

Evaluation of Proximate Composition and Phytochemical analysis of *Terminalia catappa* L. from Nagapattinam Region

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Abstract: The leaf was collected and extracted with different organic solvents and the phytochemical screening was carried out using the standard procedures. Alkaloids, flavonoids, tannins, saponins, steroids and glycosides were found to be present in the leaf extracts. The Proximate composition and Phytochemical characteristics were determined for *Terminalia catappa* leaves. The results obtained showed the moisture content 7.18 ± 0.18 moisture; ash 20.2 ± 0.48 ; crude fiber 20.47 ± 0.59 and the carbohydrate 187.4 ± 0.53 ; protein 86.73 ± 0.75 was present in the mature leaves of *Terminalia catappa* L. The present study revealed that the plant contain many phytochemicals which may be responsible for the medicinal properties.

Keywords: Phytochemical, *Terminalia catappa*, crude fiber, Proximate composition.

1. Introduction

Traditional medicine has been practiced from many centuries; especially in India by tribal and rural people for treat several diseases, due to availability and low cost and negligible side effects. It occupies great importance in many formulations and ayurvedic drugs. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine(1).

Terminalia catappa or locally known as Ketapang is widely distributed plant in tropical and subtropical area (2,3,4). The leaves and other parts of this plant have been used as a folk remedies in tropical countries to treat dermatitis, helminthiasis and hepatitis (4). From previous studies, this plant was also reported to have several bioactive compounds that can be used as antioxidant (5), antidiabetic (6,7), antifungal (8), antimicrobial (9) and antihelmintic activities.

The almond tree (*Terminalia catappa*) grows to a height of 3-8m and bears a fruit that is ellipsoid in shape with a bluntly pointed apex, and the fruit is about 7.51cm long and 5.05cm thick. On ripening, it turns from green to purplish yellow and contains a hard shell or nut, which covers the delicate edible seed. The ripe mesocarp of the fruit is mostly consumed by children neglecting the seed, which contains oil. The major countries growing this plant include Italy, Spain, Morocco, France, Greece, and Iran. It flowers appear between April and May and between September and October. The fruiting season is from October to April (10).

Terminalia catappa L. belongs to the family Combretaceae. *T. catappa* is used primarily as an ornamental shade, and salt-tolerant street tree, but the leaves provide food for the Tasar silkworm, and the seeds are edible like almonds with sim Malay peninsular and through the Canary islands this tree is known as the tropical almond. It has been claimed to

have therapeutic effects for liver related diseases(11). In India, it is used as cardiac stimulant. Its leaves are widely used as a folk medicine in Southeast Asia for the treatment of dermatitis and Hepatitis (12).

In the present study the phytochemical and proximate analysis of mature leaves (Red leaves) of *Terminalia catappa* were done for the presence of various secondary metabolites present in different organic solvents.

2. Materials and methods

A) Collection of Plant Sample: The fully mature *Terminalia catappa* (Red leaves) were collected in August – September 2012 from South Poigainallur Village in Nagapattinam district of Tamilnadu, India and the Plant was taxonomically identified by Dr.P.Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai. The Voucher Specimen of *Terminalia catappa* was (PARC/2014/2063).

B) Processing of plant sample

The Red leaves of *Terminalia catappa* were properly washed in tap water and then rinsed in distilled water. The rinsed leaves were shade dried and powdered using electrical blender. The powder was stored in air tight glass containers, protected from Sunlight until required for analysis.

C) Solvent extraction

About 10 gm of powdered plant material was soaked separately in 100ml of methanol, ethanol, hydro alcohol (1:1), ethyl acetate, chloroform and petroleum ether for 3-4 days at room temperature in dark condition. The extracts were filtered by using what man filter paper. The filtrate was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator and stored at 4 °C until further use. Each extracts was resuspended in the respective solvent and used for qualitative and quantitative analysis of phytochemicals.

D) Aqueous extraction

10 gm of dried powdered plant leaves was soaked in 100ml of distilled water for 3-4 days at room temperature in dark condition. Then the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis.

E) Proximate Analysis

Moisture, ash, crude fiber, carbohydrates, protein of the mature leaves of *Terminalia catappa* were determined as per the standard procedures.

- **Moisture content**

- Powdered plant material (2 g) was taken in a tarred silica crucible and dried in an oven at 105 °C for 30 min, cooled at room temperature in desiccator until constant weight. The powder was then weighed to calculate the moisture content based on the loss of weight on drying and the results were expressed as a percent of dry powder (13).

- **Ash content:** To determine the ash content, oven method was used. Ten grams of the sample were added to a pre weighed crucible and weighed. Then, the sample were placed in a muffle furnace at 550°C for 4 h, cooled in desiccators and reweighed. The ash content was determined (14).

- **Crude Fiber :** 2 g sample was put into 250 ml of conical flask and added 1.25% Sulfuric acid solution and then heated for about 30 min, filtered then washed until traces of acid could not be detected using pH paper. The acid extracted was transferred into 250 ml flask and added 1.25% NaOH subsequently. Heated the samples for 30 min and filtered and washed. The material was transferred into crucible and oven dried at 120°C for 12 h. After it, crucibles were placed in muffle oven for 12 h at 550°C and recorded the crucible weight(15).

- **Estimation of Carbohydrate :** 100 mg of the fruit samples were weighed and hydrolyzed with 5 ml of 2.5 N Hydrochloric acid, cooled at 37° C and neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.5ml and 1 ml aliquots were taken for analysis. Standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard glucose and the 1ml of distilled water served as the blank. 4 ml of anthrone reagent was added in all tubes and heated for 8 minutes in a boiling water bath. Then cooled rapidly and the readings were taken at 630 nm. The carbohydrate content of the fruits was calculated by comparing with the standard curve (16).

- **Estimation of Protein:** 5 g of powdered fruit samples were extracted 3 times with 50 ml of water by overnight cold percolation method. To 0.5 ml of sample, blank and standard taken in duplicate, 0.5ml of alkaline copper reagent was added, mixed and allowed to stand undisturbed for 10 minutes. Then 2 ml of phenol reagent was added to each tube; mixed immediately and placed at room temperature for 5 minutes and absorbance of samples and standard were taken at 615 nm against blank. The protein content of fruits was calculated by comparing with the standard curve (17).

F) Phytochemical Analysis

The tests were done to find the presence of the active chemical Constituents such as alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins by the following Procedures.

- **Alkaloids:** Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (18). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent(19). The extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagents. The samples were then observed for the presence of turbidity or yellow precipitation.

- **Steroids:** 1ml of the plant extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids(20).

- **Terpenoids:** To 2ml of the plant extract was added to 2ml of acetic anhydride and concentrated H₂SO₄. The formation of blue green ring indicate the presence of terpenoids(21).

- **Tannins:** 2ml of extract was added to few drops of 1% lead acetate, and the yellowish precipitate indicated the presence of tannins(22).

- **Saponins:** 5ml of Extract was mixed with 20ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of Saponins(23).

- **Anthocyanins:** 2ml of extract is added to 2ml of 2 N Hcl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins (24).

- **Coumarins:** 3ml of 10% NaOH was added to 2ml of extract and the formation of yellow colour indicates the presence of coumarins(25).

- **Flavonoids:** 1 gm of the powdered sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot and few drops of 20% sodium hydroxide solution was added to 1ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

- **Cardiac glycosides:** 5 ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride Solution. Then 1ml of concentrated sulphuric acid was added. A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A Violet colour may appear below the ring while in the acetic acid layer, a green ring may be formed. (26,27,28).

- **Glycosides:** Glycosides are compounds which upon hydrolysis give rise to one or more sugars(glycones) and a compound which is not a sugar (aglycone or genine). To 1 ml of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid was added and observed for a reddish brown colour at the junction of 2 layers and the bluish green colour in the upper layer(18).

- **Phenols:** Crude extract was mixed with 2ml of 2% FeCl₃ solution. A blue green or black colour indicated the presence of phenols & tannins(26,29,33).
- **Quinones:** Dilute sodium hydroxide was added to the 1ml of crude extract and the blue green or red coloration indicates the presence of quinones.(26,31,29).
- **Betacyanin:** About 2ml of extract was added with 1ml of 2N NaOH and heated for 5min. The Formation of bluish green colour indicates the presence of antho cyanin and Formation of yellow colour indicates the presence of beta cyanins(31).

3. Results & Discussion

The table 1 shows the proximate analysis of *Terminalia catappa* leaf. The amount of total protein and carbohydrates was found to be 86.7±0.75 & 187.4±0.53 mg/g of sample. The percentage of crude fiber, ash and moisture content in the leaf sample was 20.47±0.59, 20.2±0.48 and 7.18±0.18.

The qualitative phytochemical analysis results reveals the presence of alkaloids, flavonoids, Saponins, tannins, terpenoids, steroids, glycosides (Table-2) in the mature leaf of *Terminalia catappa* L. The hydroalcohol extract of the plant contains most of the phytochemicals, with ethanol extract in the second position with more number of phytochemicals but other solvent extracts contain the far number of the phytochemicals.

There is an urge in research on new drugs from natural sources. Therefore, now there is a need to look back towards the traditional medicine which can serve as novel therapeutics. The pharmacological value of secondary metabolites from the plants is increasing as these can act as lead chemicals for new drug development. Plant synthesize

many compounds with complex molecular structures, as a result of secondary metabolism. Some of the compounds and their derivatives such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic compounds have antimicrobial properties (32). Phytochemical analysis agree with the findings of other authors(33,34,35): Yakubu et al., reported that the presence of saponins, phenolics and glycosides may be responsible for the acclaimed anti-anaemic potential of plants used in traditional medicine. Singh *et al.*, explained that the Saponins especially are known to enhance natural resistance and recuperative powers of the body. Babayi et al., reported that the occurrence of tannins shows that the plants may be useful in various ways for example tannins are useful in food and pharmaceutical industries.

4. Conclusion

The Present study evaluated the presence of various secondary metabolites in the hydro alcohol extract of *Terminalia catappa* leaves, which may be responsible for the antioxidant efficacy and medicinal properties of the plants.

Table 1: Shows the Proximate analysis of *Terminalia catappa* leaves

S.no	Experimental studies	Quantity
1.	Total protein(mg/g)	86.7±0.75
2.	Carbohydrates(mg/g)	187.4±0.53
3.	Crude fiber(%)	20.47±0.59
4.	Ash(%)	20.2±0.48
5.	Moisture(%)	7.18±0.18

The values are expressed as mean± SD of three samples(n=3).

Table 2: Shows the qualitative analysis of different extracts of *Terminalia catappa* L.

S.No	Phytochemicals	Aqueous extract	Ethyl acetate extract	Ethanol extract	Hydro alcohol extract (1:1)	Methanol extract	Chloroform Extract	Petroleum ether
1.	Tannins	++	+	++	++	++	-	-
2.	Saponins	-	-	-	++	-	-	-
3.	Flavonoids	+	+	++	++	++	+	-
4.	Quinones	++	-	+++	+++	+	-	-
5.	Betacyanins	++	-	++	++	+	+	-
6.	Anthocyanins	-	-	-	-	-	-	-
7.	Steroids	++	-	+++	++	+	-	+
8.	Alkaloids	-	+	+	+	+	+	-
9.	Glycosides	-	-	+	+	+	-	-
10.	Terpenoids	+	-	++	++	+	-	+
11.	Cardiacglycosides	-	-	-	-	-	-	-
12.	Phenols	+	+	++	++	+	-	+
13.	Coumarins	++	-	++	+	-	-	-

Where as:(+++-Strongly present,++-Mildly present,+ Present and - Absence)

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