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CHARACTERIZATION OF PHYTOCONSTITUENTS, *IN VITRO* ANTIOXIDANT ACTIVITY AND PHARMACOLOGICAL INVESTIGATION OF THE ROOT EXTRACT OF *TYPHONIUM TRILOBATUM*

Fahim Shafa[1], Mohammad Shahriar [*2], F. A. Dain Md. Opo [2], Rumana Akhter [2], M. Mahboob Hossain[1] and Nayyium Choudhury[1]

Department of Mathematics and Natural Sciences [1], BRAC University, Dhaka, Bangladesh.
Phytochemistry Research Laboratory[2], Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh.

Key words:

Typhonium trilobatum, Phytochemical screening, Membrane stabilizing, Thrombolytic activity, *In vitro* antioxidant activity

Correspondence to Author:
Mohammad Shahriar

Associate Professor
Department of Pharmacy, University of Asia Pacific, House no. 73, Road no. 5A, Dhanmondi, Dhaka-1209

E-mail: shahriar@uap-bd.edu

ABSTRACT: In the present study, the phytochemical screening, *in vitro* antioxidant activity, thrombolytic activity, membrane stabilizing activity, acute toxicity and anti-depressant activity were evaluated using Forced Swimming Test (FST) of the root extract of *Typhonium trilobatum*. Phytochemical evaluation was done by performing different chemical tests and the presence of flavonoid, carbohydrate and phenol were detected in the root parts of the plant. Methanol, chloroform and ethanol were used as solvent in extraction process. Each extraction yield was analyzed using High Performance Liquid Chromatography (HPLC) to identify and measure the quantity of ascorbic acid. Total flavonoid content and total phenol content was also determined by UV–VIS Spectrophotometer. Ascorbic acid, flavonoid, phenol was found to be of higher concentration in ethanol extract compared with methanol and chloroform extract. Ethanol was suitable solvent in extraction process of *Typhonium trilobatum. In vitro* antioxidant activity of the root extracts was performed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, cupric reducing anti-oxidant activity, scavenging of hydrogen peroxide and nitric oxide radical scavenging assay. These four methods were used to evaluate antioxidant activity and the root extract showed good potential. The root extract showed lower thrombolytic activity. Anti-depressant test was done by using forced swim test showed low dose anti-depressant activity. Our investigation suggests that *Typhonium trilobatum* roots contain high amount of ascorbic acid, phenolic and flavonoid compound which may be responsible for its biological activities in folkloric medicine.

INTRODUCTION: Plants are good sources of natural antioxidants which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases. Actually, free radical is naturally produced in our body by the metabolism of amino acids and fats.

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Oxidation is essential to many living organisms for the production of energy to fuel biological process. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid, arthritis and arteriosclerosis as well degenerative processes associated with aging [1].

These free radicals are unstable and it can react with cells and destroy it. The binding of free radicals with DNA structure will lead to mutation and it is cause of cancer. Free radicals formed in our body are of several types such as superoxide, hydroxyl, peroxyl. The antioxidant is needed to

inhibit all the free radicals from react. They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching super oxide and singlet oxygen [2].

Typhonium trilobatum is widely distributed in tropical and subtropical area around the world. It is found from Nepal to Southeast China, North Malaysia and Sri Lanka. It is also introduced in Philippines, West Borneo, Singapore, and West Africa. In Bangladesh, the plant is distributed throughout the country, but it is mainly found in Chittagong, Chittagong Hill Tracts, Tangail, Sylhet and Dhaka [3].

Typhonium trilobatum is widely used in folk medicine for the treatment of various diseases. The herbs possess anti-diarrheal, antiseptic and analgesic qualities [4]. Thus, the present study evaluated the *in vitro* antioxidant potential of root extract of *Typhonium trilobatum.* The different antioxidant assays, including DPPH free radical scavenging, nitric oxide scavenging, hydrogen peroxide, reducing power and RBC membrane stabilization activity, were studied. Moreover, High Performance Liquid Chromatography (HPLC) was used to identify and quantify the ascorbic acid in the root extracts.

MATERIALS AND METHODS:

Collection and Processing of Plant Samples: Plant sample of *Typhonim trilobatum (L.) Schott* was collected from University of Dhaka in July, 2014 and a plant sample was submitted to the Bangladesh National Herbarium for identification (Accession number 39647)**.** Root was sun dried for seven days. The dried roots were then ground in coarse powder using high capacity grinding machine which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction Procedure:
The powdered plant parts (20 gm) were successively extracted in a soxhlet extractor at elevated temperature using 400 ml of distilled methanol (40-60)°C which was followed by ethanol, and chloroform. After extraction all extracts were kept in refrigerator at 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 [5].

Quantitative Test of Ascorbic Acid by HPLC: The quantitative estimation of root extracts of *Typhonium trilobatum* according to the standard protocols by HPLC analysis. Separation for qualitative and quantitative analysis of the ascorbic acid was performed by HPLC with a an Agilent 1100 Series HPLC syroots (Agilent, Waldbronn, Germany) with UV detector (operated at 280 nm) and injection valve with 20-µL sample loop. Compounds were separated on a 4.6 mm × 250 mm, i.e., 5-µm pore size Xterra-C18 column protected by a guard column containing the same packing. The flow rate was 1.00 ml/minutes. Data were integrated by Shimadzu class VP series software and results were obtained by comparison with standards. Samples and solutions were filtered through 0.45-µm Nylon filters (Millipore) before analysis by HPLC. Simple mobile phase was used as control for identification of blank peaks.

For calculation of assay of ascorbic acid follow the following equation was used:

$$\text{Assay of ascorbic acid} = \frac{SW \times 10 \times PA \times SP}{10 \times \text{Sample weight (mg)} \times \text{Standard peack area}}$$

Here, SW= Standard weight (mg)
PA = Sample peak area
SP = Total weight of extract (mg)

Free Radical Scavenging Assay:
The free radical scavenging capacity of the extracts was determined using DPPH [6]. 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 was added in each test tubes to make the final volume 3 ml. The mixture was incubated at room temperature for 30 minutes, the absorbance was read at 517 nm using a UV-VIS

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 UV-VIS spectrophotometer. Ethanol was used as blank. Percent of inhibition of the DPPH free radical was measured by using the following equation:

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

Here, A_1 = Absorbance of the extract or standard
A_0 = Absorbance of the control

Cupric Reducing Antioxidant Capacity (CUPRAC):
The assay was conducted as described previously [7]. 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 1 ml of copper (II) chloride solution (0.01 M prepared from $CuCl_2.2H_2O$), 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 UV-VIS spectrophotometer against blank. Ascorbic acid was used as a standard.

Determination of Total Phenolics Content:
Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method[8]. 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 20^0C temperature for 60 minutes and standard diluted solution–reagent mixture was incubated at 20^0C temperature for 30 minutes. Absorbance of samples and standard were measured at 765 nm using UV-VIS 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:

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

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 [9]. To one 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 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 were added. It was incubated at room temperature for 30 min then absorbance of the reaction mixture was measured at 415 nm using UV-VIS spectrophotometer against blank (methanol). The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated with same equation used in total content of phenol.

Nitric oxide Scavenging Assay:
Nitric oxide scavenging assay was carried by using sodium nitroprussid [10-11]. This can be determined by the use of the Griess Illosvoy reaction. Two 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 100µg/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 using UV-VIS spectrophotometer. Typical control solutions contain the same solution mixture without plant extract or standard. % inhibition was

calculated by using the equation of % inhibition of the DPPH free radical.

Hydrogen Peroxide Scavenging activity:

Scavenging of Hydrogen Peroxide Scavenging activity of extract and its sub-fractions were evaluated by Hydrogen peroxide. One ml of extract/ sub- fraction at various concentrations was taken into a test tube and added two ml of hydrogen peroxide solution in phosphate buffered saline (PBS, pH 7.4). Then finally the absorbance was measured at 230 nm after 10 minutes. Ascorbic acid was used as a standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 230 nm using a spectrophotometer [9].

The percentage of inhibition was calculated according to the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Where,

A_1 = Absorbance of the extract or standard

A_0 = Absorbance of the control

Streptokinase (SK):

Commercially available lyophilized altepase (Streptokinase) vial (Popular pharmaceutical Ltd.) of 15, 00,000 I.U, was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolytic activity evaluation.

Blood Sample:

Blood (n=5) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and one ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic Activity:

The thrombolytic activity of all extracts of the plants was evaluated by using streptokinase (SK) as the standard [12].

Membrane Stabilizing Activity:

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 [12]. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution and heat induced methods [13]. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes containing anticoagulant EDTA (3.1% Na- Citrate). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 g.

Hypotonic Solution-Induced Haemolysis:

The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetyl salicylic acid (0.1 mg/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm using UV-VIS spectrophotometer. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

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

Heat- induced Haemolysis:

Isotonic buffer containing aliquots (5 ml) of the different extracts 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 test tube and mixed properly. One pair of the tubes was incubated at 54^0C for 20 min in a water bath, while the other pair was maintained 0^0C to 5^0C in an ice bath. The reaction mixture was centrifuged for 3 min at 1500g and the absorbance of the supernatant was measured at 560nm using UV-VIS spectrophotometer. The percentage inhibition or acceleration of hemolysis in tests was calculated according to the equation:

$$\text{\% Inhibition of hemolysis} = 100 \times [1 - (OD_2 - OD_1 / OD_3 - OD_1)]$$

Where, OD_1 = optical density of unheated test sample

OD_2 = optical density of heated test sample
OD_3 = optical density of heated control sample

Experimental Animals:

Thirty Swiss Albino mice of either sex were collected for the *in vivo* investigations from the animal resource department of ICDDR, B. The age of the mice were 6-7 weeks, weighing between 10-25 gm. Animals were maintained under standard environmental conditions (temp. 27 ± 1^0C, RH 55-65% and 12 light/12 hr dark cycle) and free access to feed and water was confirmed. The animals were acclimatized to laboratory environment for one week before the experiments were performed. The mice were coded as I, II, III, IV and V on their tails. The test animals were divided into four groups of two animals in each group (**Table 1**).

TABLE 1: GROUP DISTRIBUTION AND ADMINISTERED SUBSTANCES WITH THEIR DOSES

Group	Administered extract	Dose
1	Control	Specific for each test
2	Standard	Specific for each test
3	Methanol extract	100 mg/kg
4	Ethanol extract	100 mg/kg
5	Chloroform extract	100 mg/kg

Anti-depressant Activity (Forced Swimming Test, FST):

The Forced Swimming Test (FST) is the most widely used pharmacological in-vivo model for assessing the antidepressant activity of any substance and was performed according to the method of Porsolt et al., (1977) [14] with modification[15].

A clear square water tank apparatus (30cm x 20cm x 20cm) was filled with water (temp. 25 ± 1^0C) up to 9 cm. Mice were grouped as mentioned before (

TABLE). Diazepam 2 mg/kg was used as reference drug and applied almost one hour before the final test. In the pre-test session, 24 hours before the test, every mouse was placed individually into the cubical glass apparatus for 10 min. After oral dose administration, the test was performed by letting the mice to swim for 5 min each and their duration of immobility was recorded. The period when no further attempt to escape were made (apart from the necessary to keep its head above the water) was counted as immobile time.

Acute Toxicity:

Acute toxicity describes the adverse effects of a substance which results either from a single exposure or multiple exposures in a short period of time, usually less than 24 hours. The acute toxicity test was conducted to determine the LC_{50} of the test samples. The test samples were applied orally to the test animals at different concentrations of 1000 mg/kg (as per body weight). The animals were then observed for any mortality or any other sign of toxicity for every 1 hour for next 24 hours after applying the doses. Later, the animals were also kept under observation for 1 week (Walum, 1998) [16] with slight modification [17].

Statistical Analysis:

All data are presented as a mean ±Standard deviation (SD). IC_{50} values for scavenging of free radicals by the extracts were calculated from the dose - response curve by using Microsoft Excel 2010.

RESULTS AND DISCUSSION:

Phytochemical Screening:

The root of *Typhonium trilobatum* showed either presence or absence of different phytochemicals. The results are listed below in the **Table 2**.

TABLE 2: RESULTS OF CHEMICAL GROUP TEST OF VARIOUS ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM*

Name of test	Name of the root extracts		
	Methanol	Ethanol	Chloroform
Flavonoid	+	+	+
Carbohydrate	+	+	+
Glycoside	-	-	-
Tannin	-	-	-
Steroid	-	-	-
Phenol	+	+	+
Saponin	-	-	-

[+ = presence in bioactive compound, - = absence]

Quantification of Ascorbic acid by HPLC:

The contents of the ascorbic acid in the root extract of *Typhonium trilobatum* were analyzed by HPLC. Based on the comparison of the retention times with the standard peak of ascorbic acid, three extracts were identified; retention time was 2.5 minutes respectively. The most abundant ascorbic acid obtained from the ethanol extract was 51.13 mg/0.25 gm dry extract. Methanol extract also contained 48.16 mg whereas chloroform extract

contained 44.65 mg of ascorbic acid per 0.25 gm total extract. According to this result it can be suggested that root of *Typhonium trilobatum* has good antioxidant properties. The assay has been successfully used to quantify ascorbic acid in roots and being simple and independent of other antioxidant measurement commonly employed.

Total Phenol Content:

Total phenolic content of the different extracts was determined by using the Folin-Ciocalteu reagent and were expressed as Gallic Acid Equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid ($y = 0.0027 x + 0.0832$; $R^2 = 0.9973$). The highest concentration of phenols was measured in ethanolic extracts. Methanol and chloroform extracts contain considerably smaller concentrations of phenols. The total phenolic contents in plant extracts of the Typhonium trilobatum depend on the type of extract, i.e. the polarity of solvent used in extraction. The high solubility of phenols in polar solvents provides a high concentration of these compounds in the extracts obtained using polar solvents for the extraction.

In the present investigation an ethanol extract of root of the plant was found to contain the highest amount of phenolic content (14.06 mg/gm) **(**

TABLE **3).** Phenolic contents of the extracts were found to increase in the following order: Ethanol extract > Methanol extract > Chloroform extract.

TABLE 3: TOTAL PHENOLIC CONTENTS OF DIFFERENT ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM*

Extracts	Total Phenolic Content (mg/gm, Gallic Acid Equivalents)
Methanol	12.88±2.01
Ethanol	14.06±1.70
Chloroform	12.51±2.25

[Values are Mean ± Standard deviation]

Total Flavonoid Content (TFC):

Every group of flavonoids has their capacity to act as antioxidants depending on their molecular structure. The position of hydroxyl groups in the chemical structure of flavonoids is important for their antioxidant and free radical scavenging activities. Quercetin is a potent antioxidant because it has all the right structural features for free radical scavenging activity. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage [18].

Flavonoids have several different mechanisms of antioxidant properties, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation[19]. These compounds in human diet can easily adjust antioxidants in the epithelial cell membrane walls against the attack of ROS. Depending on their structure, flavonoids are able to scavenge practically all known ROS.

The method of Aluminum chloride colorimetric was used to determine the total flavonoid contents of the different extracts of *Typhonium trilobatum.* Total flavonoid contents (TFC) was calculated using the standard curve of quercetin ($y = 0.0048 x + 0.015$; $R^2 = 0.999$) and was expressed as quercetin equivalents (QE) per gram of the root extract. Ethanol extract of *Typhonium trilobatum* leaves was found to contain the highest amount of flavonoids content (6.34 mg/gm) **Table 4.** Flavonoid contents of the extracts were found to decrease in the following order: Ethanol extract>Methanol extract>Chloroform extract. Therefore, the result suggested flavonoids may be the major contributors for the antioxidative properties and inhibitory actions toward the oxidative reaction.

TABLE 4: TOTAL FLAVONOID CONTENTS OF DIFFERENT ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM*

Extracts	Total flavonoid content (mg/gm, Quercetin equivalents)
Methanol extract	5.06 ±0.98
Ethanol extract	6.34 ±0.96
Chloroform extract	4.88±0.99

[Values are Mean ± Standard deviation]

DPPH Free Radical Scavenging Assay:

Ascorbic acid had been reported to exhibit greater potential antioxidant activity [6]. The phenomenon in this study is acceptable since ascorbic acid has the highest inhibition percentage inhibits free radical activity. The IC_{50} values of different root extracts

of *Typhonium trilobatum* are presented in table. IC_{50} of ascorbic acid was found 12.33µg/ml. In comparison to standard, methanol, ethanol and chloroform extracts showed IC_{50} values of 16.64, 18.69 and 21.22 µg/ml respectively (

TABLE 5).

TABLE 5: IC_{50} VALUES OF DIFFERENT ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM* IN DPPH FREE RADICAL SCAVENGING ASSAY

Extracts	IC_{50} Value (µg/ml)
Methanol	16.64
Ethanol	18.69
Chloroform	21.22
Ascorbic acid	12.33

Cupric Reducing Antioxidant Capacity (CUPRAC): In general the copper based assays target the thiol group containing antioxidant species. The sample which has reducing power capacity that can donate eletron and act as primary and secondary metabolites has the ability to reduce oxide intermediates [7]. Copper reduction based on a complex mixture of antioxidant and appropriate selection of reaction time. This drawback can be handled in different ways, adjusted to use a fixed reaction time and used strong complexing agent (EDTA) to stop the reaction within 3 minutes. The standard L-ascorbic acid showed the highest reducing capacity.

All the methanol, ethanol and chloroform extracts showed dose dependant reducing capacity. Among the three extracts of *Typhonium trilobatum* root of ethanol extract showed relatively better cupric reducing antioxidant capacity. Different concentration (200, 100, 50, 25, 12.5, 6.25) µg/ml of root extracts of this plant were subjected to this investigation and the methanol, ethanol and chloroform extracts showed maximum absorbance at 450 nm showed at highest concentration whereas standard L-ascorbic acid showed the highest reducing capacity at the same concentration **(Fig. 1**).

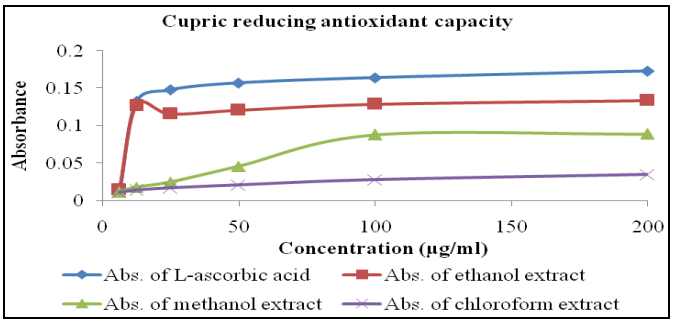

FIG.1: CUPRIC REDUCING ANTIOXIDANT CAPACITY (CUPRAC) OF ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM*

Nitric Oxide Radical Scavenging Assay:

The present study was based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions [10].

The antioxidant activity increased with an increase in concentration of the extracts. Increasing the concentration of the extracts did not result in an increase in the nitrite radical scavenging activity. Suppression of NO- release may partially be attributed to direct NO- scavenging, as all extracts decreased the amount of nitrite generated from the decrease of sodium nitroprusside *in vitro*. Different concentrations (200, 100, 50, 25, 12.5, 6.25) µg/ml of roots extracts of *Typhonium trilobatum* were subjected to investigate the nitric oxide scavenging activity and the methanol, ethanol and chloroform extracts showed maximum activity at low concentration whereas standard L-ascorbic acid showed the same.

All the three extracts showed very good activity (Methanol extract showed 64.32% inhibition, ethanol extract showed 67.08% inhibition and chloroform extract showed 63.77% inhibition) that is even slightly lower than standard (68.76% inhibition).

IC_{50} of ascorbic acid was found 119µg/ml. In comparison to standard, methanol, ethanol and chloroform extracts showed IC_{50} values of 121µg/ml, 117µg/ml and 133µg/ml respectively (**TABLE**). The result revealed that ethanol extract of *Typhonium trilobatum* roots has maximum nitric oxide scavenging capacity.

TABLE 6: IC_{50} VALUES OF DIFFERENT EXTRACTS OF *TYPHONIUM TRILOBATUM* ROOTS IN NITRIC OXIDE SCAVENGING ASSAY

Standard / Extracts	IC_{50} value (µg/ml)
L-ascorbic Acid	119
Methanol	121
Ethanol	117
Chloroform	133

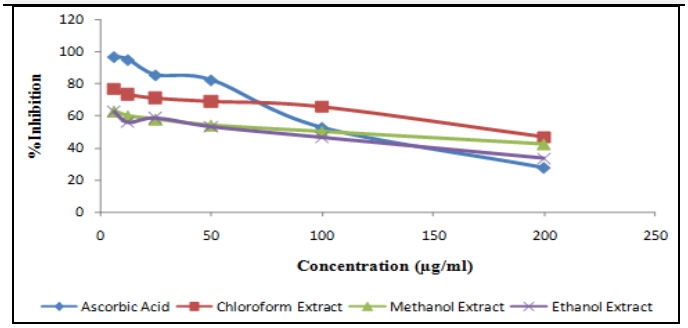

FIG.2: H_2O_2 SCAVENGING ACTIVITY OF *TYPHONIUM TRILOBATUM*

Scavenging of Hydrogen Peroxide:

H_2O_2 was considered poorly reactive because of its weaker oxidizing and reducing capabilities. Biologically, it acts as a toxicant to the cell by converting it into hydroxyl radical in the presence of metal ions and superoxide anion and also produces singlet oxygen through reaction with superoxide anion or with Hypochlorous acid (HOCl) or chloramines in living system. Hydrogen peroxide can degrade certain heame proteins, such as heamoglobin, to release Fe ions [9] and therefore the hydroxyl radical scavenging activity of phyto extracts was measured.

In this study, scavenging of hydrogen peroxide of different extracts of *Typhonium trilobatum* roots is presented in figure **(FIG.2**). Among the three extracts, chloroform extract showed good potential antioxidant activity (67.29% inhibition) compared with standard L-ascorbic acid (73.41% inhibition). Methanol extract also showed antioxidant activity (54.55% inhibition) whereas ethanol extract showed 51.78% inhibition. IC_{50} of ascorbic acid was found 128 µg/ml. In comparison to standard, methanol, ethanol and chloroform extracts showed IC_{50} values of 110, 78 and 184 µg/ml respectively. (**Table 7)** The result revealed that methanol extract of *Typhonium trilobatum* roots has maximum H_2O_2 scavenging capacity and this capacity was found to be decreased in following order: Ethanol extract> Methanol extract> Chloroform extract.

TABLE 7: IC_{50} VALUES OF DIFFERENT ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM* IN H_2O_2 SCAVENGING ASSAY

Standard / Extracts	IC_{50} value (µg/ml)
L-ascorbic Acid	128
Methanol	110
Ethanol	78
Chloroform	184

In -vitro Thrombolytic Effect:

Investigation of the thrombolytic activity of the *Typhonium trilobatum* root extracts were carried out using a simple and rapid *in vitro* clot lysis model. On the basis of the result obtained in the present study we can say that the *Typhonium trilobatum* root extracts have no thrombolytic activity compared with the standard. Streptokinase used as a standard showed 93.79% clot lysis whereas methanol extract showed 13.01% which is very much lower thrombolytic activity compared to standard. Ethanol and chloroform extracts showed

lower clot analysis of 10.91% and 12.82% respectively (**Fig.3**).

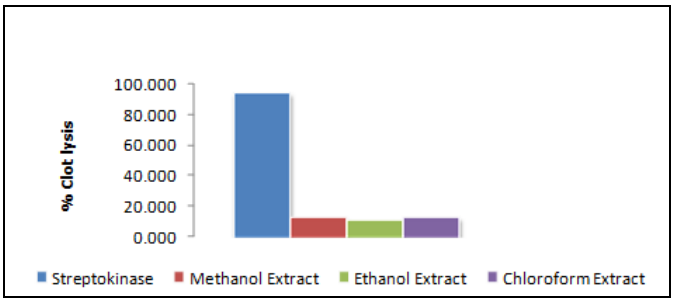

FIG.3: PERCENTAGE OF CLOT ANALYSIS OF ROOT EXTRACTS OF *TYPHONIUM TRILOBATUM*

Membrane Stabilizing Activity:

Hypotonic solution induced haemolysis and standard Acetylsalicylic Acid (0.1 mg/ml) showed 64.87% inhibition of RBC haemolysis whereas the methanol extract, ethanol extract and chloroform extract, at a concentration of 1 mg/ml, showed 57.06%, 61.06% and 56.49% inhibition of RBC heamolysis respectively (**Fig. 4**). The results revealed that although all the root extracts have very good potential of membrane stabilizing activity, ethanol extract showed higher % of inhibition of haemolysis that are even much higher than the standard. Also, it was noted that all the extract showed dose dependent membrane stabilizing activity.

The mode of action of the extract and standard anti inflammatory drugs could be connected with binding to the erythrocyte membrane with the subsequent alternation of the surface changes of the cells. This might have prevented physical interaction with aggregation agents on promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cell. It has been reported that certain flavonoids excreted profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro* while tannins and saponins posses ability cations there by stabilizing erythrocyte membrane and other biological macromolecules [20].

It was noted that ethanol extracts showed the highest membrane stabilities effect due to the presence of flavonoid. The lowest membrane stabilizing activities observed with chloroform extract due to the presence of other phytochemical constituents which mask the action of membrane stabilizing activities by other phytoconstituents. On the basis of these results, it could be inferred that the extracts of *Typhonium trilobatum* capable of stabilizing bovine red blood cells membranes against hypotonic induced lyses.

The plant therefore could be regarded as a natural source of membrane stabilizers and was capable of providing an alternative remedy for the management and treatment of inflammatory related disorders and diseases. 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 heamolysis respectively whereas standard Acetylsalicylic Acid showed 46.87% inhibition of RBC haemolysis. Methanol and chloroform extracts showed better potential in heat induced haemolysis that is even much higher than the standard (**Fig. 5**).

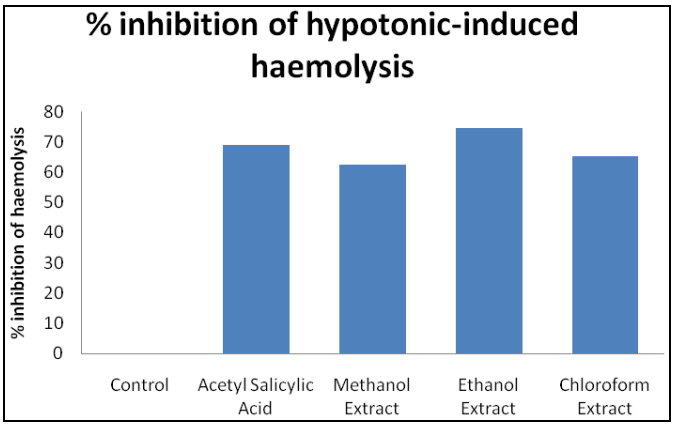

FIG.4: PERCENTAGE INHIBITION OF HYPOTONIC INDUCED HAEMOLYSIS OF *TYPHONIUM TRILOBATUM*

As lysosomal membrane stabilization contributes to protect cells from inflammation, the present investigation suggests that the membrane stabilizing activity of *Typhonium trilobatum* root extracts may play a very significant role in development of anti-inflammatory drugs.

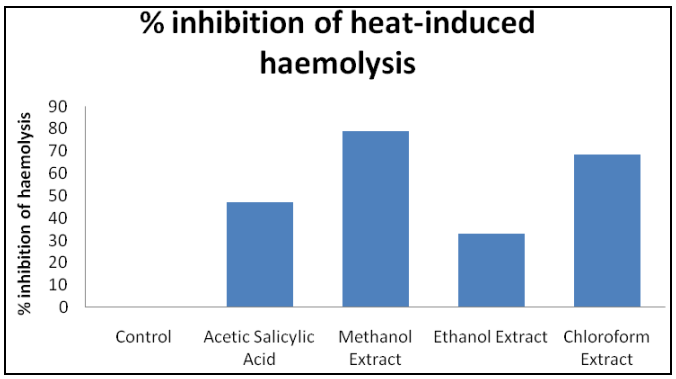

FIG. 5: PERCENT INHIBITION OF HEAT INDUCED RBC HAEMOLYSIS OF *TYPHONIUM TRILOBATUM*

Anti-depressant Activity (Forced Swimming Test): FST is still one of the best models of antidepressant test. This is of low cost, fast, reliable model. The prevention and management of stress disorders remain a major clinical problem. Hence it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. In this work, it was demonstrated that the administration of 100 mg/kg doses of the each extract of *Typhonium trilobatum* in mice was able to induce antidepressant effects. In the forced swimming test, the extract can decrease the immobility time in rats with mild sedative effect. It was found that *Typhonium trilobatum* can produce anti-depressant like activity at a dose of 100 mg/kg body weight in a dose dependent manner (**Fig.6**). The decrease in the immobility time is accompanied by the increase in swimming time.

An antidepressant drugs reduce the exploratory behavior depending upon the concentration. At present, the study revealed that all extracts of methanol, chloroform and ethanol significantly reduced the number of head dips and the number of line crossings where the indicator of exploratory behavior. The findings from the present investigation indicate that *Typhonium trilobatum* possesses antidepressant activity as shown by its mitigating effects on different experimentally induced stress models in mice.

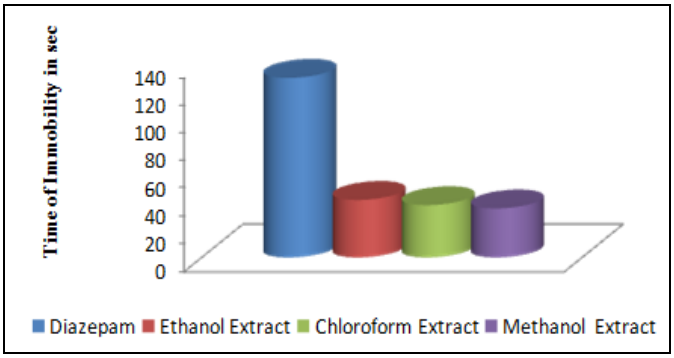

FIG. 6: IMMOBILE TIME IN SWIMMING TEST AFTER ADMINISTRATION OF SAMPLES

Acute Toxicity:

In this study, the mice in the treated groups were administrated with crude extract, respectively. The mice were monitored each hour until 12 hours for any toxic signs and mortality. The clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups [17]. During the 6 hour and 12 hours of period acute toxicity evaluation, mice which are orally administrated with each extract at single dose 1000 mg/kg showed no overt signs of distress and there were no observable symptoms of either toxicity or deaths (**Table 8**). All of the mice displayed no significant changes in behavior. Apart from that, the physical appearance features such as skin, fur and eyes were found to be normal, this indicates that the administration of the crude extract has negligible levels of toxicity.

TABLE 8: GENERAL APPEARANCE AND BEHAVIORAL OBSERVATIONS FOR CONTROL AND TREATED GROUPS

Observation	6 Hour	12Hour
Skin and fur	Normal	Normal
Eyes	Normal	Normal
Mucous membrane	Normal	Normal
Behavioural patterns	Normal	Normal
Salivation	Normal	Normal
Lethargy	Normal	Normal
Sleep	Normal	Normal
Coma	Normal	Normal
Diarrhea	Normal	Normal

CONCLUSION: Root 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 carbohydrate in root parts

of the plant. *In vitro* antioxidant activity determined the potential of root extracts by assessing its scavenging capacity. Ascorbic acid, total phenolic and flavonoid contents were determine which has antioxidant activity. The root extracts has no thrombolytic activity but also have good membrane stabilizing capacity. After pharmacological studies with root extracts of *Typhonium trilobatum* has mild anti depressant activity.

The result clearly indicates that the extracts of *Typhonium trilobatum* may be a very important contributor in different drug discovery including antioxidant 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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