

## QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL INVESTIGATION OF *TRAPA BISPINOSA ROXB.* LEAVES EXTRACT

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### ABSTRACT

Leaves of *Trapa bispinosa roxb.* were shade dried, powdered, and was extracted using solvents petroleum ether by successive solvent extraction method and the extracts were tested for the confirmation of phytoconstituents (carbohydrates, polyphenols, alkaloids, flavonoids, tannins, saponins, steroids etc.) The literature has suggest that the preliminary phytochemical investigation of crude extracts revealed the presence of alkaloids, flavonoids, steroids, phenols, glycosides, tannins, saponins, carbohydrates in the different parts of plant *Trapa bispinosa roxb.* such as fruits, fruit kernels, stem, roots and leaves which has been reported their use as Antimicrobial, Antibacterial, Anti-inflammatory, Antidiabetic, Neuroprotective effect and immunomodulatory activity. The phytochemical investigation of

leaves of *Trapa bispinosa roxb.* shows the presence of carbohydrates, phenols, saponins, carboxylic acid, fixed oils and fats in petroleum ether extract. These findings provided the evidences that solvent extract of this plant have important phytoconstituents which may show their similar use in the traditional medicines for the treatment of various diseases as well as nutritive purpose.

**KEYWORDS:** *Trapa Bispinosa Roxb.* Phytochemical Analysis, Plant Extracts.

### INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest sources of medicinal plants. In India, the use of medicinal plants is century's old tradition and approximately two millions tradition health practitioners still use medicinal plants for curing various ailments. Medicinal plants have bioactive compounds which are used for curing of various human

diseases and play an important role in healing.<sup>[1]</sup>

The world health organization (WHO) has reported that around 21,000 plants have been used for medicinal purposes in the world. About 500 higher species have been thoroughly investigated as potential sources of new drugs nearly 119 pure chemicals were extracted from 90 plant species. There is a growing tendency all over the world to shift from synthetic to natural hared products including medicinal plants among the estimated 250,000 – 500,000 plant species, only small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of given plant will reveal only a very narrow spectrum of its constituents.<sup>[2,3]</sup>

The plant kingdom has proven to be the most useful in the treatment of diseases and they provide important source of all the world's pharmaceuticals.

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemical). These phytochemical often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others. Nearly 50% of drugs used in medicine are of plant origin and only a small fraction of plants with medicinal activity has been assayed.<sup>[7]</sup>

Therefore, much current research devoted to the phytochemical investigation of higher plants which have ethanobotanical information associated with them.

Phytochemicals are naturally occurring in the medicinal plants leaves, vegetables, and roots that have defense mechanism and protect from various diseases.<sup>[1]</sup>

Phytochemicals are primary and secondary compounds chlorophyll, proteins, and common sugars are involved in primary constituents and secondary compounds have terpenoids, alkaloids, and phenolic compounds.

The most important of these bioactive constituents of plant are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, and glycosides. Plants in all facet of life have served a valuable starting material for drug development.

The chemical constituents of plant medicines are a part of the physiological activities of living plants and hence they are believed to have a better compatibility with the human body.

Knowledge of the chemical constituents of plant is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources of such a economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plant would further be valuable in discovering the actual value of folkloric remedies;<sup>[4,5]</sup> chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and inactive ones are called inert chemical constituents.<sup>[6]</sup> *Trapa bispinosa roxb.* (Water chestnut) is an annual, floating leaved aquatic plant which belongs to the family Trapaceae<sup>[9]</sup> found in freshwater wetlands, lakes, ponds, and sluggish reaches of rivers in India. In addition to being important for aquatic ecosystem.<sup>[8]</sup>



***Trapa bispinosa roxb:*** *Trapa bispinosa* species are also food for humans and animals in India, china, and Southeast Asia. It is grown throughout the Asia and tropical Africa in lakes and ponds and is often cultivated across different parts of India for its consumable seasonal fruit commonly known as singhara which is a good source of nutrition having considerable amount of carbohydrates, proteins and vitamins. The medicinal values for the whole herb and fruit have long been recognized in folklore medicine as a cure for various diseases. This herb having two type of leaves finely divided feather-like submerged leaves borne along the length of the stem, and undivided floating leaves borne in a rosette at the water surface, the floating leaves are rhomboid, fan shaped and have toothed edges, 2-6.5 cm diameter, broader than long, denticulate, denate, serrate or incised with entire base, apex acute, red and densely pubescent or villous beneath. Traditionally, the plant has been used in India for several important medicinal purposes. It has been used as nutritive, astringent, aphrodisiac, cooling,

appetizer, tonic, anti-diarrheal etc. It is a good source of nutrition having carbohydrates, protein and vitamins.<sup>[10]</sup>

This study looks into the fundamental scientific bases for the use of aquatic plant by determining the phytochemical constituents present in these plant.

## MATERIALS AND METHODS

**Collection of plant material:** The leaves of *trapa bispinosa roxb.* were collected from western region of Madhya Pradesh, in the month of August 2014.

Plant material was collected as per standard procedure. Infected parts were carefully discarded from plant sample. The samples were thoroughly wash with water to remove foreign organic matter, debris and dried in the shade. Plant materials were authenticated by Taxonomist, Department of Botany, S. S. V. P. S College of science, Dhule, Maharashtra, India.

The dried leaves were powdered by using pulveriser and sieve no.20 and kept in airtight container until used. The label stating name, part of plant date, collection site, weight etc. was appropriately pasted to respective samples.

**Chemicals:** Petroleum ether, Methanol, Drangendorff's reagent, Liebermann's burchard reagent, Fehling's A and B, Phenol Ciocalteu reagent, indigo sulphonic acid etc.

**Extraction of plant materials:** The air dried leaves (500gm) of *Trapa bispinosa roxb.* were crushed. The crushed leaves extracted with solvents of increasing polarity viz. petroleum ether by hot percolation method by using soxhlet apparatus. The extract was evaporated till dryness to obtain residue. The extracts were concentrated under reduced pressure.

**Extractive values:** It is employed for material to which as yet no suitable chemical or biological assay exists. Extract were prepared with various solvents by standard methods,<sup>[11]</sup> percentage of dry extract calculated in terms of air dried leaf powder.

**Alcohol soluble extractive values:** Accurately weighed 5 gm of powdered drug placed in glass stoppered conical flask and macerated with 25ml of petroleum ether for 6 hours with frequent shaking, and mixture allowed to stand for 18hr. after completion of 18 hr, filtered rapidly taking care not to lose any solvent. transferred the filtrate in tarred flat bottom

porcelain dish. Filtrate was evaporated to dryness on water bath, dried at 105°C for 6 hr cooled desiccator for 30 min and weighed. The extractive values were calculated and expressed as a milligram per gm of air dried material.<sup>[11]</sup>

**Table 1: Extractive value of fresh plant sample.**

| Sr No. | Extracts                | Color  | % yield (w/w) |
|--------|-------------------------|--------|---------------|
| 1      | Petroleum ether extract | Yellow | 10%           |

## Phytochemical Investigation

### 1. Qualitative Chemical Estimation

The proximate analysis was performed according to the established protocol. Petroleum ether was subjected to qualitative chemical investigation.<sup>[11]</sup>

### Test for carbohydrates

#### 1. Molisch's test (general test)

To 2-3 ml aqueous extract, add few drops of alpha naphthol solution in alcohol shake and add conc. H<sub>2</sub>SO<sub>4</sub> from sides of test tube. Violet ring is formed at the junction of two liquids.

#### 2. Tests for reducing sugars

A. Fehling test: mix 1 ml Fehling's A and 1 ml Fehling's B solution, boil for 1 min. add equal volume of test solution. Heat in boiling water bath for 5-10 min. first yellow, then brick red ppt. is observed.

B. Benedict's test: mix equal volume of Benedict's reagent and test solution in a test tube. Heating in boiling water bath for 5 min. solution appears green, yellow, and red depending on amount of reducing sugar present in test solution.

#### 3. Test for hexose sugar

##### Cobalt chloride test

Mix 3ml test solution with 2 ml cobalt chloride. Boil and cool. Add few drops of NaOH solution. Solution appears greenish blue (glucose) or purplish (fructose) or upper layer greenish blue and lower layer purplish (mixture of glucose and fructose).

#### 4. Tests for non-reducing sugars

A. Test solution does not give response to Fehling's and benedicts test.

B. Hydrolyze test solution, Fehling and Benedict's tests are negative.

**Tests for fats and oils****Solubility test**

1. Oils are soluble in ether, benzene and chloroform but insoluble in 90% ethanol and water.
2. Filter paper gets permanently stained with oils.

**Tests for glycosides****I. General test**

**TEST A:** extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on a water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1ml Fehling's solution A and B until it becomes alkaline (Test with PH paper) and heat on water bath for 2 minutes.

Note the quantity of red precipitate formed and compared with that of formed in Test B.

**TEST B:** extract 200 mg of the drug using 5ml of water instead of sulphuric acid. After boiling add equal amount of water as used for sodium hydroxide in the above test. Add 0.1ml Fehling's A and B until alkaline (test with PH paper) and heat on water bath for 2 minutes note the quantity of red precipitate formed.

Compare the quantity of precipitate formed in Test B with that of formed in Test A. if the precipitate in Test A greater than in Test B then glycosides may present. Since Test B represents the amount of free reducing sugar already present in the crude drug, Whereas Test A represents free reducing sugar plus those related on acid hydrolysis of any glycosides in the crude drug.

**II. Test for saponins glycosides****1. Foam test**

Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

**2. Haemolytic test**

Add drug extract or dry powder to one drop of blood placed on glass slide. Haemolytic zone appears.

**Test for steroids****1.Salkowski reaction**

To 2 ml extract, add 2ml chloroform and 2ml conc. H<sub>2</sub>SO<sub>4</sub>. Shake well. Chloroform layers appears red and acid layer shows greenish yellow fluorescence.

**2.Liebermann burchard reaction**

Mix 2 ml extract with chloroform add 1-2 ml acetic anhydride and 2 drops of conc. H<sub>2</sub>SO<sub>4</sub> from side of test tube first red then blue and finally green color appears.

**3.Liebermann's reaction**

Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. H<sub>2</sub>SO<sub>4</sub>, blue color appears.

**Test for flavonoids****1.Shinoda test**

To dry powder or extract add 5 ml 95% ethanol, few drops conc. HCL and 0.5gm magnesium turnings, orange, pink, red to purple color appears.

**2.Sulphuric acid test**

On addition of sulphuric acid (66% or 80%) flavones and flavonols dissolve into it and give a deep yellow solution chalcones and aurones give red or red bluish solutions. flavanes give orange to red colors.

**Tests for alkaloids**

Evaporate all the aqueous, alcoholic, chloroform extracts separately. To residue, add dilute HCL. Shake well and filter. With filtrate, perform following tests:

**1.Drangendorff's test**

To 2-3 ml filtrate, add few drops of Drangendorff's reagent. Orange brown ppt is formed.

**2.Mayer's test**

2-3 ml filtrates with few drops Mayer's reagent gives precipitate.

**3.Hager's test**

2-3 ml filtrate with few drops Hager's test reagent gives yellow precipitate.

**4. Wagner's test**

2-3 ml filtrate with few drops Wagner's reagent gives reddish brown precipitate.

**5. Murexide test**

To 3-4ml test solution, add 3-4 drops of conc. HNO<sub>3</sub>, evaporate to dryness. Cool and add 2 drops of NH<sub>4</sub>OH. Purple color is observed.

**6. Tannic acid test**

Test solution treated with tannic acid solution gives buff colored precipitate.

**Tests for tannins and phenolic compounds**

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagent

1. 5% FECL<sub>3</sub> solution :deep blue black color.
2. Lead acetate solution: white precipitate.
3. Gelatin solution: white precipitate.
4. Bromine water: decolouration of bromine water.
5. Acetic acid solution: red color solution.
6. Potassium dichromate: red precipitate.
7. Dilute iodine solution: transient red color.
8. Dilute potassium permanganate solution: decolouration.

**Table. 2: Qualitative chemical test for various extracts of *Trapa Bispinosa roxb.***

| Sr. No. | Chemical tests                           | Pet. Ether extract |
|---------|--|--------------------|
| 1.      | Test for carbohydrates                   |                    |
|         | A. Molisch's test                        | +                  |
|         | B. Fehling's test                        | +                  |
|         | C. Benedict's test                       | +                  |
|         | D. Barfoed's test                        | +                  |
|         | E. Cobalt chloride test for hexose sugar | +                  |
| 2.      | Test for proteins                        |                    |
|         | A. Biuret test                           | -                  |
|         | B. Xanthoprotein test                    | -                  |
| 3.      | Test for amino acid                      |                    |
|         | A. Million's test                        | -                  |
|         | B. Ninhydrine test                       | -                  |
| 4.      | Test for starch                          |                    |
|         | A. Iodine test                           | -                  |
| 5.      | Tests for steroids and triterpenoids     |                    |
|         | A. Salkowski reaction                    | -                  |
|         | B. Liebermann-Burchard reaction          | -                  |
|         | C. Liebermann's reaction                 | -                  |

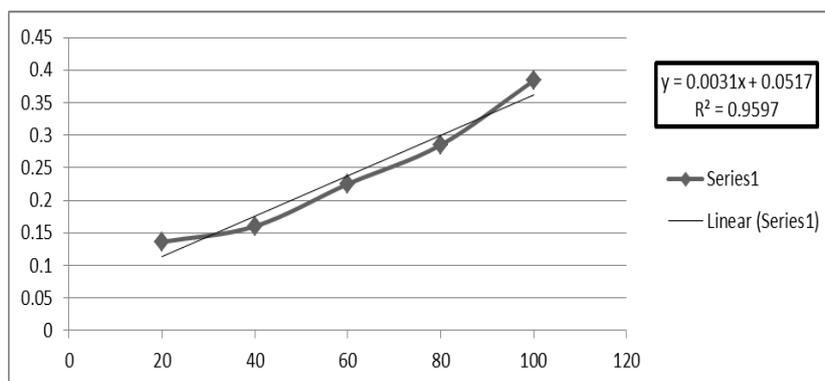
|   |  |   |
|---|--|---|
| 6.<br>I.                                  | Tests for glycosides                     |   |
|   | General test                             |   |
|   | A. Tests for specific glycosides         | - |
|   | B. Tests for anthraquinone glycosides    | - |
|   | a. Borntrager's test                     | - |
| b. Modified Borntrager's test             | -  |   |
| c. Test for hydroxyl anthraquinone        | -  |   |
| II.                                       | Tests for coumarin glycosides            | - |
| III.                                      | Cynogenetic glycosides                   | - |
| IV.                                       | Saponins glycosides                      |   |
|   | A. Foam test                             | + |
|   | B. Haemolytic test                       | + |
| 7.  | Test for flavonoids                      |   |
|   | A. Shinoda test                          | - |
|   | B. Sulphuric acid test                   | - |
| 8.  | Tests for alkaloids                      |   |
|   | A. Drangendorff's test                   | - |
|   | B. Mayer's test                          | - |
|   | C. Hager's test                          | - |
|   | D. Wagner's test                         | - |
|   | E. Murexide test for purine alkaloids    | - |
| F. Tannic acid test                       | -  |   |
| 9.  | Tests for tannins and phenolic compounds |   |
|   | A. 5% FeCl <sub>3</sub> solution         | + |
|   | B. Lead acetate solution                 | - |
|   | C. Gelatin solution                      | - |
|   | D. Bromine water                         | - |
|   | E. Acetic acid solution                  | - |
|   | F. Potassium dichromate                  | - |
|   | G. Dilute iodine solution                | - |
|   | H. Dilute HNO <sub>3</sub>               | - |
| I. Dilute potassium permanganate solution | -  |   |
| 10.                                       | Tests for fats and oils                  |   |
|   | A. Solubility test                       | + |
|   | B. Staining test                         | + |

(+) – indicates the test is positive it shows the presence of respective phytoconstituents

(-) - indicates the test is negative it shows the absence of respective phytoconstituents

## 2. Quantitative Estimation

1. **Total Polyphenolic Content:** The total polyphenolic content of extracts determine by folin-ciocalteu's reagent. The extract 0.1ml was mixed with the folin ciocalteu phenol reagent 0.2ml, water 2ml, and sodium carbonate 15% w/v, 2ml, and absorbance was measured at 660 nm using spectrophotometer (SHIMADZU 2405) after 2 hr incubation period at 50°C for 10 min. all the experiment was performed in triplicate. The total phenolic content is expressed as µg Gallic acid equivalents (GAE).<sup>[12]</sup>



**Figure. 1: Linearity curve of gallic acid for total Polyphenolic content.**

## 2. Total Saponins Content

5g of sample were taken and weighed, extracted with 90% v/v methanol by refluxing for half an hour. The residues were extracted two more times by taking 25 ml methanol. The methanolic extract was combined and distilled off the solvent. The soft extract treated left after distillation of alcohol, with petroleum ether 60-80°C, 25 ml by refluxing for an half an hour. Cooled and removed the solvent by decantation.

Then, treated the same with ethyl acetate 25ml and poured the solvents after cooling, the soft extract kept in the same flask. The residue extracted with n-butanol (25ml) successively 3 times. The n-butanol extract were combined and evaporated under vacuum to a thick paste in 5ml 90% methanol and poured drop wise to 25 ml solvent ether with stirring. The precipitate was collected on pre weighed filter paper. The precipitate washed with 5ml ether and dry to a constant weight.

## RESULTS AND DISCUSSION

**Table. 3: Quantitative estimation of phytoconstituents.**

| Sr No. | Phytoconstituents | Standard    | Total content (mg/g) |
|--------|-------------------|-------------|----------------------|
| 1      | Polyphenols       | Gallic acid | 67                   |
| 2      | Saponins          | -           | 70                   |

Phytochemicals are important chemicals found virtually in plants and their different parts at different concentrations. The extractive value was reported as shown in Table 1. The qualitative phytochemical screening of plant extract was done by confirmed the chemical test based on its color of the reaction and precipitating chemical reagents to gives the information about the phytoconstituents present in the plant extract. The qualitative chemical test revealed the presence of Carbohydrates, polyphenols, saponins, and fats, fixed oils in pet. ether extract

of *Trapa bispinosa roxb.* The results of preliminary qualitative phytochemical investigation are tabulated in Table 2.

The quantitative estimation of Polyphenolic content (67 $\mu$ g/gm of GAE) by using UV spectrophotometer, and Estimation of saponins content (70 $\mu$ g/gm) by solvent extraction in petroleum ether extract of *Trapa bispinosa roxb.* as shown in Table 3.

## CONCLUSION

From the findings of qualitative and quantitative investigation of leaves of *Trapa bispinosa roxb.* The active constituents present in the studied plant may show the positive pharmacological effect. So further study needed for the identification, isolation and purification of active phytochemical constituent responsible for therapeutic properties may lead to a new drug development from this plant. Further in vitro and in vivo studies are recommended to harvest the benefits of natural medication from this plant.

## CONFLICT OF INTREST

To our knowledge, the present study is the first one which systematically reports the phytochemicals present in petroleum ether extract of leaves of *Trapa bispinosa roxb.*

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