Research Article

A Preliminary Study on Phytoanalysis, Antioxidant Potential of *Terminalia catappa* l. Fruit Flesh

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ABSTRACT

Terminalia catappa L. is a common tree but its fruits are eaten only by birds, children and is not available in the shops like ordinary, common fruits. This means *Terminalia catappa* fruits are underutilized. Hence, preliminary study was performed to know better about the phytochemicals, secondary metabolites and antioxidant activities of fruit flesh. The observed phytochemical results show that it contains alkaloid, flavonoid, saponin, anthroquinone, glycoside. In addition to these phytochemicals qualitatively tested, it also found to contain phenolics and flavonoids when tested quantitatively. The results of invitro antioxidant studies assessed shows, which it is able to have higher reducing power, nitric oxide scavenging, total antioxidant activity but metal chelating activity was moderate with *Terminalia catappa* fruit flesh. Since, *Terminalia catappa* fruit flesh possess phytochemicals with good antioxidant activity, it can be utilized by everyone like all fruits as it finds enormous pharmaceutical applications. Let's be aware of commonly available fruits and its values to preserve our nature.

Keywords: Free radical, Fruit flesh, Medicinal value, Phyto analysis, *Terminalia catappa*.

INTRODUCTION

Terminalia catappa L. is a Combretaceous plant, leaves are widely used as a folk medicine in Southeast Asia to treat dermatosis and hepatitis.[1] This tree is tolerant to strong winds, salt spray and moderately high salinity in the root zone. It grows in a freely drained, well aerated, sandy soil. *Terminalia catappa* fruit contain cyanidin-3-glucoside, corilagin (Topoisomerase I and II inhibitor[2,3] and Xanthin oxidase inhibitor,[4] ellagic-acid (anti-HIV),[5,6] anti asthmatic compound, gallic-acid,[7] and pentosans. *Terminalia catappa* fruit also contains phytochemicals which are indicative of it's potential in treatment of DB e.g. brevifolin-carboxylic-acid, an aldose reductase inhibitors, euginic acid having anticataract activity.[8] This fruit is antidiabetic[9] in nature as it is rich in tannins. The extract of *T.catappa* leaves and fruits have been reported for anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic and hepatoprotective activity.[10-13] *Terminalia catappa* red variety pulp (100g) contain 11.05 to 14.05% carbohydrate, vitamin C - 95.9 to 138.6mg and β-carotene - 754-2090µg.[14] Saponin, glycoside, flavonoid, alkaloid, anthraquinone, anthraquinone glycoside are present in this fruit.[15,16] Different kinds of plants and its parts like leaf, bark, seed, stem, flowers, fruits, twigs, and peel individually or totally exhibit therapeutic properties, each part exhibiting different biological activity and antioxidant potency.[17-21] Cosidering the therapeutic importance of *Terminalia catappa* fruit, it is important and essential to promote processing and consumption of this tropical fruit. Hence, the present study was planned to analyse the phytochemicals, secondary metabolites, antioxidant activities of *Terminalia catappa* fruit flesh.

MATERIALS AND METHODS

Sample collection

The *Terminalia catappa* fruits (red variety) were collected from tree located in front of the campus of Navodaya academy senior secondary school (CBSE), Namakkal, Namakkal District, Tamil Nadu, India during November-December, 2013. The flesh from fruits along with the skin were separated and allowed to shade dry. After drying it completely, the dried flesh of fruits was powdered in a blender. The powdered fruit flesh was used for further studies.

PHYTOCHEMICAL ANALYSIS

Qualitative analysis was done for the presence of alkaloid, flavonoid, Saponin, anthroquinone, terpenoid and steroid, glycoside.[22,23]

Test for alkaloid

This test was performed by adding few drops of saturated solution of picric acid to a drop of extract. Positive test shows the presence of yellow colour precipitation.

Test for flavanoid

To a drop of extract add magnesium turnings followed by 1/2 drops of concentrated hydrochloric acid. Positive result gives red color.

Test for saponin

Foams produced when the extract was shaken with water for positive result.

Test for free anthraquinone

5 ml of chloroform was added to the powdered sample and filtered after shaking it for 5 mins. To this add equal volume of 10% ammonia solution. Positive result shows the appearance of bright pink colour in the aqueous layer.

Test for Steroid and Terpenoid

Dissolve a small portion of extract in 1ml chloroform and then filtered. The filtrate was kept on ice, to this 1 ml of acetic acid and few drops of conc. sulphuric acid was added. The appearance of a pink colour indicates the presence of terpenoids. The appearance of blue colours indicates the presence of steroids. A mixture of pink and blue colour indicates the presence of both.

Test for Glycoside

Powered sample was boiled with 1.0 ml of sulfuric acid in a test tube, filtered while hot and then cooled. Then equal volume of chloroform was added.10ml ammonia was added to the separated chloroform layer of mixture. The presence of reddish brown precipitate in the filtrate shows positive result.

Aqueous extract preparation

Aqueous extract was prepared by taking different concentrations of *Terminalia catappa* fruit flesh powder (50, 75, 100mg). Each concentration was dissolved in 10ml water mixing with a magnetic stirrer at 4°C for 4h. The mixture was filtered through nylon cloth and centrifuged at 20,000g for 30min. 0.1ml of supernatant was used for the analysis. Each experiment was performed three times.

DETERMINATION OF SECONDARY METABOLITES

The phenol and flavonoid content of aqueous extract was analysed.

Determination of total phenol content

Total phenolic content were determined by Folil-ciocalteau method. The extract (0.1ml) was mixed with folin-ciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5min and added aqueous $NaCo_3$ (4ml, 1M). The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765nm. The 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.[24, 25]

Estimation of flavonoids

The aluminium chloride method was used for the determination of the total flavonoid content. Extract solution were taken and to this 0.1ml of 1M potassium acetate, 0.1ml of $AlCl_3$ (10%), 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30min 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.[26]

DETERMINATION OF ANTIOXIDANT ACTIVITIES

Reducing power assay, Total antioxidant assay, Nitric oxide scavenging assay, Metal chelating activities were performed.

Reducing power assay

Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°c for 20min. 1.0 ml of Trichloro acetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (1.5ml) was mixed with distilled water (1.5ml) and $FeCl_3$ (0.1ml, 0.1%) after mixing, the contents were incubated for 10min and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control.[27]

Total antioxidant capacity

Total antioxidant capacity by 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 4ml reagent solution containing 0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM Ammonium molybdate. The tubes were incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to RT, the absorbance of mixture was measured at 695nm against blank. The phosphomolybdenum method is quantitative, since, the total antioxidant activity is expressed as the number of equivalents of ascorbic acid.[28]

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 (10mM), in phosphate buffered saline, was mixed with extract and incubated at room temperature for 150min. After the incubation period, 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. Ascorbic acid was used as a positive control.[29]

Metal chelating activity

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

water (160µl) instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution.[30]

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

STATISTICAL TOOL

Each experiment was carried out in triplicate and the results are given as the mean ± standard deviation. The Mean and Standard deviation (S) 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

The results of Phytochemicals present in *Terminalia catappa* fruit flesh is shown in Table 1.

The fruit flesh is found to be devoid of terpenoid and steroid but found to contain alkaloid, flavonoid, saponin, glycosides, steroids, anthroquinone and glycosides. The medicinal value of the plant is determined by the phytochemicals present.

Table 1: Phytochemicals in aqueous extract of *Terminalia catappa* L. fruit flesh

Phytochemicals	Present(+) /Absent (-)
Alkaloid	+
Flavonoid	+
Saponin	+
Anthroquinone	+
Terpenoid, Steroid	+
Glycosides	+

Secondary metabolites and Antioxidant activities

The results of secondary metabolites and antioxidant activities of *Terminalia catappa* fruit flesh is shown in Table 2.

Table 2: Secondary metabolites and Antioxidant activities in aqueous extract of *Terminalia catappa* L. fruit flesh.

Terminalia cattappa Fruit flesh (mg)	Total phenolics (mg/g)	Total flavonoids (mg/g)	Reducing power activity (mg/g)	Total antioxidant activity (mg/g)	Nitric oxide scavenging activity (mg/g)	Metal chelating activity(mg/g)
50	9.3±2.7	30.2±2.02	64.40±2.02	14.2±2.76	54.2±2.02	20.16±1.40
75	28.66±1.15	61.33±1.15	91.33±2.30	49.33±1.15	78.0±2.0	29.0±1.73
100	121.33±6.11	193.33±2.30	233.33±2.30	84.0±8.0	204.0±6.92	65.33±3.05

Values are Mean±SD for Three experiments

Table 2 shows the results of *Terminalia catappa* fruit flesh. Total flavonoids were found to be higher when compared to total phenolics at all concentrations studied. Among the antioxidants studied, the reducing power activity, Nitric oxide scavenging activity was found to be higher. But, total antioxidant activity as well as metal chelating activity was lower with fruit flesh. (Table.2) Flavonoids are hydroxylated phenolic substances are synthesized by plants during stress eg. microbial infection[31] and influence the quality, stability of foods by acting as flavorants, colorants, and antioxidants.[32,33]Functional hydroxyl groups in flavonoids enhances the antioxidant effects by scavenging free radicals or by chelating metal ions.[34,35] The metal chelation is crucial in preventing free radical generation.[36,37]

The antioxidant activity of several polyphenolic extracts, compounds of leaves, fruits, seeds, bark, roots and oilseeds are well documented.[38,39] Antioxidants defend by contributing an electron of their own to neutralize free radicals and help prevent cumulative damage to body cells and tissues.[40] The antioxidant activity of phenolic constituents might be due to their redox properties, by acting as reducing agents/hydrogen-atom donors, their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals.[41] The reducing power decreases inversely to polarity of the solvent used [42] and the phytochemicals, containing non polar secondary metabolites are inactive.[43] Compared to fresh fruits, polyphenol and antioxidant activity of dried fruits are higher because of low moisture content in it which increases shelf life.[44]

CONCLUSION

The research results showed that, *Terminalia catappa* fruit flesh contains sufficient amount of phenolics and flavonoids which activates antioxidant activity. The antioxidant activities present are due to the phytochemicals contained in it. From the obtained results, it is concluded that it can be used like common fruits to get the pharmacological benefits free of cost as we find the tree everywhere.

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