

EVALUATION OF NUTRITIONAL, PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF *TRAPA BISPINOSA* ROXB. FRUITS

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ABSTRACT

The water caltrop, water chestnut or Singhara (*Trapa bispinosa* Roxb.) belonging to family Trapaceae is one of the most ancient fruit indigenous to India growing as floating annual aquatic plants. Singhara fruits have been traditionally used for their high nutritious value, as a culinary delight and for several medicinal properties. Singhara ka atta (water caltrop flour) is used for fasting in many Indian religious rituals. In the present work, we have attempted to study different characteristics of the fruit so as to understand its potential for value addition and nutraceutical development. This work demonstrated that fruit of *Trapa bispinosa* under study possess high protein, carbohydrate, flavonoid content and antioxidative potential thus can be a potential source of nutrition. Inductively coupled plasma optical emission spectrometry (ICP-OES) analysis presented this fruit as a good source of minerals and some trace metals. Acetone extract of this fruit contains higher phenolics and antioxidant property.

Keywords: *Trapa bispinosa*, Antioxidant activity, Polyphenols, Phytochemical analysis, Mineral analysis.

INTRODUCTION

Antioxidant intake in the human diet is considered important. Higher manufacturing costs, lesser effectiveness and toxicity bound the use of synthetic antioxidants than natural antioxidants. Natural antioxidants, specially phenolics and flavonoids are protected and bioactive¹. Antioxidant scavenge free radicals and allow cells to renew the process of life². In recent years underutilized species have become choice of interest for the researchers. Water chestnut belongs to the family Trapaceae, free floating plant grown in shallow water fields. Because of the high activity of enzymes and phenolics content, color of water caltrop hulls changes from the original pink color to dark brown during transportation and processing³. It is a potent drug in Indian subcontinents says Yunani and Ayurvedic medicine⁴. The acrid juice of *Trapa* is used for diarrhea and dysentery⁵. The fruits are used as intestinal astringent, aphrodisiac and anti-inflammatory and in leprosy, urinary discharges, fractures, sore throat, bronchitis and anemia^{6,7}.

Research studies demonstrate correlation between the antioxidant properties of plant polyphenolic compounds and their disease-preventing property⁸. Polyphenols demonstrated antibacterial, anticarcinogenic, antiviral and vasodilator activity^{9,10}. Studies show that flavonoids exhibit antioxidant activities in addition to inhibition of enzymes related to tumorigenesis and induction of detoxifying enzymes¹¹. Earlier studies reported that *Trapa bispinosa* possesses antimicrobial and cytotoxic activity¹². Medicinal properties of plants have been investigated in recent years compared to synthetic drugs as a potential source of pharmacological activity, less toxicity and economic viability¹³.

Trapa flour is eaten during fasting in India. So, the aim of the present study was to determine various nutritional phytochemicals present in underutilized Indian *Trapa* fruit and their use as a potential source of natural antioxidants in order to fully utilize the local fruits for expansion as functional food which possess various health benefits.

MATERIALS AND METHODS

Chemicals

DPPH (Diphenylpicryl-hydrazyl) was purchased from Sigma Aldrich, ABTS (2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) was purchased from Sigma Life Science, FRAP (2,4,6-Tri(2-Pyridyl)-s-triazine) was purchased from Fluka Analytical, DCM (dichloromethane) was purchased from SRL, MeOH (methanol) was purchased from Loba chem, Acetone was purchased from SRL, PE (petroleum ether) was purchased from Loba Chem, Trolox was

purchased from Aldrich chemistry, Gallic acid was purchased from HiMedia and Rutin was purchased from Aldrich Chemistry.

Sample preparation

Fruits of *T. bispinosa* Roxb. were collected from Delhi, India. The samples were identified and authenticated by Dr. H. B. Singh and deposited under accession number NISCAIR/RHMD/Consult/-2011-12/1800/100 at Raw Materials Herbarium & Museum, NISCAIR, CSIR, New Delhi, India. The sample of dried fruit was crushed partially and was then analyzed for various parameters.

Extraction

T. bispinosa fruits were crushed partially and extracted using two different solvent systems: DCM: MeOH (1:1) and acetone. Extraction was carried out on an orbital shaker for 24 h at room temperature. Solvents were evaporated under vacuum and resulting extracts were stored at 4 °C.

Determination of nutritional attributes

T. bispinosa fruits were dried in an oven at 105°C overnight for 17 h to obtain moisture content¹⁴. The ash content was analyzed by weighing the samples before and after burning at 500 °C¹⁵. Macro Kjeldhal method was used for evaluation of total nitrogen and crude protein content (N x 6.25)¹⁶. Soxhlet extraction was used to measure fat content of *T. bispinosa* fruit, using petroleum ether as a solvent¹⁶. Total carbohydrate was estimated using the formula. A total carbohydrate was also measured¹⁵.

The mineral components of the *T. bispinosa* were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). 1 g of crushed *T. bispinosa* fruit was digested by 5 ml of concentrated HNO₃ in microwave. After digestion, sample was cooled and volume made up to 25 ml with double distilled water. Set plasma conditions for analysis were: Argon on 151/min, auxiliary 0.21/min, nebulizer flow at 0.851/min, RF power on 1300 W and chiller at 15 °C. Set of standards were run and then samples were analyzed against the standard.

Phytochemical analysis

Total phenolic content of *T. bispinosa* in the two solvent systems were determined by Folin Ciocalteu reagent method¹⁷ and expressed in terms of mg Gallic acid equivalents (GAE) /g of dry extract. Total flavonoids content was also determined using aluminium chloride colorimetric method¹⁸ and expressed in terms of mg rutin equivalents (RE) /g of dry extract. Concentration of crude alkaloids¹⁹ and saponins were also measured²⁰.

Determination of antioxidant activity

DPPH radical scavenging assay

The stable DPPH radical was used for determination of free radical scavenging activity of the extracts according to the modified method of Blois²¹. 0.1 ml of extract was mixed with 1 ml of 0.1 mM of DPPH. Absorbance was measured at 517 nm after 30 min of incubation. Ascorbic acid was used as a standard.

ABTS radical scavenging assay

The total antioxidant capacity of extracts was determined as ABTS radical scavenging activity. ABTS radicals are generated through a chemical oxidation reaction with potassium persulfate²². 10 µl of extract was mixed with 990 µl of ABTS reagent. After 10 min of incubation absorbance was measured at 734 nm and trolox was used as standard.

FRAP assay

The FRAP assay was carried out as described by Benzie and Strain²³ and FRAP values were calculated as mg of Trolox equivalents/g extract (TE). 100 µl of extract was mixed with 900 µl of FRAP reagent. Absorbance was measured at 593 nm after 4 min of incubation.

Metal chelating assay

The chelating activity of *T. bispinosa* extracts on ferrous ions was estimated using the method of Dinis²⁴. 1 ml of extract at various concentrations was mixed with 0.05 ml of 2 mM FeCl₂. 0.2 ml ferrozine was added to the reaction mixture and absorbance was measured at 562 nm after 10 min at room temperature. EDTA was used as positive control.

Statistical analysis

All data are presented as means ± SD. The mean values were calculated based on the data taken from at least three independent experiments conducted on separate days using freshly prepared reagents. Statistical analyses were performed using student's t-test with the help of origin 6.1. The statistical significances were achieved when $p < 0.05$.

RESULTS AND DISCUSSION

Nutrition analysis

Nutritional studies have demonstrated potential benefits of *T. bispinosa*. Moisture content and dry matter analysis during nutrition reporting is very important because it directly affect its nutritional content, its stability and storage. Proximal values were calculated and are depicted in the Table 1.

Table 1: Nutritional composition of *T. bispinosa* fruits on dry weight basis (g /100 g)

Ash	Moisture	Crude fat	Total protein	Total carbohydrate
1.5 ± 0.10	11.3 ± 0.25	0.12 ± 0.005	16.65 ± 0.20	70.43 ± 0.50

Data are mean±SD values of triplicate determinations.

In our studies, *T. bispinosa* was found to be rich in proteins and carbohydrates. Moisture content was also very high. Crude fat was found to be in negligible amounts. ICP-OES studies demonstrated *T.*

bispinosa to be highly rich in Ca, K and an important source of microelement Zn (Table 2). Heavy metals and trace elements were under acceptable limits.

Table 2: Mineral composition of *T. bispinosa* on concentration basis (ppm)

Analyte	<i>T. bispinosa</i>
Ca	365 ± 0.23
K	98.2 ± 1.23
Na	37.24 ± 0.36
Zn	6.926 ± 0.12
Ba	0.482 ± 0.32
Cr	0.106 ± 0.02
Cd	0.011 ± 0.002

Data are mean±SD values of triplicate determinations.

Phytochemical analysis

The phytochemical content of *T. bispinosa* was analyzed and high quantity of saponins (36.92±0.67%) was found in *T. bispinosa*. Alkaloids present in the plants, function as spasmolytic, anticholinergic and anesthetic agents. The alkaloid content in *T. bispinosa* was found to be 0.775±0.33%. Reports suggest that phenols antioxidant activity is due to their redox properties, H-

donation, prevention of chain initiation by donating electrons or by binding of transition metal ion catalysts and singlet oxygen quenchers²⁵. Flavonoids are important for their pharmacological activities as scavengers²⁶. Flavonoids prevent platelet stickiness and hence platelet aggregation. Colorimetric study of the two extracts of *T. bispinosa* showed that acetone solvent system was able to extract more phytochemicals in comparison to DCM:MeOH (Table 3).

Table 3: Total phenolic and total flavonoid content of extracts of *T. bispinosa*

Extracts	Total phenolic content (µg GAE/ mg extract)	Total flavonoid content (µg RE/ mg extract)
Acetone	7.924±0.03	743.38±0.35
DCM:MeOH	3.924±0.01	491.37±0.56

Data are mean±SD values of triplicate determinations. GAE, Gallic acid equivalent; RE, Rutin equivalent.

Antioxidant activity

DPPH scavenging activity

DPPH is a free-radical generating compound and has been widely used to evaluate the free-radical scavenging ability of various antioxidant compounds²⁷. Presence of antioxidant in sample leads to the disappearance of DPPH radical chromogens which can be

detected spectrophotometrically at 517 nm. Fig. 1 shows the percentage inhibition of DPPH radicals by *T. bispinosa* extracts. The DCM:MeOH extract showed higher radical scavenging activity (IC₅₀= 491.37±0.65 µg/ml) than acetone extract (IC₅₀= 743.38 ±0.35 µg/ml). However, standards ascorbic acid (IC₅₀= 0.055 ± 0.001 mg/ml) showed higher radical scavenging than both extracts. Both the extracts were significantly different ($p < 0.05$).

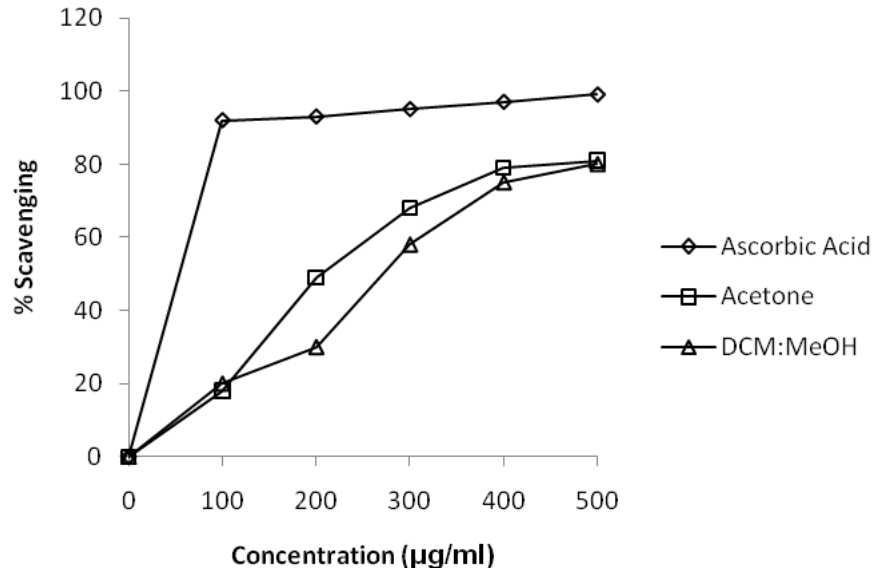


Fig. 1: DPPH free radical scavenging activity of extracts of *T. bispinosa* fruit

FRAP assay

FRAP assay measures the reducing ability of antioxidant that react with ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ)²³. The intensity of the colour is related to the amount of antioxidant reductants in the samples. Trolox was used as the standard curve for FRAP. The values of Trolox equivalents for the samples acetone and DCM:MeOH were calculated by extrapolation of the standard curve ($y=0.343x-0.341$, $R^2=0.971$). FRAP value for Acetone and DCM:MeOH extracts were found to be 7.183 ± 0.55 and 3.084 ± 0.12 µg TE/g respectively.

ABTS scavenging activity

ABTS scavenging assay is applicable for screening both lipophilic and hydrophilic antioxidants. Fig. 2 shows the percentage inhibition

of ABTS radical by *T. bispinosa* extracts and standard trolox. Acetone extract ($\text{IC}_{50}=5 \pm 0.24$ µg/ml) and DCM:MeOH ($\text{IC}_{50}=7 \pm 0.76$ µg/ml) showed less scavenging than that of standard trolox ($\text{IC}_{50}=1 \pm 0.01$ mg/ml). There was significant difference ($p < 0.05$) in ABTS scavenging activity of both the extracts.

Metal chelation activity

Lipid peroxidation by the Fenton reactions is initiated by ferrous iron. Thus, minimizing Fe^{2+} concentrations in Fenton reactions by metal chelation affords protection against oxidative damage. The chelating of Fe^{2+} ions by the extracts was estimated by the method of Dinis²⁴. In this assay, both extracts interfered with the formation of ferrous and ferrozine complex in an almost similar manner, suggesting that they have chelating activity and capture Fe^{2+} ion before ferrozine (Fig. 3).

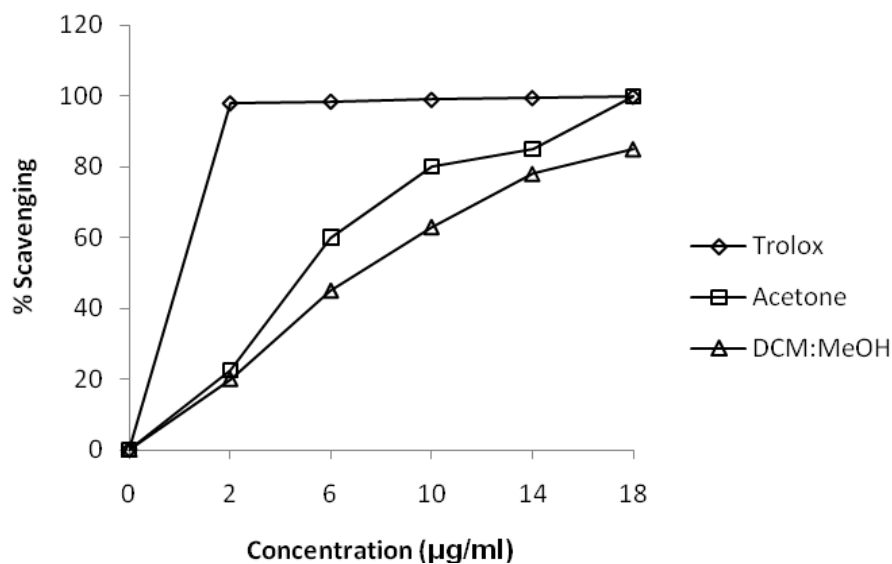


Fig. 2: ABTS free radical scavenging of the extracts of *T. bispinosa* fruit

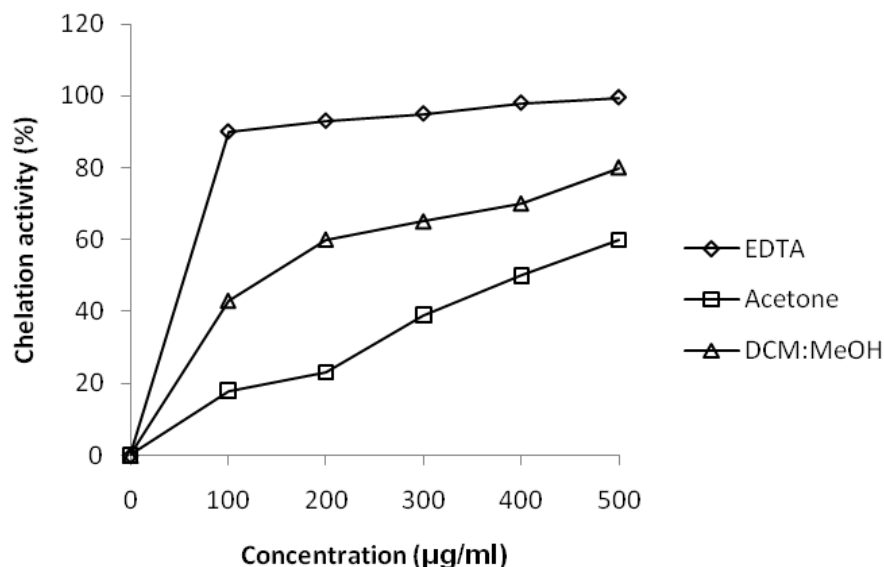


Fig. 3: Metal chelation activity for the extracts of *T. bispinosa* fruit

CONCLUSION

The dried fruits of *T. bispinosa* were analyzed in terms of nutritional, phytochemical and antioxidant properties. *T. bispinosa* was found to be rich in proteins, carbohydrates and minerals. Its negligible fat content contributes it to be a very interesting constituent for fat free diets. It proved to be good in terms of nutrition and antioxidant potential making it ideal for a balanced diet and also for the diet of people who have relied significantly upon wild water chestnut to supplement their normal. In our study, the acetone extract of *T. bispinosa* showed the highest total phenolics, total flavonoids and radical scavenging activity among the two extracts and proved to be the better solvent for extraction of polyphenols. Thus, utilization of *Trapa* fruits as source of phytochemicals tender varied opportunities for nutraceutical, functional food application and therapeutic that it offer and make it more useful in dietary constitution of common public in India and world over.

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