

PHYTONUTRIENT ANALYSIS IN *TERMINALIA CATAPPA* FRUIT FLESH, NUT, SHELL

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ABSTRACT

Terminalia catappa fruit is rich in phytochemicals and nutrients which contribute to the therapeutic potential. Fruit as a whole is very tasty but the curiosity in analyzing the flesh and seed separately made an attempt to do this work. The present study was carried out for the presence of phytochemicals, phytonutrients, behavior with different chemicals, fluorescence as well as percent yield. Phytochemicals such as carbohydrate, alkaloids, glycosides, saponin, flavonoid, tannin and phenolic compounds, Protein and amino acids whereas, yellow and green fluorescence was observed with nut, flesh. The percent yield observed was 12.912, 09.086, 08.122. The carbohydrate content calculated was high with flesh 94.66 ± 9.23 mg/g and protein was higher in 13.95 ± 0.30 mg/g and nut contains moderate amount of amino acids. From the result it is concluded that *Terminalia catappa* fruit flesh, nut might be a potential source for the generation of herbal drug.

Key words: Nutrients, Percent, Secondary metabolites, *Terminalia catappa*, Yield.

INTRODUCTION

Terminalia catappa is a large tree able to tolerate strong winds, salt spray and salinity in the root zone. It could grow up in a freely drained, aerated, sandy soil. 3yr old tree produces nutritious, tasty fruits and the seed kernels may be eaten immediately after extraction. In India, it is used as cardiac stimulant. Nutritional value, biological activity of the kernel of *Terminalia catappa* exhibit very good digestibility, antioxidant activity, anti-HIV, anti-asthma, anti-inflammatory, anti-carcinogenic, antibacterial and hepatoprotective properties.¹⁻⁸ Daily consumption of fruits and vegetable supplies macronutrients, micro nutrients, non nutrient compounds that have protective role against human diseases. The vegetable and fruit peels normally not used and considered as waste but different phytochemicals present in them makes it an important alternative source of natural antioxidants. Hence, it was planned to compare the phytochemicals, nutrients, secondary metabolites in fruit flesh, nut, shell.

MATERIALS AND METHODS

Sample collection

The samples *Terminalia catappa* fruits were collected from Navodaya academy senior secondary school located at Namakkal, Namakkal District, Tamil Nadu, India, during the month of November- December, 2013. The collected fruits

were cleaned thoroughly and its fruit flesh, were peeled off and the nuts were taken out with the help of nut cracker, the shell was also collected after taking the nut from it. The fruit flesh, nuts, shells were allowed to dry under shade. Once the drying process is complete, the dried fruit flesh, nuts, shells were ground to powder using blender for further use. The plant was identified by Dr. B. Sankar, Head and Assistant Professor, Department of Botany, Poompuhar College, Tamil Nadu, India.

Aqueous extract preparation

Aqueous extract was prepared by dissolving 15g of powdered Terminalia catappa fruit flesh, nuts, shells were dissolved in 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage yield, and the remaining marc left was extracted with water and used for Qualitative analysis.

Phytochemical analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures^{9,10} fluorescence analysis,¹¹ behaviour of drugs powder with different chemical reagents.¹²

Test for carbohydrate

Molisch's test: To the extract added few drops of alcoholic alpha naphthol solution, few drops of concentrated sulphuric acid along the sides of test tube. Positive result gives purple or violet colored ring at the junction.

Fehling's test: To the extract added equal amount of Fehling's A and B solution and then the tubes were kept in a boiling water bath. Brick red precipitation of cuprous oxide formation confirms the presence of reducing sugar.

Benedict's test: To the extract added few drop of Benedict's reagent and the contents were heated in a boiling water bath. Red precipitation indicated positive result.

Test for alkaloids

Wagner's test: To the extract added few drops of iodine solution in potassium iodide. Reddish brown precipitate signified positive result.

Hager's test: To the extract added few drops of saturated solution of picric acid. Positive result was indicated by yellow color precipitation.

Test for steroids and sterols

Libermann-Burchard test: To the extract added 2ml chloroform followed by 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. Positive result is indicated by bluish red to cherry red color ring at chloroform layer.

Salvoski test: To the extract added few drops of chloroform followed by concentrated sulphuric acid. Bluish red to cherry red color change showed positive result.

Test for Glycoside

Legal test: To the extract added pyridine, sodium nitroprusside. Positive result showed pink red color.

Baljet test: To the extract added picric acid. Appearance of orange color was confirmed as a positive result.

Test for saponin

Foaming test: Foam produced when the extract was mixed with water.

Test for flavonoid

Shinoda test: To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

Zinc hydrochloride test: To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicated positive result.

Test for tannin and phenolic compound

Ferric chloride test: To the extract added ferric chloride. Formation of greenish black color showed positive result.

Potassium dichromate test: To the extract added potassium dichromate solution. Positive result was confirmed by brown precipitate formation.

Gelatin test: To the extract added 1% gelatin solution containing 10% sodium chloride. White precipitate signified positive result.

Test for protein and amino acid

Biuret test: To the extract added 4% sodium hydroxide and few drops of 15% copper sulphate. Development of purple color signifies presence of protein, amino acid.

Ninhydrin test: Bluish violet color formation confirmed positive result, when a solution of ninhydrin was heated with a test solution.

Heat test: Protein coagulation showed positive result when test solution was heated on a boiling water bath.

Test for fixed oil

Copper sulphate test: Blue color formation denoted positive result when the extract was mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Quantitative analysis of phytonutrients

Total carbohydrates,¹³ proteins,¹⁴ amino acids¹⁵ were performed according to standard prescribed methods.

Estimation of carbohydrate

The total carbohydrate was estimated by Anthrone method. 1mg of *Soymida febrifuga* seed powder was hydrolyzed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N HCl and then cooled to room temperature. After neutralizing, the contents were centrifuged and 0.1 ml of supernatant was used for further analysis. To the extract added 4ml of anthrone reagent and the contents were heated in a boiling water bath for 8 minutes. The tubes were then cooled and read at 630nm using spectrophotometer shimadzu Model - UV 1800. Standards were developed with glucose. Standard graph plotted was used to find out the concentration of glucose present in unknown sample.

Estimation of protein

Total protein content was estimated by Lowry's method. To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of Folin Ciocalteau reagent (diluted in the ratio of 1:2) was added and incubated for 30minutes. The blue color developed was read at 660nm using spectrophotometer shimadzu - model UV 1800. Standards were developed with bovine serum albumin and standard graph was plotted and the concentration of protein present in unknown sample was calculated.

Estimation of amino acids

The amino acid present was estimated by Ninhydrin method. To 0.1 ml of sample added 1ml of ninhydrin solution dissolved in ethanol. The test tubes were covered with a piece of paraffin film to avoid loss of solvent due to evaporation. With gentle stirring, allow the contents to react at 80-100°C for 4-7 minutes. Cool the test tubes and the color developed was read at 570nm. Tyrosine was used for developing standards. From the standard graph obtained the amino acid content in the extract was calculated.

STATISTICAL TOOL

Each experiments were 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 percentage recovery of the aqueous extract obtained was calculated, expressed in Table 1.

Table.1 Percentage yield of Aqueous extract of Terminalia catappa fruit flesh, nuts, shells

S.No	Name of the seed powder	Weight taken for extraction	weight of the beaker (gm)		Weight of extract (gm)	% recovery
			Initial	Final		
1.	Fruit flesh	15g in 200ml	147.9810	149.9178	1.9368	12.912
2.	Nut	15g in 200ml	179.9658	181.3287	1.3629	09.086
3.	Shell	15g in 200ml	179.9266	181.1450	1.2184	08.122

Table.1 Depicts the results of percent yield The final weight of the dry extract was found to be 1.9368,1.3629,1.2184gm for fruit flesh, nut, shell and their respective percent recovery was found to be 12.912, 9.086,8.122.

S.No	Tests	Observation			Inference		
		<i>Terminalia catappa</i>			<i>Terminalia-catappa</i>		
		Fruit flesh	Nut	Shell	Fruit flesh	Nut	Shell
1.	Powder+ Picric acid	Yellow color	Yellow color	-	Presence of alkaloid	Presence of alkaloid	Absence of alkaloid

2.	Powder+ Conc. H ₂ SO ₄	Reddish brown color	Reddish brown color	-	Presence of steroids	Presence of steroids	Absence of steroid
3.	Powder+ Aq. FeCl ₃	Green color	Green color	Brown color	Presence of flavonoids	Presence of flavonoids	Absence of flavonoids
4.	Powder+ Aq.5% KOH	No yellow color	No yellow color	-	Presence of anthroquin-one	Presence of anthroquin-one	Absence of anthroquin-one
5.	Powder + NaOH	Yellow color	Brown color	Brown color	Presence of flavono -ids	Presence of flavonoids	Absence of flavonoids
6.	Powder+ Aq. AgNO ₃	White precipitate	White precipitate	-	Presence of protein	Presence of protein	Absence of protein

Analysis of powders for its behavior

Table.2 Terminalia catappa fruit flesh, nuts, shells powder behaviour with different chemicals

The results of behavior of the samples studied were shown in Table.2. Only fruit flesh, nut showed positive results for alkaloid, flavonoid, protein, anthroquinone. Antioxidant activities were reported by Krishnaveni et.al for Terminalia catappa fruit flesh¹⁶ and nut¹⁷.

FLUORESCENCE ANALYSIS

Table.3 Fluorescence analysis of aqueous extract of Terminalia catappa fruit flesh, nuts, shell

S.No	Name of <i>Terminalia catappa</i> aqueous extract used	Day light	UV light
1.	Fruit flesh	Pale brown	Green fluorescence
2.	Nut	Milky white	Yellow fluorescence
3.	Shell	Half white	Yellow fluorescence

Fluorescence analysis

The results of fluorescence analysis were shown in Table.3. The aqueous extract of fruit flesh, nut, shell was observed under day light for its color, it exhibited pale brown, milky white, half white color, when the same was observed under UV light it showed green and yellow fluorescence.

Phytochemical analysis

The results of phytochemical analysis were expressed in Table.4. All the phytochemicals tested were found to be positive with fruit flesh and nut while shell contain very traces of phytochemicals except steroid and protein. The presence of tannin in the shell might be due to the attachment of flesh to shell.

Table.2 Phytochemicals in aqueous extract of *Terminalia catappa* fruit flesh, nuts, shell

S.No	Name of the Qualitative test	<i>Terminalia catappa</i>		
		Fruit flesh	Nut	Shell
1.	Carbohydrate	+++	+++	+
2.	Alkaloids	+++	+++	+
3.	Steroids and sterol	++	-	-
4.	Glycosides	+++	+++	+
5.	Saponins	++	++	+
6.	Flavonoids	++	-	-
7.	Tannin and phenolic compounds	++	++	+
8.	Protein and amino acids	++	++	-
9.	Fixed oil	-	++	-

+++Stronger reactions, ++ Moderate, +Slight changes, - Absence

PHYTONUTRIENT ANALYSIS

The phytonutrients estimated were tabulated in Table.5.

Table.5 Phytonutrients in aqueous extract of *Terminalia catappa* fruit flesh,Nut,Shell

S.No	Phytonutrients	Nutrient content (mg/g)
1.	<i>Terminalia catappa</i> fruit flesh	
	Total carbohydrate	94.66±9.23
	Total protein	02.04±0.15
2.	<i>Terminalia catappa</i> Nut	
	Total carbohydrate	44.66±2.30
	Total protein	13.95±0.30
3.	<i>Terminalia catappa</i> Shell	
	Total carbohydrate	16.66±1.15

Total protein	00.44±0.15
Amino acids	00.39± 0.11

Values are Mean ± SD for three experiments

Table.5 shows the results of phytonutrients assessed. Among the nutrients studied, Carbohydrate was higher in fruit flesh when compared to nut, shell. Likewise, protein was rich in nut and amino acid level was high on comparison with fruit flesh, shell.

CONCLUSION

Plants naturally able to synthesize secondary metabolites according to the climatic condition they grow and also various other environmental factors. Hence, studying parts of the plant from a particular place is a must to ensure its specific properties. In the present study, stronger reactions were observed for alkaloid, flavonoid, protein, phytosteroid with fruit flesh, nut. Higher carbohydrate content was higher with flesh. Among the *Terminalia catappa* fruit flesh, nut, shell studied, except shell the other two showed significant phytochemicals, nutrients. Hence, this nutritious fruit, nut containing secondary metabolites must be used for herbal drug preparation after clinical trials.

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