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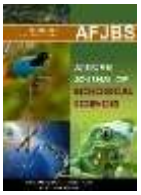

TYPHONIUM TRILOBATUM, AN MIRACLE PLANT FROM TRIPURA, NORTH EAST INDIA

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Abstract:

In recent times the traditional system of medicine giving the competitive aspects to the modern medicine as its having less side effects. Compared to synthetic molecules natural bioactive compounds show more safe as well as root cause of the diseases can be removed. The active compounds present in plants are responsible for various kind of therapeutic activities in human body without harming the major organs responsible for metabolism. The present investigation is aimed to evaluate the important phytochemicals and find out active compounds present in the roots of *Typhonium trilobatum* belonging to the family Araceae. The findings of the present investigation will provide a detailed referential information on the important bioactive compounds of *Typhonium trilobatum* for future aspects in pharmacological activity.

Keywords: *Typhonium trilobatum, Active compounds, medicinal properties, Traditional medicine, Tripura*

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Introduction

The *Araceae* (Arum) family comprises of monocotyledonous flowering plants (aroids), which are born as inflorescence termed spadix. This family includes as many as 114 genera and 3,750 species. The genus *Typhonium* is belong to the family *Araceae* and native to Australia and southern Asia. In traditional system of medicine, *Typhonium* is reported for its anti-inflammatory, analgesic, wound-healing and antidiarrheal properties. Typhonium trilobatum is a herbaceous plant with tuberous root up to 4 cm in diameter. leaf lamina hastate and subtrisect, segments are acuminate, middle segment ovate and lateral segments obliquely ovate, short in size and subbilobed at the base and Petiole 25-32 cm long. Peduncle 5-7 cm long and thin. Spadix nearly 15 cm long. Female inflorescence usually cylindrical, 7 mm long and male inflorescence 1.25-1.5 cm long, located above the female inflorescence and rose-pink in color. Flowering and fruiting time is April- October (Ghani, 2003). Typhonium comprises of approximately 50 species and considered as the largest genus of the family Araceae which are endemic to Australia, tropical Asia, and the South Pacific. Typhonium trilobatum is widely distributed plant in tropical and subtropical area around the world. It is found from Nepal to Southeast China, Sri Lanka and North Malaysia. This plant is 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 (Ghani, 2003). The genus Typhonium, consists of total 69 species of perennial herbs with mostly edible tubers. The plant is well spread in North Australia. China, Bangladesh, Ceylon, Malaysia and the tropical region of India. Typhonium trilobatum is abundantly found in the forest areas of Tripura and grown wildly and is locally known as “Kharkan gass” . The plant contains many important photochemical which are popularly used by the traditional healers of Tripura.

It contains several phytochemical and nutraceutical constituents. Research studies reveals that the plant is very useful as it having anti-inflammatory activity, analgesic activity, antimicrobial activity, anti-diabetic activity, anti-oxidant activity and anti-depressant properties.

Material and methods:

Typhonium trilobatum were collected from different places of Tripura during the rainy season and washed in running water to remove the soil particles and other extraneous materials and the field data of the plant was noted in the field book. It was then shade dried and then pulverized. For authentication purpose, the selected plant samples were collected in flowering condition and herbarium was prepared and submitted to the Botany Department of Assam down town University, Guwahati, Assam.

1. Morphological analysis: The macroscopical observations were carried out and the microscopical investigations i.e, stomatal index and moisture content determination were performed as per procedures mention below.

a) Stomatal index: The fragments of the leaf from the middle of lamina were cleared by boiling with chloral hydrate solution. The uppermost epidermis was peeled separated by means of a forceps. The mount of upper epidermis was separately prepared in glycerin water. A square of known dimensions was drawn by means of a stage micrometer and camera lucida on a drawing paper. The stage micrometer was replaced by means of cleared leaf preparation, focused under the same magnification and the epidermal cells and stomata were traced by looking through the microscope when a superimposed image of the leaf is seen at the same time. The number of stomata and the epidermal cells within the square were counted, cell being counted if at least half of its area lies within the square, provided two adjacent sides are considered for purpose of calculation. Successive adjacent field were examined until about 400 cells were counted and calculate the stomatal index using the following formula

$$\text{Stomatal Index} = \frac{S}{S+E} \times 100$$

Where S: Number of stomata; E: Number of Epidermal cells, S.I: Stomatal index

b) Determination of moisture content

Moisture is an inevitable component of dried medicinal plant materials, which must be eliminated as far as practicable. Drying in scientific way plays an important role in maintaining the quality of material. Moisture enhances the enzymatic actions and provides favorable condition, to grow living micro-organisms.

Method: The roots of *Typhonium trilobatum* plants were collected, dried properly and analyzed at different stages of development. Two gms of the dried material was accurately weighted in a tarred watch glass. The dried materials was kept in hot air oven at 105 °C and

dried for a period until constant weight obtained. The difference in weight determines the moisture content in the dried material.

c) Powder analysis

The roots of the plants were collected and cleaned with water to remove undesired particles. This was further sun dried for one week and after that oven dried for 60 hours at 40 degree centigrade. After complete drying, it was powdered. Hot solvent extraction procedure was applied for extraction of the plant material (Soxhlet apparatus) at elevated temperature. The temperature was maintained between 400C to 60 0C. As solvent methanol, chloroform and ethanol were used.

2. Determination of Ash Value

Ash values are useful in the determination of the quality of a drug or dried medical plant material especially in a powered form. The objective of making ash of the dried roots of *Typhonium trilobatum* is to removal all the unwanted particles which can interfere in the analytical examination. This measures the amount of silica present, especially as sand and siliceous earth.

a) Total ash

3 gms of the dry roots powder of *Typhonium trilobatum* was accurately weighted and taken in a silica crucible which was previously ignited and weighted. The powdered material was spread in a fine layer at the bottom of the tarred crucible. The crucible was kept inside the muffle furnace and the temperature increased to make it dull red hot until free from carbon. The crucible was cooled and weighted. The procedure was repeated to get the constant weight. The percentage of total ash was measured and the value was noted.

a) Acid insoluble ash. The ash was combined with 25 cc of dilute HCL and heated for 4
5 minutes. The insoluble ash was collected using ashfree filter paper and rinsed with hot water. T
he insoluble matter was placed to a tarred silica crucible, fired, and weighed. The operation was r
epeated to get a constant weight. The proportion of acid insoluble ash was estimated in relation t
o the air-dried medication.

b) Water soluble ash

The total ash obtained was boiled with 25ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried material.

3. Determination of extractive Values

Extractive values of a dried medicinal plant material determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which no suitable chemical or biological assay exists.

a) Alcohol soluble extractive

Five gm of the coarse powder (60-80 mesh) of the dried plant material was macerated with 100 ml of 90 % alcohol, in a closed flask for a duration of 24 hrs, shaking the flask frequently during the process and finally allowed to stand for 18 hrs. The solution was filtered rapidly, taking precaution against loss of alcohol. The filtered solution of 25 ml was evaporated to dryness at 105°C in a tarred flat bottomed petridish. The percentage of alcohol soluble extract was determined with reference to the shade dried material.

Materials and Methods

The collected fresh leaves of wild variety of *Typhonium trilobatum* were air dried properly in the shade beds. The dry leaves were ground in a grinder into a fine powder. These powder leaves were used for further experiment. Two methods are used to prepare leaves extracts.

In the first method 500 gram of air leaf power soaked in methanol (5 Litre) at normal room temperature. Methanolic extracts were prepared and concentrated using rotary evaporator at

45°C. The crude methanolic extract were used for further investigation (El-Khateeb et al.,2014). The yield of extract was 24.02% for the wild variety of *Typhonium trilobatum.*

In the second method 500 grams of dried powdered leaves soaked in distilled water and boiled at 100°C for 3 hours to prepare an initial extract and kept for cooling to room temperature. After that the extract was filtered using conical flask and filter paper. After that the extract was Iyophilized in a low temperature and preserved at 20°C for further use (Kim et al., 2011). The yield of aqueous extract was 13.84% for wild variety of *Typhonium trilobatum.*

Laboratory investigation were performed to evaluate the biometabolites present in the wild variety of *Typhonium trilobatum* by boiling the crude methanolic extracts and aqueous extracts for 3 hours to estimate the presence of: proteins, alkaloids, flavonoids, phenolic glycosides and reducing sugar etc.

1**.** Determination of Total Protein concentration in the different extracts was evaluated by Folin-Lowry method (Lowry et. al., 1951). Total soluble proteins were estimated using 1 gram fresh grinded leaves with 10 ml extraction buffer (0.5 M Tris pH 6.8, 10% SDS) (El-Adl, 0 2012). The extracts were centrifuged for 10 minutes at 4°C at 10,000 rpm and the material was applied for SDS-Page analysis.

2. Determination of alkaloids concentration were performed according to the procedure of Harborne (1988). 1 ml of leaves extracts was taken and added 2ml of diluted hydrochloric acid. Then five drops of Wagner's reagent were added to 1ml of the previous solution and shacked properly after addition of each drop and kept carefully and precipitation formed in the solution showed the presence of alkaloids

3. Determination of flavonoids were conducted according to the procedure of Harborne (1988). 1 ml of leaves extract was soaked in 1% hydrochloric acid over night and filtered then added 10% sodium hydroxide solution to the filtrate, the occurrence of yellow color proved the presence of flavonoids.

4. Determination of phenol were carried out according to Harborne (1988). A few drops of strong sulfuric acid were applied to 1ml of leaves and fruit extracts, immediately the solution turned into red in color and when water added the red colour disappeared.

5. Determination of Reducing Sugars: The carbohydrate content in the different extracts were carried out by anthrone method (Yemm and Willis, 1954). The carbohydrate content was expressed as mg/g of the sample as calculated from sucrose standard graph.

Table 3.1: Different fractions of extracts of Typhonium trilobatum

Plant	Solvent	Yield (%)
Typhonium trilobatum	Methanol	22.05%
	Ethanol	24.2%
	Chloroform	10%

Table 4.1: Results of chemical group test of various root extracts of Typhonium trilobatum

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

(+ = presence in bioactive compound, - = absence)

Conclusion

Typhonium trilobatum root extracts were taken for phytochemical screening as well as in vitro and in vivo pharmacological evaluations in order to justify the ethnomedicinal use and determine the plants other medicinal properties.

The scavenging ability of root extracts was assessed in vitro to identify their antioxidant activity. Ascorbic acid, flavonoid and total phenolic content were determine which has antioxidant activity.The root extracts show no thrombolytic action, however they do have good membrane st abilisingproperties. Pharmacological research with Typhonium trilobatum root extracts revealed modest antidepressant effect. The findings clearly show that Typhonium trilobatum extracts coul d play a key role in the discovery of several medications, including antioxidants and antidepressa nts. The current study indicated a better likelihood of the plant's antitumor capability, which may be shown in the near future. Therefore, additional work on Typhonium trilobatum to identify ne w bioactive chemicals may be the next step to be taken.

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Conflict of interests

The author declares no conflict of interest.

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Characterization of Phytoconstituents, In vitro Antioxidant Activity and Pharmacological Investigation of the Root Extract of Typhonium trilobatum

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