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Determination of Total Phenolic Content and Total Antioxidant Activity in Various Parts of *Trapa bispinosa*

Chandana Majee, Avijit Mazumder, Rupa Mazumder, Alka N. Choudhary,
Noida Institute of Engineering & Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh

Abstract

From ancient times, human beings are dependent on nature. 80% of the population living in rural areas of developing countries depends on traditional medicines for their health care needs. Many of the powerful drugs used in modern medicines have originated from plants. Today's plant-based drugs treat a verity of diseases range from headache to cancer`. In the present study, antioxidant activities of the alcoholic extracts of *Trapa bispinosa* have been carried out by various models. The total phenolic content has been determined by spectrophotometric method following the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Antioxidant activity has also been determined by three different methods, such as nitric oxide scavenging method, hydroxyl radical scavenging capacity method and DPPH free radical scavenging method. Alcohol extracts of leaves and fruit peels have shown significant dose dependent free radical reducing potentiality. The result of antioxidant activity performed by hydrogen peroxide scavenging method revealed that fruit peels extract have shown 20% peroxidation inhibitin activity, where as leaf extract have shown 15% peroxidation inhibiting activity. Leave and fruit peel extracts have shown dose dependent free radical reducing activity. How ever when tested by nitric oxide radical inhibition assay method the reducing ability of leaf and fruits peel extract were found 46.02and 52.09 respectively. In DPPH assay method fruits peel extract give better result as compared to the leaf extract.

Key ward: *Trapa bispinosa* , antioxidant activity**,** DPPH

INTRODUCTION

Antioxidants are those compounds, which inhibit or delay the oxidation process. For this reason, antioxidants are used in food industries, cosmetic industries as well as, in pharmaceutical industries[1]. Researchers have shown high levels of interest to work on naturally obtained phenolic compounds due to good antioxidant properties particularly to be used in management of threatening diseases, like heart failure, cancer, liver injury and so on[2]. There are two types of antioxidants, endogenous antioxidants and exogenous antioxidants. Endogenous antioxidants are enzymes, such as albumin, globulin, uric acid and so on[3]. When endogenous antioxidants are unable to protect the organisms from the reactive species, the exogenous antioxidants are to be delivered from outside, such as vitamin A, vitamine E, flavanoids, minerals, β-carotene and so on[4]. Nature is a big source of all remedies that prevents and cures most of the diseases of human beings. This study highlights the antioxidant property of a magical plant, *Trapa bispinosa*, commonly known as singhara or water chest nut, belong to the Trapaceae family [5]. The plant has wide range of therapeutic uses. *Trapa bispinosa* is extensively used for the treatment of anemia, diarrhea and stomachache. From the various articles, it has been confirmed that this plant contains high amounts of phenolic compounds .[6]

MATERIALS AND METHODS

Collection of plant materials

Various plant materials (leaves and fruit peels of *Trapa bispinosa*) were collected from the herbal garden of Noida Institute of Engineering and Technology, Greater Noida, UP, India, during October, 2017. Plant materials were shade dried at room temperature, powdered with blender and passed through sieve no. 20, followed by its storage in air tight containers.

Preparation of extracts

The shade dried powdered materials were extracted by methanol in Soxhlet apparatus at 70°C for 72 h. The solvent of the extracts was evaporated under reduced pressure. The extracts were then subjected to estimation of their total phenolic contents and antioxidant potentials using various chemical methods.

Determination of percentage yield of *Trapa bispinosa*

Shade dried leaves and fruit peels were extracted by methanol and the percentage yields were calculated using the following equation:

% Yield= Weight of extract/Weight of powder drug taken x100

Determination of total phenolic content

Total phenolic contents (*TP*) of the extracts were determined using Folin–Ciocalteu reagent (FCR), following modified method of Kumar *et al*. (2008)[7]. Gallic acid was used as standard. The solution of each extract (0.5 ml, 1 mg ml^{-1}) was diluted to 10 ml with distilled water in a volumetric flask. FCR (1 ml) was added and mixed thoroughly and then sodium carbonate solution (3 ml, 2%) was added. The absorbance was measured at 760 nm after 2 h. The total phenolic content was determined and compared with the standard (gallic acid). The results were recorded in micrograms of gallic acid equivalents (mg of GAE) per gram of dry weight (g DW) of the powder. All tests were conducted in triplicate.

Antioxidant activity

Antioxidant activities of the prepared extracts were quantified using the following methods

Hydrogen Peroxide Scavenging Capacity

The ability of the *Trapa bispinosa* extracts to scavenge hydrogen peroxide was determined. Hydrogen peroxide solution (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/ml) in distilled water were added to hydrogen peroxide solution (0.6 ml, 40mM). After 10

minutes absorbance was determined at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging effects of both the extracts and standard compounds were calculated as per the fallowing formula [8, 9]

% Scavenged $[H_2O_2]$ = [(AC – AS)/AC] x 100

Nitric oxide scavenging activity: In this method, the reaction mixture (3 ml) containing sodium nitropruside (10mM 2 ml), phosphate buffer saline (0.5) and the extract or the standard solution (0.5 ml) was incubated at 25°C for 2.5 h. After incubation, 0.5 ml of the reaction mixture were withdrawn and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min. for completion of diazotization. 1 ml of 1-naphthylaimne (5%) was added, mixed and allowed to stand for 30 min., when a pink coloured chromophore was formed in diffused light. The absorbance of that solution was measured at 540 nm for each of the extracts and standard against the corresponding blank and subsequently, the IC_{50} values (concentration of sample required to inhibit 50% of the nitric oxide radical) were determined. [10-11]

DPPH free radical scavenging activity The spectrophotometric method with DPPH was applied to antioxidant capacity determination in fruits peel and leaves extracts. Ethanolic solution of DPPH (300 ml, 0.05mM) was added to 40 ml of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol (2.7 ml, 96%) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. The ethanol without extract (blank) was used to set the absorbance at zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. [12, 13]

RESULTS AND DISCUSSION

The percentage yields, phenolic contents and antioxidant activities of the methanolic extracts of *Trapa bispinosa*, as determined by various methods mentioned above, have been reported in the following sections.

Percentage yields of various extracts of *Trapa bispinosa*

The percentage yields of the methanolic extracts of leaves and fruit peels of *Trapa bispinosa* have been reported in Table 1.

Table 1: Percentage Yields of Methanolic Extracts of Various Parts of *Trapa bispinosa*

Sl. No.	Plant Parts	Solvent	Initial Weight(gm)	Percentage Yield(%)
1	Leaves	Methanol	50.3	12.34
2	Fruit peel	Methanol	55.0	17.35

Total phenolic (TP) content

The average values of the total phenolic contents of the extracts of leaves and fruit peels of the tested plant as recorded in Table 2, depicts that the phenolic content of the fruit peel extract is higher than that of the leaves.

Table 2: Total Phenolic Contents of Various Extracts of *Trapa bispinosa* (Results are expressed as mean ± SD)

Sl. No.	Methanolic Extract of Plant Parts	Total Phenolic Content (mg GAE g^{-1} DW)
1	Leaves	69.2±0.25
2	Fruit peel	73.6±0.86

Values are expressed Mean ± SEM (Standard error mean)

Hydrogen Peroxide Scavenging Capacity

The scavenging abilities of the methanolic extracts of leaves and fruit peels of *Trapa bispinosa* on hydrogen peroxide have been compared with that of ascorbic acid in Fig. 1.

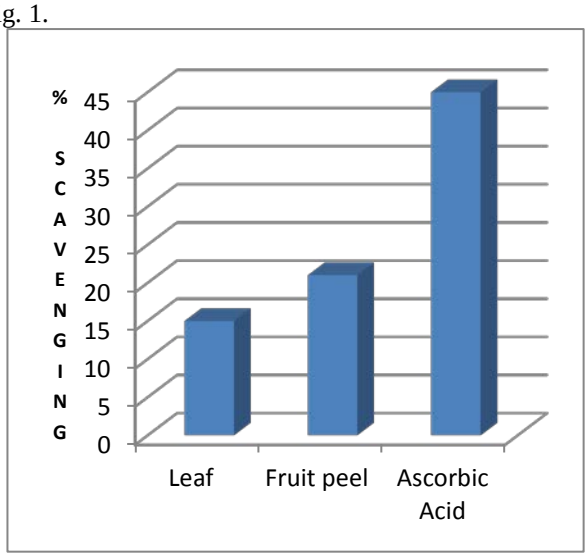

Fig. 1 Comparative Hydrogen Peroxide Scavenging Activities of Leave and Fruit Peel Extracts of *Trapa bispinosa*

Nitric oxide scavenging activity

The results of the nitric oxide scavenging activities of leaf and fruit peel of extracts of *Trapa bispinosa* have been depicted and compared with that of ascorbic acid in Table 3.

Table 3: Antioxidant Activities of Leaf and Fruit Peel of Extracts of *Trapa bispinosa* by Nitric Oxide Radical Inhibition Assay Method

Sample	IC_{50} value ± SE *(µg/mL)
Leaf extract	46.02 ± 0.57
Fruit peel extract	52.09 ± 1.23
Ascorbic acid	22.66 ± 0.98

These are represented as mean ± SEM (Standard Error Mean).

DPPH free radical scavenging activity: DPPH free radical scavenging activities of different tested doses of leaf and fruit peel extracts of *Trapa bispinosa* have been reported in **Table 4**.

Table 4: Antioxidant Activity of Leaf and Fruit Peel Extracts of *Trapa bispinosa* Determined by DPPH Assay Method

Extracts	% of DPPH Scavenging Activity
Leaf extract (50 µg/ml)	55.25 ± 0.65
Leaf extract (100 µg/ml)	63.34 ± 0.57
Fruit peel Extract (50 µg/ml)	64.56 ± 0.85
Fruit peel Extract (100 µg/ml)	82.23 ± 0.78

Value are represented as mean ± SEM (standard error mean).

Antioxidants are very important substances having unique ability to protect the body from free radical induced oxidative stress.[14] The antioxidant potentials of methanol extracts of the tested parts of *Trapa bispinosa* have been investigated as a part of search for new bioactive compounds from natural resources.[15]

Plant polyphenols act as reducing agents, antioxidants and free radical-scavengers by the hydrogen donating property of their hydroxyl groups[16] Hence, there should be a close correlation between the content of phenolic compounds and antioxidant activity of any plant part.[17] The TP contents in the different extracts (leaves and fruit peel) of the plant under study have shown to have values of 69.2±1.25, 73.6±2.86 mg GAE g^{-1} DW, respectively.

From the various studies conducted , it was concluded that as such DPPH is a very stable free radical. In the maximum absorbance at 517 nm, DPPH can easily undergo scavenging by an antioxidant and quickly converted in to 1,1-diphenyl-2-picrylhydrazine. It has been found that the methanolic extract of fruit peel showed higher values of % of DPPH scavenging activity as compared to that of the leaf extract, but less than that of standard compound, ascorbic acid. Generally, free radical scavenging activity associated with the concentration and nature of phenolics compounds which capture the DPPH free radical .[18]

H_2O_2 is highly important because of its ability to penetrate into biological membranes.[19] The hydroxyl radical is an enormously reactive species and generally reacts at a very high rate with all adjacent molecules such as nucleic acid proteins, lipids and carbohydrates.[20-21] Fruits peel extract was found to be the powerful scavenger of the hydroxyl radical, with an inhibition of up to 20.5 % at and leaves extract showed free radical scavenger activity up to 15**%** when compared with standard drug which has scavenging capacity upto 40%.

CONCLUSION

From the study , it may be concluded that the methanol extract of *Trapa bispinosa*, fruits peel and leaves extract demonstrated good antioxidant activities when compare with the standard compounds . This finding substantiates its traditional uses in treating various disorders and increases the interest and potential use of this sample as nutraceutical and pharmacological agent. Further isolation and purification of compounds from this extract and study of their biological effects may provide further information of their medicinal value.

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