water	**Working solution;** mix solution A and solution B	Reducing sugar
Fehling's solution	**Solution A;** 34.66gm copper sulphate + distilled water to make final volume 100mL **Solution B;** 173gm potassium sodium tartarate + 50gm NaOH + ditilled water to make 100mL.		Carbohydrate, Reducing sugar

Determination of antibacterial activity

Six bacterial strains used in the present study include *Shigella bodydii*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aureus*, *Sarcina lutea* and *Salmonella paratyphi*. These organisms were maintained on nutrient agar slopes and the organisms were confirmed by biochemical test. Agar media was prepared by adding 5.6 g of nutrient agar to 200ml distilled water and then autoclaved at 121°C and pressure of 15 lbs. /sq. inch for 15 minutes. Agar slants were prepared by pouring sterilized agar media into petri dishes with inoculation of bacteria and then incubated at 37°C (typically 37.5-37.8°C) for 16-18 hours to observe the zone of inhibition. To observe the zone of inhibition, standard ciprofloxacin concentration was 5 µg and crude extract concentration were 1000 µg and 2000 µg[7, 8].

Determination of Total Phenolic Content

The total phenolic content of the extract of *Typhonium trilobatum* was determined by using Folin-ciocalteu reagent. 1 ml of plant or standard of different concentration solution was added in 5 ml of Folin-ciocalteu (diluted 10 times with water) reagent and 5 ml of sodium carbonate (7.5%) solution. The mixture was incubated for 20 minutes at 25℃ and the absorbance of the final mixture was measured at 760 nm. Gallic acid was used as reference standard and the result was expressed as mg gallic acid equivalent (GAE/gm) of the dried extract [7, 8].

Absorbance of different concentrated solution mixtures were measured at 760 nm using spectrophotometer against blank and the TPC in root extract in Gallic Acid Equivalence (GAE) was calculated using the following equation.

$$\boldsymbol{C = (C \times \mathcal{V})/m}$$

Here, C= total content of phenolic compounds, mg/gm root extract, in GAE
c= the concentration of Gallic acid established from the calibration curve (mg/ml)
$\boldsymbol{\mathcal{V}}$ = the volume of extract in ml
m = the weight of root extract in gm.

Determination of Total Flavonoid Content

For the determination of total flavonoid of the extract of *Typhonium trilobatum*, quercetin was used to make the standard calibration curve. Using methanol (5-200 g/mL), the standard solutions of quercetin were prepared by a series of dilutions. Separately, 0.6 mL of diluted standard quercetin extracts or solutions was combined with 0.6 mL of 2% aluminum chloride. The mixture was then left to sit at room temperature for 60 minutes. Using a UV-Vis spectrophotometer, the absorbance of the reaction mixtures was measured against a blank at 420 nm. The calibration plot was used to compute the amount of total flavonoid content in the test samples, which was then reported as mg of quercetin equivalent (QE)/g of dried plant material [7, 8].

The total content of flavonoid compounds in plant methanol extracts in Quercetin equivalents was calculated using the following equation:

$$C = (C \times V)/m$$

Here, C= total content of phenolic compounds, mg/gm root extract, in Quercetin equivalent,
c= the concentration of Quercetin established from the calibration curve (mg/ml),
V= the volume of extract in ml
m = the weight of root extract in gm.

Result:

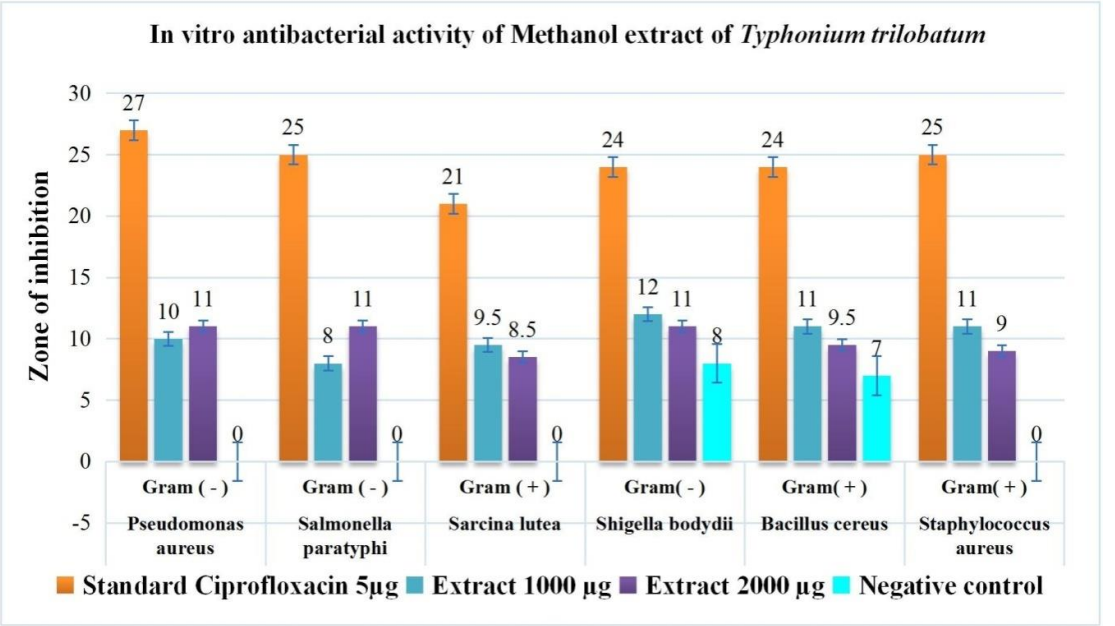
For centuries, *Typhonium trilobatum* has been utilized as a traditional medicine, not just in Bangladesh but also in other areas of the world. Under this experiment a number of different tests have been done. The tests have been summarized in the following graphs.

Phytochemical test results

Test	Observation
Alkaloid	+
Carbohydrate	+
Reducing sugar	-
Cardiac glycoside	-
Proteins and amino acids	-
Flavonoids	+
Phenolic compounds	+
Saponins	+

(+) indicates presence, (-) indicates absence

Evaluation of antibacterial activity result



Determination of Total Phenolic Content

Graph for the determination of phenolic content (Standard)

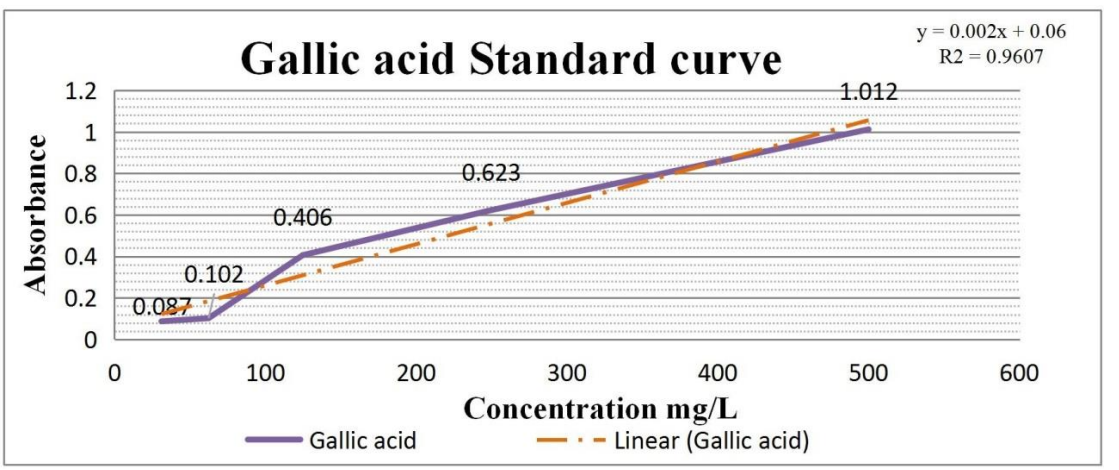
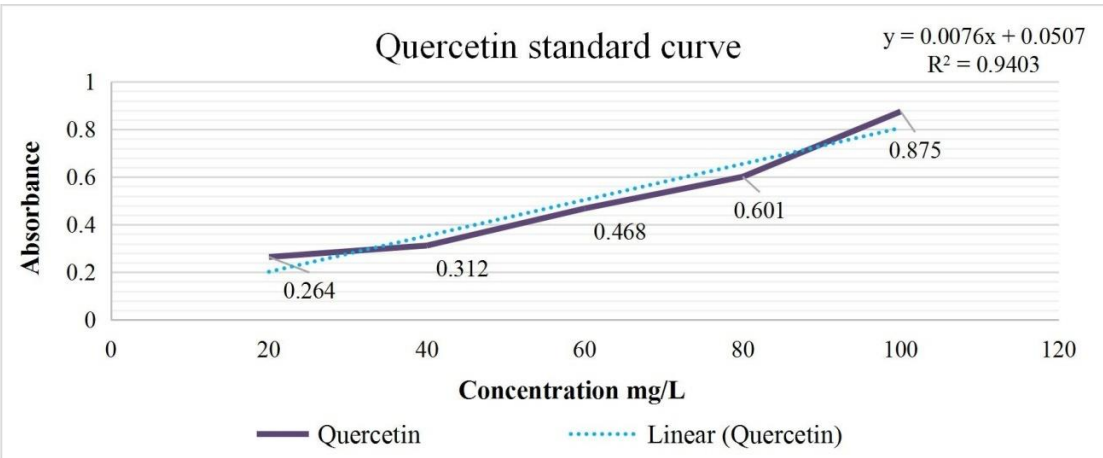


Table 1: Data for the determination phenol content of *Typhonium trilobatum* samples

Sample solution (µg/ml)	**GAE conc. (µg/ml)**	**TPC as GAE equivalent, C= (mg/gm)**

1000	9.6	**9.6**
1000	12.15	**12**

Determination of Total Flavonoid Content
Graph of determination total flavonoid content (Standard).



Determination of Total Flavonoid Content
Graph of determination total flavonoid content (Standard).

Table 2: Data for determination flavonoid content of *Typhonium trilobatum* samples

Sample solution (µg/ml)	**Quercetin conc. (µg/ml)**	**TFC as Quercetin Equivalent, C= $\frac{c \times v}{m}$ (mg/gm)**
1000	**5.96**	**6**
1000	**6.75**	**7**

DISCUSSION

The crude extracts of *Typhonium trilobatum* were subjected to phytochemical screening, determination of total phenolic content, total flavonoid content and *in vitro* antibacterial activity. The phytochemical screening tests revealed the presence of phenols, flavonoids and alkaloids in this plant. *In vitro* antioxidant activity of leaf and stem extracts were determined by assessing its total phenolic and flavonoid content. Polyphenol have been shown to decrease the formation of atherosclerotic plaques, block LDL oxidation and reduce arterial stiffness, leaving arteries more responsive to endogenous stimuli of vasodilation. Ethanol extract of leaves of the plant was found to contain the highest amount of phenolic content (13.3. mg/gm) [8]. Our investigation also confirmed similar result, a total of 10.8mg GAE/gm of phenolic content with reference to gallic acid.

Plants' antioxidant systems depend heavily on flavonoids. The scavenging of free radicals, chelation of metal ions like iron and copper, and inhibition of enzymes that produce free radicals are only a few of the processes by which flavonoids possess antioxidative characteristics. Depending on their structural

makeup, flavonoids can scavenge nearly every known ROS. Previous study with *Typhonium trilobatum* leaf ethanol extract found to have the greatest flavonoid concentration (14.26 mg/gm) [8]. In compare with this, our study with *Typhonium trilobatum* methanol extract has found average 6.5 mg quercetin equivalent (QE/gm) flavonoid concentration. This may be due to natural variations. Flavonoid contents of the extracts were found to decrease in the following order: Ethanol Extract > Methanol Extract [8].

The zone of inhibition for the antibacterial activity of *Typhonium trilobatum* methanolic extract at concentrations 1000µg/10µl were recorded for *Shigella bodydii* (12±0.29 mm), *Bacillus cereus* (11±0.29 mm), *Staphylococcus aureus* (11±0.29 mm), *Pseudomonas aureus* (10±0.29 mm), *Sarcina lutea* (9.5±0.29 mm) and *Salmonella paratyphi* (8±0.29 mm). Our result suggested that this extract has antibacterial properties against both the Gram positive and negative bacteria. Study reported active growth inhibition of S. aureus by crude methanolic, ethyl acetate and chloroform extracted fractions of the aerial parts of *Typhonium trilobatum* Linn [15].

The medicinal value of the plant extract may be related to their phytochemical constituent. So further investigations are needed to isolate and identify the active component present in the plant extract and its various fractions, and their efficacy. It will help in the development of novel and safe drugs for the treatment of various diseases.

Conclusion

Phytochemical screening and *in-vitro* pharmacological evaluation of crude extract *of Typhonium trilobactum* were done to investigate different therapeutic activities of this plant. Phytochemical screening tests confirmed the presence of phenols, flavonoids and alkaloids in the leaf extract. *In-vitro* antioxidant activity test determined the scavenging activity of *Typhonium trilobactum* including total flavonoids and phenolic contents. The leaf extract also revealed significant antibacterial activity. This study result indicates that *Triphonium trilobactum* may be an important source of new drug discovery including antioxidant, antibacterial agents and cardioprotective agents. Therefore, further study on *Typhonium trilobactum* might be required to find out other therapeutic effects and to isolate new medicinal compounds.

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