

**PHYTOCHEMICAL AND PHARMACOLOGICAL INVESTIGATION  
OF THE CRUDE EXTRACT OF *TYPHONIUM TRILOBATUM* (L.)  
SCHOTT**

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Article Received on  
30 Nov 2014,

Revised on 24 Dec 2014,  
Accepted on 19 Jan 2015

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**ABSTRACT**

*Typhonium trilobatum* (L.) Schott of the family Araceae has been considered as an important plant in traditional medicine practices in Bangladesh. The aim of the present study was to evaluate the phytochemical constituents of *Typhonium trilobatum* along with *in-vitro* antioxidant activity, *in-vitro* thrombolytic and membrane stabilizing activities, *in-vivo* anti-spasmodic activity, *in-vivo* anti-depressant activity and acute toxicity were investigated. Standard qualitative chemical tests were performed as phytochemical screening and the presence of flavonoids, phenols, and alkaloids was detected in various parts of the plant. Six different assays were performed to evaluate antioxidant activity and the leaf extracts showed good

potential. The presence of flavonoid and phenolic contents was remarkable in the ethanolic leaf extract of the plant. Methanol (% clot lysis 30.15%) and chloroform (% clot lysis 22.64%) extracts of leaves of the plant showed significant ( $p < 0.05$ ) thrombolytic effect and good human RBC membrane stabilizing effect. Chloroform extracts of 200 mg/kg showed very significant ( $p < 0.01$ ) result in GI motility. All the leaf extracts of 100 and 200 mg/kg demonstrated very potent dose dependent anti-depressant effect. Thus the present study validated the traditional uses of *Typhonium trilobatum* and demonstrated its potential to contribute in drug discovery from natural resources.

**KEYWORDS:** *Typhonium trilobatum*, Membrane stabilizing, *in-vitro* antioxidant activity, GI Motility, Acute Toxicity.

## INTRODUCTION

Typhonium is a genus in the Araceae family endemic to tropical Asia, the South Pacific, and Australia. It consists of approximately 50 species that are typically found growing in wooded area. Seven species of Typhonium grow in Bangladesh among them Typhonium available in Bangladesh, *Typhonium trilobatum* (L.) Scott is selected for the current study. It is a neglected species of Typhonium genus on which very few scientific investigations have been conducted although it is widely used as traditional medicine. There remains a possibility that the plant may contain some bioactive compounds essential to treat diseases and so this plant is considered under the current phytochemical and pharmacological studies. *Typhonium trilobatum* is a small tuberous herb, with subglobose tuber up to 4 cm diam. Petiole 25-30 cm long; lamina hastate-subtrisection, segments all acuminate, front segment ovate, 8-18 cm long, lateral ones obliquely ovate, shorter, subbilobed at base. Peduncle thin, 5-7 cm long; tube of spathe oblong, 2.5 cm long, lamina oblong-ovate-lanceolate, acuminate, 15 or more cm long, 5-7 cm broad, inside rose-purple. Spadix nearly 15 cm long. Female inflorescence short-cylindric, about 7 mm long; male inflorescence 1.25-1.5 cm long, rose-pink, situated above the female. Flowering and fruiting time: April- October.<sup>[1]</sup> As a part of our continuing studies on medicinal plants of Bangladesh the organic soluble materials of the crude extract of *Typhonium trilobatum* were evaluated for phytochemical screenings, *In-vitro* bioassay study of leaf extracts of the plant which are antioxidant activity, thrombolytic activity, membrane stabilizing activity (both hypotonic solution induced and heat induced RBC hemolysis), *In-vivo* bioassay study of leaf extracts of the plant; antispasmodic activity or effect on GI motility; anti-depressant activity (forced swim test); acute toxicity were performed for the first time.<sup>[2-9]</sup>

## MATERIALS AND METHODS

**Plant Collection and Identification:** Whole plant of *Typhonim trilobatum* (L.) Schott was collected during June, 2014, from the premises of University of Dhaka. Then the plant sample was submitted to the National Herbarium of Bangladesh, Mirpur, Dhaka. One week later its voucher specimen was collected after its identification (Accession No.39584) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka.

**Extraction Procedure:** The powdered plant parts (22 gm) were successively extracted in a soxhlet extractor at elevated temperature using 250 ml of distilled methanol (40-60) °C which

was followed by ethanol and chloroform. After extraction all extracts kept in refrigerator 4°C for future investigation with their necessary markings for identification.

**Photochemical Screening:** Different extracts were screened for the presence of phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate by using standard protocols.<sup>[10]</sup>

### **In-vitro Bioassays**

#### **DPPH Free radical scavenging activity**

The free radical scavenging capacity of the extracts was determined using DPPH.<sup>[11]</sup> 1 ml of plant extract or standard of different diluted (6.25 µg/ml to 800 µg/ml) concentration solutions was taken in test tube and freshly prepared 2 ml of 0.004% DPPH solution is added in each test tubes to make the final volume 3 ml. Incubate the mixture in room temperature for 30 minutes, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard. The absorbance was read at 517 nm using a spectrophotometer. Ethanol was served as blank. % inhibition of the DPPH free radical was measured by using the following equation:

$$\% \text{ inhibition} = (1 - A_1/A_0) \times 100\% \dots\dots\dots [\text{Equation 1}]$$

where,

A<sub>1</sub> = Absorbance of the extract or standard

A<sub>0</sub> = Absorbance of the control

#### **Cupric reducing antioxidant capacity (CUPRAC)**

The assay was conducted as Demiray *et al.*, 2009.<sup>[12]</sup> To 0.5 ml of plant extract or standard of different diluted (5 µg/ml to 200 µg/ml) concentrations solutions was taken in test tube and added 1 ml of copper (II) chloride solution (0.01 M prepared from CuCl<sub>2</sub>.2H<sub>2</sub>O), 1 ml of ammonium acetate buffer at pH 7.0, 1 ml of neocaproin solution (0.0075 M) were mixed. The final volume of the mixture was adjusted to 4.1 ml by adding 0.6 ml of distilled water and the total mixture was incubated for 1 hour at room temperature. Then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank. Ascorbic acid was used as a standard.

**Total phenolics analysis:** Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method.<sup>[13]</sup> All of extracts and standard were diluted by serial

dilutions as (6.25 µg/ml to 200 µg/ml) then, on each test tube containing 1ml of diluted solution of sample and standard, following reagent solutions were added 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (7.5% sodium carbonate) of sodium carbonate. Samples were incubated at 20<sup>0</sup>C temperature for 60 minutes and standard diluted solution–reagent mixture was incubated at 20<sup>0</sup>C temperature for 30 minutes. Absorbance of samples and standard were measured at 765 nm using spectrophotometer against blank. A typical blank solution contained the solvent used to dissolve the plant extract. The total content of phenol compounds in plant extracts in Gallic acid equivalents (GAE) was calculated using the following equation:

$$C = (c \times V)/m \dots\dots\dots [\text{Equation 2}]$$

Where; C = total content of phenol compounds, mg/gm plant extract, in GAE, c = the concentration of Gallic acid established from the calibration curve (mg/ml), V = the volume of extract in ml, m = the weight of crude plant extract in gm.

#### **Determination of total flavonoids content**

Aluminum chloride colorimetric method was used for flavonoids determination.<sup>[13]</sup> To 1 ml of plant extract or standard of different diluted (6.25 µg/ml to 200 µg/ml) concentrations solutions was taken in a test tube and added 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It incubated at room temperature for 30 min then absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated with equation 2.

**Determination of total antioxidant capacity:** The total antioxidant capacity was evaluated by the phosphomolybdenum method.<sup>[14]</sup> 0.3 ml of extract and sub-fraction in methanol, ascorbic acid used as standard (12.5-200 µg/ml) and blank (methanol) were combined with 3 ml of reagent mixture separately and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A = (c \times V)/m \dots\dots\dots [\text{Equation 3}]$$

Where, A = total content of antioxidant compounds, mg/gm plant extract, in ascorbic acid equivalent c = the concentration of ascorbic acid established from the calibration curve, mg/ml, V = the volume of extract in ml, m = the weight of crude plant extract, gm.

### **Nitric oxide scavenging assay**

Nitric oxide scavenging assay was carried by using sodium nitroprussid.<sup>[15]</sup> This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub- fraction of different diluted (6.25 µg/ml to 100 µg/ml) concentrations solution and the mixture was incubated at 25°C for 150 minutes. From the mixture 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 minutes. Finally, 1.0 ml Naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 minutes. The absorbance was measured at 546 nm. Typical control solutions contain the same solution mixture without plant extract or standard. % inhibition was calculated by using equation 1.

### **Hydrogen peroxide scavenging assay**

The scavenging activity of extract towards hydrogen peroxide radicals was determined by the modified method.<sup>[16]</sup> Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560nm using UV spectrophotometer. 0.1mg/ml of the extract was added to hydrogen peroxide solution and absorbance measured at 560nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated using equation 1.

### **Thrombolytic Activity**

#### **Blood sample**

Blood was drawn from healthy human volunteers (n=5) without a history of oral contraceptive and anticoagulant therapy. 500 µl of blood was transferred to previously weighted micro-centrifuge tubes and was allowed to form clots.

**Streptokinase (SK):** Commercially available lyophilized Altepase (Streptokinase) vial (Trade name: S-Kinase from Popular Pharmaceuticals Ltd.) of 15,00,000 I.U. was collected and 5ml of 0.9% NaCl was added and mixed properly to prepare the concentration 3,00,000 I.U. This suspension was used as stock from which 100 µl was used for *in-vitro* thrombolysis.

The thrombolytic activity of all extracts were evaluated by using *in-vitro* clot lysis method developed by Prasad *et al.*, 2006 and Ratnasooriya *et al.*, 2008<sup>[17,18]</sup> with slight modification, using streptokinase (SK) as the standard substance. Human blood was allowed to clot and different extracts of *Typhonium trilobatum* leaf were added to the clotted blood. Thrombolytic activity was evaluated by determining % clot lysis using the following equation.

$$\% \text{ of clot lysis} = (W_2 - W_3 / W_2 - W_1) \times 100$$

Where,  $W_1$  = Weight of microcentrifuge tube alone,  $W_2$  = Weight of microcentrifuge tube with blood clot and  $W_3$  = Weight of microcentrifuge tube after clot lysis.

### Membrane Stabilizing Activity

#### Membrane stabilizing activity

The membrane stabilization by hypotonic solution and heat-induced haemolysis method was used to assess anti-inflammatory activity of the plant extracts by following standard protocol.<sup>[19, 20]</sup> Since the erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane.<sup>[21]</sup> The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced human erythrocyte haemolysis. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant 3.1% Na-citrate). The blood was centrifuged and blood cells were washed three times with solution (154mM NaCl) in 10mM sodium phosphate buffer (pH 7.4) through centrifugation for 10min at 3000g.<sup>[22, 23]</sup>

#### Hypotonic solution-induced haemolysis

The test sample consisted of stock erythrocyte (RBC) suspension (0.5mL) mixed with 5mL of hypotonic solution (50mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0mg/mL) or acetyl salicylic acid (ASA) (0.1mg/mL). The control sample consisted of 0.5mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10min at room temperature, centrifuged for 10min at 3000g and the absorbance of the supernatant was measured at 540nm. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

$$\% \text{ inhibition of haemolysis} = 100 \times (OD_1 - OD_2) / OD_1$$

Where,  $OD_1$  = optical density of hypotonic-buffered saline solution alone (control).

$OD_2$  = optical density of test sample in hypotonic solution.

### Heat-induced haemolysis

Isotonic buffer containing aliquots (5ml) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 56°C for 30min in a water bath, while the other pair was maintained at (0-5) °C in an ice bath. The reaction mixture was centrifuged for 5min at 2500g and the absorbance of the supernatant was measured at 560 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation.

$$\% \text{ Inhibition of hemolysis} = 100 \times [1 - (\text{OD}_1 - \text{OD}_2) / (\text{OD}_3 - \text{OD}_1)]$$

Where, OD<sub>1</sub>= optical density of unheated test sample

OD<sub>2</sub>= optical density of heated test sample.

OD<sub>3</sub>= optical density of heated control sample.

### In-Vivo Bioassays

#### Experimental animals

Swiss albino mice (*Mus musculus*) aged around 4 to 5 weeks and weighing 20-30 g of either sex was used for the research. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) Mohakhali, Dhaka. The animals were maintained under standard hygienic conditions (temperature 27<sup>0</sup>C ± 2<sup>0</sup>C, relative humidity 55-65% and natural day night cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments.

#### Gastrointestinal (GI) motility or anti-spasmodic activity

Forty eight Swiss Albino mice, weighing between 10-20 g were selected and housed properly for 10 days before performing the experiment. On the test day, the animals were divided into eight groups of six mice each. They were weighed and deprived of food, with free access to water. Three hours after food deprivation, the animals in group 1 received orally by gavages 5 ml/kg body weight of 0.9% NaCl (normal saline) as control group, while those in group 2 received 50 mg/kg body weight of butapen as standard group. The other six groups received their respective doses as shown in the table. After 90 min, 0.3 ml of an aqueous suspension of 5% charcoal in normal saline was administered to each animal orally by gavages (time 90 min). Sixty minutes later they had free access to food (time 150 min). The animals were observed at 5 min intervals until feces with charcoal were eliminated (maximum time of

observation was 300 min). Charcoal was observed on the feces using normal light when it was easily visible, or using a microscope to help the identification of the black spots. The results were based on the time for the charcoal to be eliminated.<sup>[24]</sup>

### **Anti-depressant activity (forced swimming test, FST)**

According to Porsolt *et al.*, 1977<sup>[25]</sup> swimming test was performed with slight modification. Animals were randomly divided into 8 groups with 6 mice on each group. Group 1 (control) received 1% Tween 80, 10ml/kg orally. Group 2 received diazepam of 10 mg/kg body weight which served as standard. Groups 3, 4 received sample where methanol extract of *Typhonim trilobatum* 100 mg/kg and 150 mg/kg of body weight. Groups 5, 6 received sample where ethanol extract of *Typhonium trilobatum* 100 mg/kg 150 mg/kg of body weight. Group 7, 8 served as sample where chloroform extracts of *Typhonim trilobatum* has given to mice as dose of 100mg/kg & 150mg/kg of body weight. The forced swim test was carried out on mice individually forced to swim in an open acquire water tank apparatus (29cm x 19cm x 20cm), containing 9 cm of water at 25±1 °C. The total duration of immobility during the 6-min test was scored as described. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility was recorded. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity.

### **Acute toxicity**

Acute toxicity describes the effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). The acute toxicity study was conducted to find out LC<sub>50</sub> of the test samples. The test samples were administered orally to the test animals at different concentration (100, 500 & 1000 mg/kg body weight). After administration of the extract solutions mortality or sign of any toxicity was observed for one hour. Then the test animals were observed every 1 hour for next 5-6 hours. The animals were kept under observation for 1 week.<sup>[26-30]</sup>

**Statistical Analysis:** Data was expressed as Mean ±SEM (Standard Error of Mean). Significance was measured by t-test (two sample assuming unequal variances). Results below p<0.05 and p<0.01 were considered as statistically significant.

## RESULTS AND DISCUSSIONS

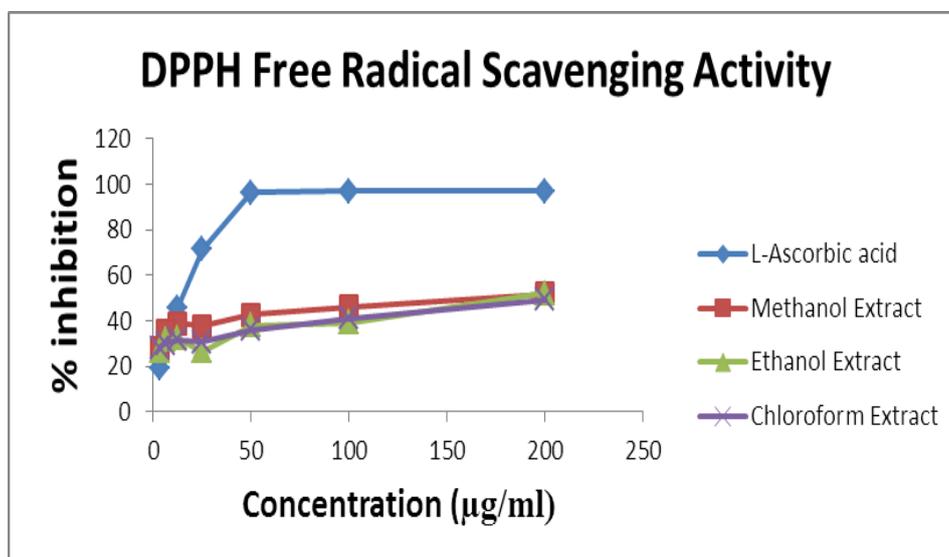
**Phytochemical Screening:** Preliminary phytochemical screening of crude extracts of different parts of *Typhonium trilobatum* (L.) Schott revealed the presence of different bioactive chemical components. Different crude extracts of *Typhonium trilobatum* have been shown to contain the presence of various chemical groups including flavonoids, carbohydrates, phenols and alkaloids. Different parts of the plant possess these components in different concentrations. Results show the presence of flavonoids in methanol extract of leaves and ethanol extract of root in moderate concentration. Methanol extract of root shows high concentration of carbohydrates whereas chloroform extract of leaves indicates the moderate presence of phenols and alkaloids. Methanol extract of root showed the presence of very low concentration of glycosides. No tannin, steroid and saponin were detected in this plant. These phytochemicals present in the crude extracts may account for their various pharmacological activities.

***In-vitro* Bioassays:** Oxidation is an imperative process for the generation of energy through metabolism to sustain our lives. But ironically the oxidation process exerts a great deal of oxidative stress which is usually managed by our endogenous antioxidants. If the oxidative stress is not balanced by the endogenous antioxidants, the oxidative damage may be caused by Reactive Oxygen Species (ROS) and this may lead to initiation of various diseases like aging, cancer, inflammation, and heart disease.<sup>[31]</sup>

Medicinal plants may contain bioactive substances that act as naturally occurring antioxidants; hence protective biochemical functions of these naturally occurring antioxidants in plants are gaining more and more attention of scientists as a key to manage oxidative stress.<sup>[32]</sup> A great number of *in-vitro* methods have been developed to measure the efficacy of natural antioxidants. It is essential to use more than one method to evaluate antioxidant capacity of plant extracts because of the complex structures of phytochemicals.<sup>[33]</sup> Different crude extracts of *Typhonium trilobatum* leaves were subjected to six different methods of antioxidant activity evaluation.

***DPPH free radical scavenging activity:*** DPPH radical scavenging is a popular and reliable method for screening the antioxidant activity of plant extracts.<sup>[34]</sup> DPPH free radical contains an odd electron and shows maximum absorption at 517 nm. When this odd electron of DPPH becomes paired with an electron donated by an antioxidant compound, its molar absorptivity reduces and the color turns from deep purple to yellow or becomes decolorized. This visual

color change confirms the DPPH free radical scavenging by the antioxidant molecule. The DPPH free radical scavenging activity is usually described as  $IC_{50}$  (concentration of sample to produce 50% reduction of free radical). The  $IC_{50}$  values of different leaf extracts of *Typhonium trilobatum* are presented in (Table 1). Different concentration (200, 100, 50, 25, 12.5, 6.25, 3.125  $\mu\text{g/ml}$ ) of leaf extracts of this plant were subjected to this investigation and the methanol, ethanol and chloroform extracts with standard L-ascorbic acid showed % inhibition at the same concentration in “Fig. 1”.  $IC_{50}$  of ascorbic acid was found 14.08  $\mu\text{g/ml}$ . In comparison to standard, methanol, ethanol and chloroform extracts showed  $IC_{50}$  values of 168.22, 183.54 and 200.13  $\mu\text{g/ml}$  respectively (Table 1). The result revealed that methanol extract of *Typhonium trilobatum* leaves has maximum DPPH free radical scavenging capacity and this capacity was found to decreased in following order: Methanol Extract > Ethanol Extract > Chloroform Extract.



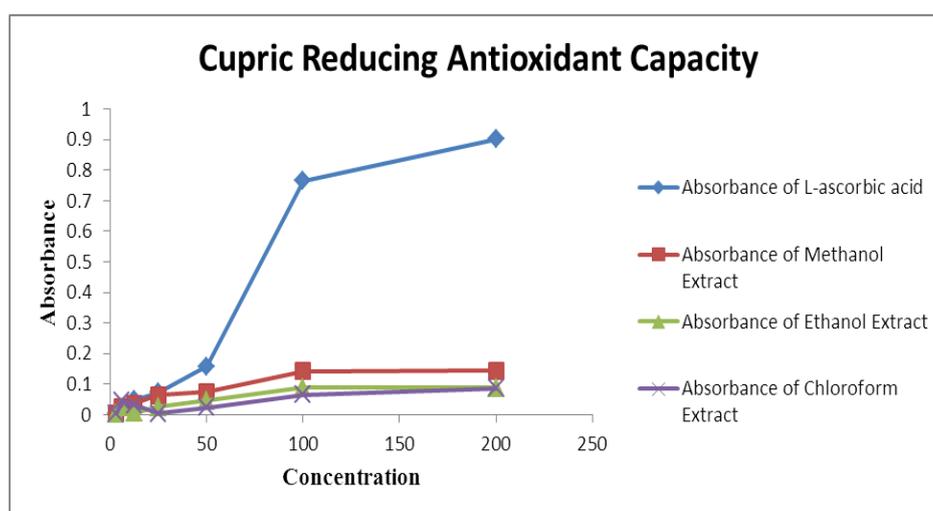
**Figure 1:** DPPH free radical scavenging activity of different leaf extracts of *Typhonium trilobatum*.

**Table 1:**  $IC_{50}$  values of different leaf extracts of *Typhonium trilobatum* in DPPH free radical scavenging assay

Sample	$IC_{50}$ value ( $\mu\text{g/ml}$ )
L-ascorbic acid	14.08
Methanol Extract	168.22
Ethanol Extract	183.54
Chloroform Extract	200.13

**Cupric reducing antioxidant capacity (CUPRAC):** This method is based on the principle of increase in the absorbance of reaction mixtures. Increase in absorbance indicates the increase in antioxidant capacity, thus increase in reducing power of the sample.<sup>[12]</sup> Compounds with reducing power indicates that they are electron donor and can reduce the oxidized intermediates, thus they can act as primary and secondary antioxidants.<sup>[35]</sup>

The standard L-ascorbic acid showed highest reducing capacity. The entire methanol, ethanol and chloroform extracts showed dose dependant reducing capacity but that is not significant as compared to the standard L-ascorbic acid. Among the three extracts of *Typhonium trilobatum* leaves methanol extract showed relatively better cupric reducing antioxidant capacity “Fig. 2”.



**Figure 2: CUPRAC of leaf extracts of *Typhonium trilobatum***

### Total phenolic content (TPC)

Polyphenols have been shown to block LDL oxidation, decrease the formation of atherosclerotic plaques and reduce arterial stiffness, leaving arteries more responsive to endogenous stimuli of vasodilation.<sup>[36-38]</sup> Moreover, polyphenols have been shown to exert anticarcinogenic effects and inhibit the cytochrome P<sub>450</sub> superfamily of enzymes that metabolizes many pro-carcinogens to reactive compounds, thus reducing the formation of reactive intermediates.<sup>[39]</sup> In addition, they have been shown to inhibit lipoxygenase and cyclooxygenase activity leading to lower aggregation of platelets and a reduction of thrombotic tendency.<sup>[36]</sup> The results strongly suggest that phenolics are important components of the tested plant extracts. Total phenolic content of the different extracts of was determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per

gram of plant extract. Ethanol extract of leaves of the plant was found to contain the highest amount of phenolic content (13.3 mg/gm) (Table 2). Phenolic contents of the extracts were found to decrease in the following order: Ethanol Extract > Methanol Extract > Chloroform Extract.

**Table 2: Total phenolic contents of different leaf extracts of *Typhonium trilobatum***

Extracts	Total Phenolic Content (mg/gm, Gallic Acid equivalents)
Methanol Extract	12.74
Ethanol Extract	13.3
Chloroform Extract	12.64

**Total flavonoid content (TFC):** Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation.<sup>[40]</sup> Depending on their structure, flavonoids are able to scavenge practically all known ROS. Aluminum chloride colorimetric method was used to determine the total flavonoid contents of the different extracts of *Typhonium trilobatum*. Ethanol extract of *Typhonium trilobatum* leaves was found to contain the highest amount of flavonoids content (14.26 mg/gm) (Table 3). Flavonoid contents of the extracts were found to decrease in the following order: Ethanol Extract > Methanol Extract > Chloroform Extract.

**Table 3: Total flavonoid contents of different leaf extracts of *Typhonium trilobatum***

Extracts	Total Flavonoid Content (mg/gm, Gallic Acid equivalents)
Methanol Extract	13.51
Ethanol Extract	14.26
Chloroform Extract	13.09

**Nitric oxide scavenging assay:** Nitric oxide (NO) is a physiologically important chemical mediator generated by endothelial cells and involved in the regulation of various biochemical processes. Excess generation and accumulation of NO are implicated in cytotoxic effects observed in various disorders like AIDS, cancer, Alzheimer's disease and arthritis.<sup>[41]</sup> Overproduction of NO can mediate toxic effects such as DNA fragmentation, cell damage and neuronal cell death. Different concentration (200, 100, 50, 25, 12.5, 6.25, 3.125 µg/ml) of leaf extracts of *Typhonium trilobatum* were subjected to investigate the nitric oxide

scavenging activity and the methanol, ethanol and chloroform extracts showed maximum activity of at low concentration whereas standard L-ascorbic acid showed the same. All the three extracts showed very good activity (methanol extract showed 90.40% inhibition, ethanol extract showed 86.91% inhibition and chloroform extract showed 89.53% inhibition) (Table 5) that is even higher than standard (76.16% inhibition). IC<sub>50</sub> of ascorbic acid was found 40.2 µg/ml. In comparison to standard, methanol, ethanol and chloroform extracts showed IC<sub>50</sub> values of 145.8, 165.67 and 155.45 µg/ml respectively (Table 4). The result revealed that methanol extract of *Typhonium trilobatum* leaves has maximum nitric oxide scavenging capacity and this capacity was found to decrease in following order: Methanol Extract > Chloroform Extract > Ethanol Extract.

**Table 4: IC<sub>50</sub> values of different extracts of *Typhonium trilobatum* leaves in nitric oxide scavenging assay**

Sample	IC <sub>50</sub> value (µg/ml)
L-Ascorbic Acid	40.2
Methanol Extract	145.8
Ethanol Extract	165.67
Chloroform Extract	155.45

**Table 5: % inhibition of samples in nitric oxide scavenging activity**

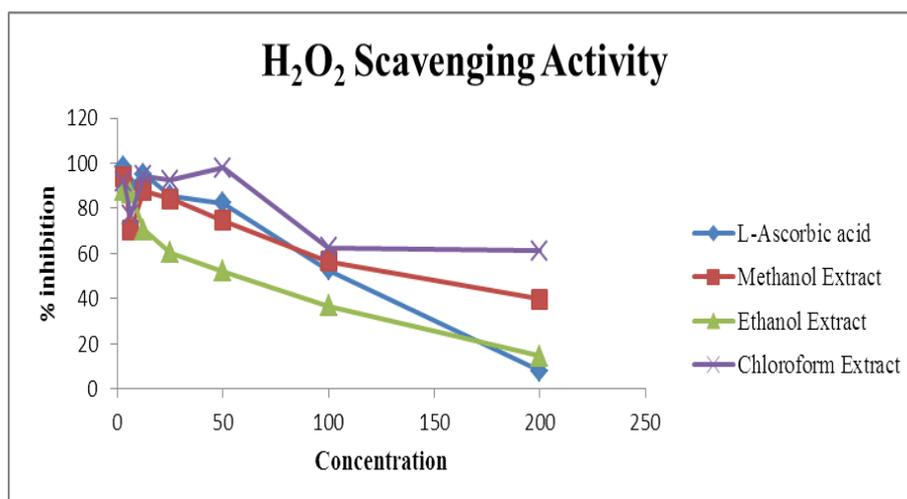
Samples	% inhibition
L-Ascorbic Acid	76.16
Methanol Extract	90.40
Ethanol Extract	86.91
Chloroform Extract	89.53

**Hydrogen peroxide scavenging activity:** Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H<sub>2</sub>O<sub>2</sub> in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H<sub>2</sub>O<sub>2</sub> in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects. % inhibition of hydrogen peroxide of different extracts of *Typhonium trilobatum* leaf is presented in figure "Fig. 2". Among the three extracts, Chloroform extract showed very good activity that is even higher than standard L-ascorbic acid. All these extracts possessed with an IC<sub>50</sub> value of 141.65, 58.58, and 187.78 µg/ml respectively whereas standard showed IC<sub>50</sub> value of 108.36 µg/ml (Table 6). The result revealed that ethanol extract of *Typhonium trilobatum* leaves has maximum H<sub>2</sub>O<sub>2</sub>

scavenging capacity and this capacity was found to decrease in following order: Ethanol Extract > Methanol Extract > Chloroform Extract.

**Table 6: IC<sub>50</sub> values of different leaf extracts of *Typhonium trilobatum* in H<sub>2</sub>O<sub>2</sub> scavenging assay**

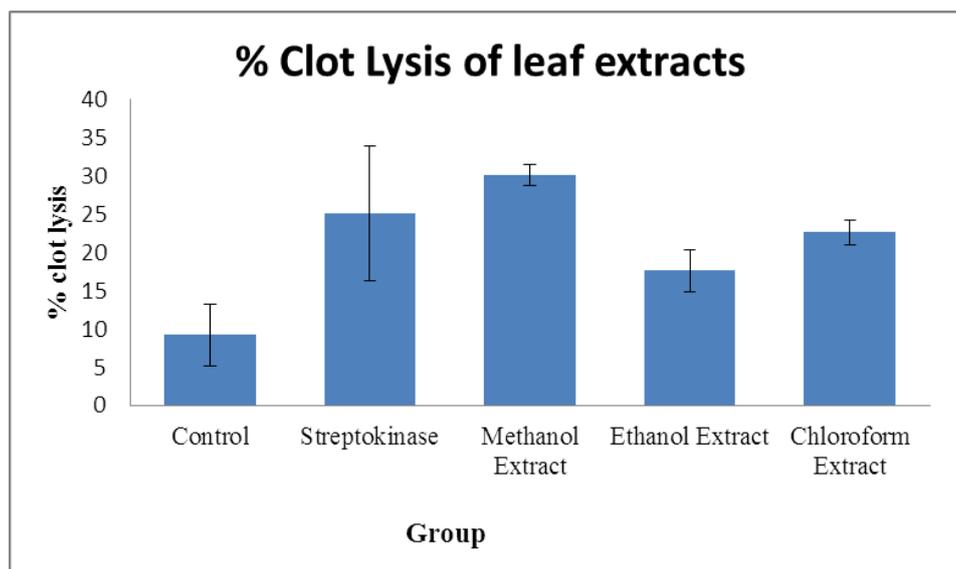
Sample	IC <sub>50</sub> value (µg/ml)
L-Ascorbic Acid	108.36
Methanol Extract	141.65
Ethanol Extract	58.58
Chloroform Extract	187.78



**Figure 2: H<sub>2</sub>O<sub>2</sub> scavenging activity of different leaf extracts of *Typhonium trilobatum***

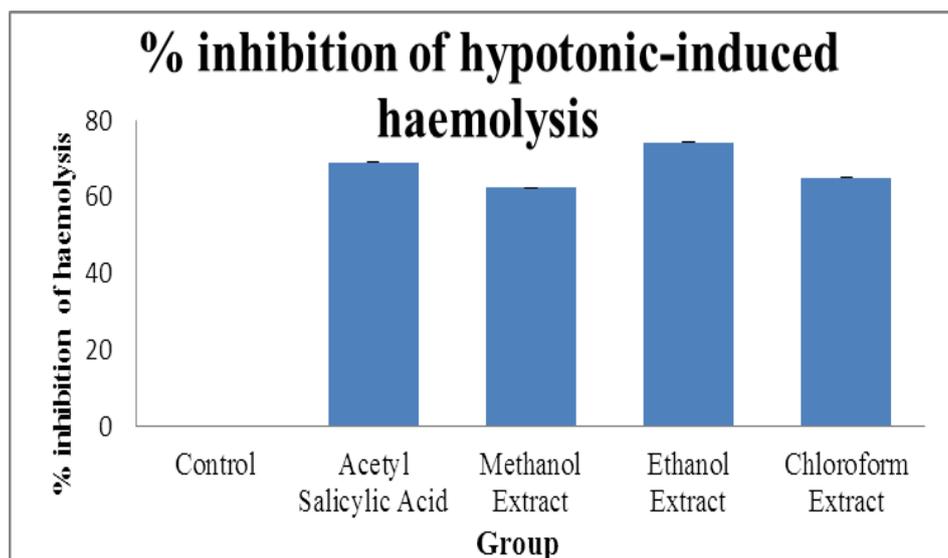
**Thrombolytic Activity:** The result revealed all the leaf extracts of *Typhonium trilobatum* exert better thrombolytic activity.

Blood clot treated with distilled water as control showed negligible clot lysis (9.23%) whereas Streptokinase as standard showed 25.07% clot lysis. Significant ( $p < 0.05$ ) clot lysis were visually seen in case of 20mg/ml methanol, ethanol and chloroform extracts of leaves of *Typhonium trilobatum*. Methanol extract showed maximum clot lysis (30.15%) that is even higher than standard activity (25.07%). Ethanol and chloroform extracts also showed better clot lysis of 17.62 % and 22.64% respectively “Fig. 3”. This is an important finding which may have important implications in cardiovascular health through the discovery of cardio-protective drugs from *Typhonium trilobatum*. In addition, this finding may indicate the possibility of developing novel thrombolytic agent from *Typhonium trilobatum*.



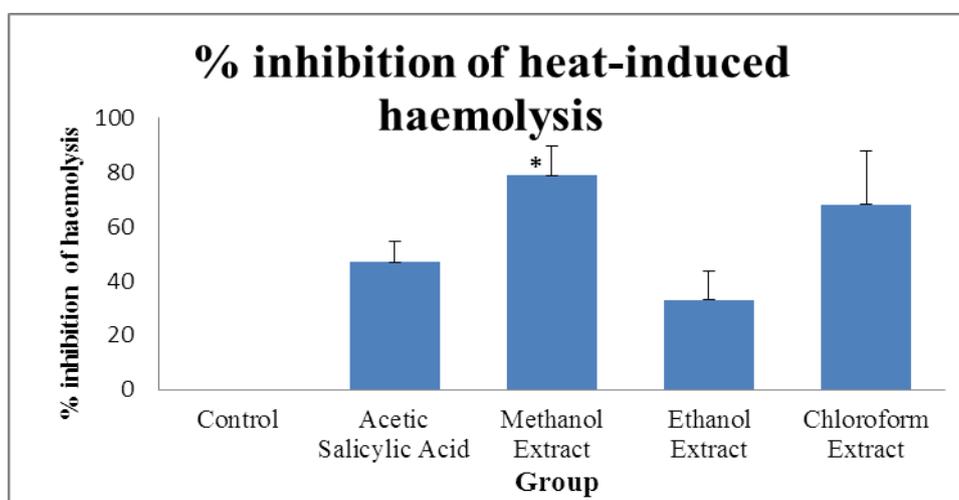
**Figure 3: % clot lysis of different leaf extracts of *Typhonium trilobatum* [Values are mean ± SEM; n=5;]**

**Membrane Stabilizing Activity:** In hypotonic solution induced haemolysis, standard acetic salicylic acid (0.1 mg/ml) showed 68.99% inhibition of RBC haemolysis whereas methanol extract, ethanol extract and chloroform extract, at a concentration of 1 mg/ml, showed 62.39%, 74.41% and 65.15% inhibition of RBC haemolysis respectively “Fig. 4”. On the other hand, in heat induced haemolysis, methanol extract, ethanol extract and chloroform extract showed 78.81%, 33.06% and 68.17% inhibition of RBC haemolysis respectively whereas standard acetic salicylic acid showed 46.87% inhibition of RBC haemolysis “Fig. 5”. The results revealed that although all the leaf extracts have very good potential of membrane stabilizing activity, ethanol extract showed good potential in hypotonic induced haemolysis and methanol and chloroform extracts showed better potential in heat induced haemolysis that are even much higher than the standard. No significant ( $p < 0.05$ ) value was observed in hypotonic solution induced haemolysis, but methanol extract showed significant ( $p < 0.05$ ) result in heat induced haemolysis. As lysosomal membrane stabilization contributes to protect cells from inflammation, the present investigation suggests that the membrane stabilizing activity of *Typhonium trilobatum* leaf extracts may play a very significant role in development of anti-inflammatory drugs.



**Figure 4:** % inhibition of hypotonic solution induced RBC haemolysis of *Typhonium trilobatum* leaves.

[Values represent Mean  $\pm$  SEM; n=5]



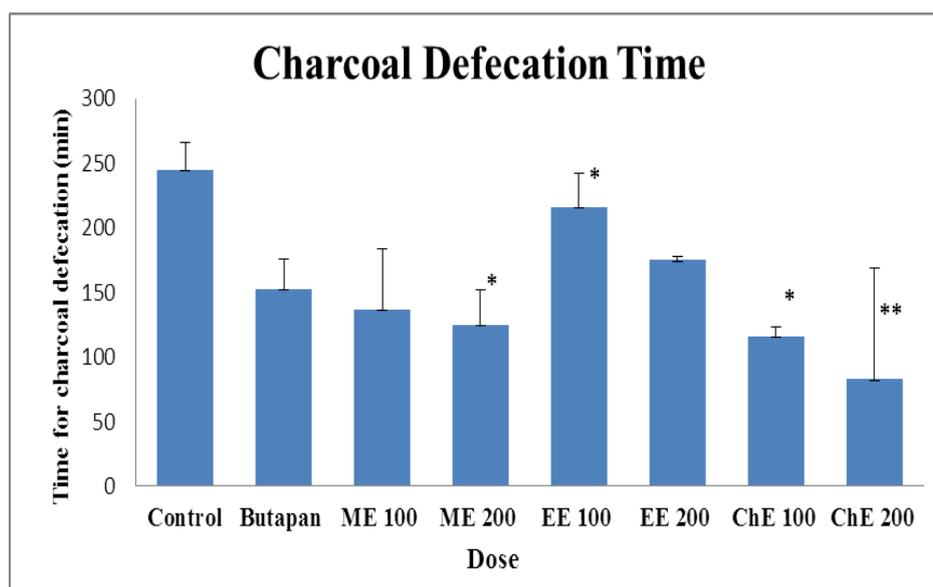
**Figure 5:** % inhibition of heat induced RBC haemolysis of *Typhonium trilobatum* leaves.

[values are mean  $\pm$  SEM (n=6), \* (p< 0.05), \*\* (p< 0.01), \*\*\* (p< 0.001) significantly different when compared with the corresponding value of standard group, done by independent sample t-test]

### In-Vivo Bioassays

**Anti-spasmodic activity / GI motility test:** Among all the leaf extracts, methanol extracts of 100 mg/kg and 200 mg/kg and chloroform extracts of 100 mg/kg and 200 mg/kg showed better GI motility or antispasmodic effect as compared to standard "Fig. 6". Charcoal defecation time after administration of methanol extracts of 100 mg/kg and 200 mg/kg were

137 minutes and 125 minutes respectively whereas standard butapan took 153 minutes. Defecation time decreased significantly ( $p < 0.05$ ,  $p < 0.01$ ) in case of chloroform extracts administration. Chloroform 100 mg/kg and 200 mg/kg extracts needed 116 and 83 minutes for charcoal defecation. The result revealed that the stimulating effect of the methanol and chloroform extracts of the plant on GI motility is dependent on its concentration. This increase in GI motility indicates the potential of antispasmodic effect of the extracts. The presence of alkaloid in *Typhonium trilobatum* leaf might be responsible for its increased GI motility effect.

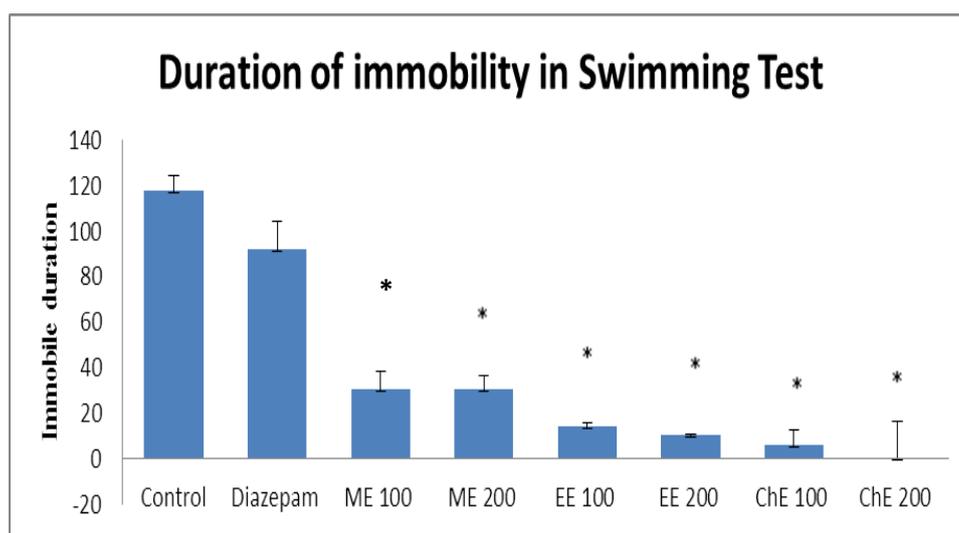


**Figure 6: Charcoal defecation time after administration of samples**

[Methanol Extract = ME, Ethanol Extract = EE, Chloroform Extract = ChE, values are mean  $\pm$  SEM (n=6), \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), \*\*\* ( $p < 0.001$ ) significantly different when compared with the corresponding value of standard group, done by independent sample t-test]

**Anti-depressant activity (forced swimming test, FST):** The swimming test has been validated as a suitable tool to evaluate drugs with anti-depressant effects.<sup>[25]</sup> When the subject is forced to swim in a confined place, they tend to become immobile after struggling. The depressant behavior of the subject in this stressful inescapable situation can be evaluated by assessing its immobile period. The development of immobility in that situation reflects the cessation of persistent escape-directed behavior.<sup>[42]</sup> In the present study, methanol, ethanol and chloroform extracts of 100mg/kg and 200 mg/kg showed immobile duration of 30.33, 30.67, 14.0, 10.67, 6.33 and 0.33 seconds respectively whereas standard diazepam 2mg/kg

showed 92 seconds of immobile time. The results revealed that all the extracts of *Typhonium trilobatum* leaves possessed significant ( $p < 0.05$ ) stimulant effect rather than anti-depressant effect. These extracts showed their capability to stimulate Central Nervous System (CNS) depending on their dose. The presence of polyphenols, flavonoids and alkaloids in *Typhonium trilobatum* leaf might be responsible for this potent stimulant effect because flavonoids are responsible for the decrease in immobile phase in the swim test<sup>[43]</sup> and so do alkaloids as well.<sup>[44]</sup> This important finding leads to conclude that the leaf of *Typhonium trilobatum* has a great potential to contribute in the development of potent CNS stimulant drug.



**Figure 7: Immobile time in swimming test after administration of samples.**

[Methanol Extract = ME, Ethanol Extract = EE, Chloroform Extract = ChE, values are mean  $\pm$  SEM (n=6), \* ( $p < 0.05$ ) significantly different when compared with the corresponding value of standard group, done by independent sample t-test]

**Acute toxicity:** No death or toxic reaction was observed in mice during the test period. This indicated the absence of any toxic material in the leaf of *Typhonium trilobatum*. The leaf extract of this plant is completely safe upto 1000 mg/kg.

## CONCLUSION

Different crude extracts of *Typhonium trilobatum* were subjected to phytochemical screening and *in-vitro* and *in-vivo* pharmacological evaluations to validate the traditional use and to find out any other therapeutic activity of the plant. Phytochemical screening revealed the presence of phenols, flavonoids, and alkaloids in different parts of the plant. *In-vitro* antioxidant activity determined the potential of leaf extracts by assessing its scavenging capacity and

total phenolic and flavonoid contents. The leaf extracts also revealed significant ( $p < 0.05$ ) thrombolytic activity and good membrane stabilizing capacity. Effect of leaf extracts on GI motility was evaluated and it showed significant ( $p < 0.05$ ,  $p < 0.01$ ) anti-spasmodic or laxative effect. The leaf extracts demonstrated very potent CNS stimulating or anti-depressant effect ( $p < 0.05$ ). The result clearly indicates that the crude extracts of *Typhonium trilobatum* may be a very important contributor in different drug discovery including cardioprotective, antioxidant, anti-spasmodic and anti-depressant drugs. The present study indicated a better chance of anti-tumor potential of the plant that might be revealed in near future. Therefore, further investigation on *Typhonium trilobatum* to isolate new bioactive compounds might be the next step to be followed.

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