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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *TERMINALIA CATAPPA* NUTS

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ABSTRACT

Objective: The main objective of this study is to assess the secondary metabolites and antioxidant activities of *Terminalia catappa* nut as it is not a commercialized nut.

Materials and Methods: The secondary metabolites and antioxidant activities were experimented by adopting recommended standard procedures using aqueous extract of *T. catappa* nut powder 25, 50, 75, 100 mg.

Results: The total phenolics content was higher on comparison with flavonoid content. Similarly, among the antioxidant activities, nitric oxide scavenging (257.33±2.30 mg/g) as well as reducing power activity (142.66±6.11 mg/g) was higher compared with total antioxidant (82.66±6.11 mg/g) and metal chelating activity (64.66±2.30 mg/g).

Conclusion: The results obtained shows that it contained phenolics and flavonoid, which in turn induces antioxidant activities. Hence, this might act as a potential antioxidant, and needs further characterization and purification.

Keywords: Antioxidant, Aqueous extract, Nuts, Secondary metabolites, *Terminalia catappa.*

INTRODUCTION

Nuts have been the food of man from the earliest times and still are the mini-articles of diet in many parts of the world [1]. From the 2000s, some farmers have become interested in the popularization of this tree for the sale of its dried fruits. From the scientific point of view, various studies, mainly on the chemical composition and nutritional value of *Terminalia catappa* kernel and pulp, have been carried out [2]. *T. catappa* Linn (combretaceae) is present in the warmer parts of India. It is also known as Indian almond, Malabar almond, and tropical almond [3]. The various extracts of leaves and bark of the plant have been reported to be anticancer, antioxidant [4], anti-HIV reverse transcriptase [5], hepatoprotective [6], anti-inflammatory [7], anti-hepatitis [8], aphrodisiac [9], antimicrobial [10,11], nephroprotective [12], antitumor [13]. The fallen leaves of *T. catappa* have been used in the management of sickle cell disorders [5]. The moderate consumption of the seed kernel is useful in the treatment of men with sexual dysfunctions, primarily from premature ejaculation [9]. Meanwhile, *T. catappa Linn*, a medium sized tree has been identified with potent antioxidant activity which has been exploited as curative agents against a number of pathological conditions [14]. Its fruits have been used for the treatment of asthma and diabetes [15]. Nuts are very nutritious and contain a significant amount of high-quality proteins and vital minerals [16]. The nuts are good sources of edible oils and fats. Considering the significance, it was decided to study the secondary metabolites and antioxidant activities of *T. catappa* nuts.

MATERIALS AND METHODS

Sample collection

The *T. catappa* fruits were collected from the tree located in-front of the campus Navodaya Academy Senior Secondary School (CBSE), Namakkal, Namakkal District, Tamil Nadu, India. The collected fruits were cleaned thoroughly, and the flesh was removed by cutting it separately. The nut covered by thick shell was allowed to dry under the shade. After that the dried fruits are crushed with a nut cracker and the inner portion, i.e., nuts were removed. The removed nuts were allowed to shade dry until complete dryness occurs. Then, the completely dried nuts were ground to powder for further use.

Aqueous extract preparation

Aqueous extract was prepared by taking different concentrations of powdered *T. catappa* nuts (25, 50, 75, 100 mg). Each concentration weighed was mashed into paste in a mortar and pestle and dissolved up to the mark with distilled water in 10 ml standard flask, and 0.1 ml was used for each experiment. Each experiment was repeated thrice.

Determination of secondary metabolites

The phenol and flavonoid content of aqueous extract were analyzed.

Determination of total phenol content

Total phenolic content were determined by Folil–Ciocalteau method. The extract (0.1 ml) were mixed with Folin–Ciocalteau reagent (5 ml, 1:10 diluted with distilled water) for 5 minute and aqueous $NaCo_3$ (4 ml, 1 M) were added. The mixture was allowed to stand for 15 minute, and the phenols were determined by colorimetric method at 765 nm. A standard curve was prepared. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound [17,18].

Estimation of flavonoids

The aluminum chloride method was used for the determination of total flavonoid content. Extract solution were taken and to this 0.1 ml of 1 M potassium acetate, 0.1 ml of $AlCl_3$ (10%), 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated using known concentration of quercetin. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample [19].

Determination of antioxidant activities

Nitric oxide scavenging assay, reducing power assay, total antioxidant assay, metal chelating activities were performed.

Reducing power assay

Aqueous extract was mixed with phosphate buffer (2.5 ml, 0.2 M, $_p$H 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°c for 20 minutes. 1.0 ml of trichloro acetic acid (10%) was added

to stop the reaction, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (1.5 ml) was mixed with distilled water (1.5 ml) and $FeCl_3$ (0.1 ml, 0.1%) after mixing, the contents were incubated for 10 minute and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control [20].

Total antioxidant capacity

Total antioxidant capacity by the phosphomolybdenum method assay is based on the reduction of Mo (V1) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH by adding 4 ml reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate, 4mM Ammonium molybdate. The tubes were incubated in the water bath at 95°C for 90 minutes. After the samples had been cooled to RT, the absorbance of the mixture was measured at 695 nm against blank. The phosphomolybdenum method is quantitative, since, the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [21].

Nitric oxide scavenging activity

This procedure is 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. For the experiment, sodium nitroprusside (10 mM), in phosphate buffered saline, was mixed with extract and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Ascorbic acid was used as a positive control [22].

Metal chelating activity

Add extract (0.1 ml) to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM Ferrozine (160 µl), the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Absorbance of the solution was then measured spectrophotometrically at 562 nm. Standard curve was plotted using ascorbic acid. Distilled water (1.6 ml) instead of sample solution was used as a control. Distilled water (160 µl) instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution [23].

For all estimations, readings were taken using UV-visible spectrophotometer - Shimadzu, Japan make. Model UV 1800. Standard graph was plotted for all experiments using their respective standards, and samples were plotted against the standard by taking concentration in X axis and OD in Y axis.

Table 1: Secondary metabolites of *T. catappa* nuts

Serial number	*T. catappa* nut powder (mg)	Total phenolics GAE mg/g dry material	Total flavonoids QE mg/g dry material
1	25	10.66±1.52	26.33±20.51
2	50	15.96±2.65	44.43±30.37
3	75	65.33±2.30	109.33±1.15
4	100	128.0±6.9	129.33±2.30

Values are mean±SD for three experiments. GAE: Gallic acid equivalents, QE: Quercetin equivalents, *T. catappa: Terminalia catappa*, SD: Standard deviation

Statistical tool

Each experiment were carried out in triplicate, and the results are given as the mean±standard deviation (SD). The mean and SD was calculated by using the following formula:

Mean = Sum of x values/n (Number of values), $S=\frac{\sqrt{\sum(X-M)^2}}{n-1}$

RESULTS AND DISCUSSION

Secondary metabolites

Secondary metabolites like polyphenols have properties, including antioxidant, antimutagenic, anticarcinogenic, antiinflammatory, and antimicrobial effects that might potentially be beneficial in preventing diseases [24]. The concentration of phenolic compounds in fruits, vegetables are regulated by genetic, environmental, physiological and chemical factors such as temperature, light, rainfall, soil, chemicals and plant growth regulators [25]. Plants carry number of classes of active compounds, but the presence of phenolic compounds in plants defines their medicinal importance [26].

The results of secondary metabolites are shown in Table 1. In our study also, the concentration of phenolics is higher compared to flavonoids. When experimented with 25, 50, 75, 100 mg of aqueous extract of *T. catappa* nuts showed varying phenolic contents like 26.33±2.51 mg/g, 44.43±3.37 mg/g, 109.33±1.15 mg/g, 129.33±2.30 mg/g. Similarly, the flavonoid content was studied, the results are as follows: 10.66±1.52 mg/g, 15.96±2.65 mg/g, 65.33±2.30, 128.0±6.9mg/g. Flavonoids are nontoxic, manifest a diverse range of beneficial biological activities.

Antioxidant activities

Spices have higher antioxidant activity as compared to fruits, cereals and nuts [27]. An ideal antioxidant should be readily absorbed and quench free radicals, chelate redox metals at physiologically relevant levels. Few antioxidants can interact with other antioxidants regenerating their original properties, this mechanism is often referred to as the "antioxidant network" [26]. The importance of natural antioxidants for medical and food application is commonly performed through extraction procedures [28].

The results of various antioxidant activities studied are shown in Table 2. Among the various antioxidant activities tested, reducing power activity, nitric oxide scavenging activity was more effective compared with other activities. Variation observed in antioxidant activities are solely depends on varieties, location and growth conditions [29]. The presence of antioxidant activity, total phenol, total flavonoid content of medicinal plants help in identifying plants as new sources of therapeutical and industrial utilization [30].

CONCLUSION

Therapeutic potential of medicinal plants is mainly due to their phenolic compounds present in fruit, vegetables, nuts, seeds, stems and flowers. Hence, medicinal plants are used by humans since the beginning of human life on earth. From the present study, it is concluded, that *T. catappa* nuts contain phenolics and flavonoid, which induces various antioxidant activities. Thus, *T. catappa* nuts might act as a good antioxidant.

Table 2: Antioxidant activities of *T. catappa* nuts

Serial number	*T. catappa* nut powder (mg)	Ascorbic acid equivalent mg/g dry material			
		Reducing power	Total antioxidant	Nitric oxide	Metal chelating
1	25	32.66±2.51	50.0±1.0	27.66±2.51	4.66±0.76
2	50	55.96±1.35	24.86±3.37	53.73±4.06	15.30±2.0
3	75	94.66±2.30	34.66±2.30	116.66±1.15	52.66±1.52
4	100	142.66±6.11	82.66±6.11	257.33±2.30	64.66±2.30

Values are mean±SD for three experiment. SD: Standard deviation, *T. catappa: Terminalia catappa*

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