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Phytochemical analysis of the leaf extract of *Terminalia catappa* L.

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ABSTRACT

The present study involves extraction and phytochemical investigation of *Terminalia catappa L*. belonging to the family Combretaceae, for its medicinal value. A qualitative phytochemical analysis of four solvent extract methanol, ethanol, petroleum ether and acetone was performed for the presence of carbohydrate, protein, starch, steroid, glycosides, alkaloids, saponins and amino acids. Observation revealed the presence of maximum biological compounds in methanol extract. Therefore, leaf extract of this plant serve as a good source of useful drugs.

Key words: Terminalia catappa, phytochemical analysis, medicinal plants, bioactive compounds.

T*erminalia catappa* (tropical almond) is a large spreading tree belonging to family Combretaceae. It has a vast natural distribution in near coastal areas of Indian ocean through tropical Asia into the pacific ocean. It is commonly used as shade tree in Srilanka & India. *Terminalia catappa* is easily propogated from seeds and is fast growing and flourish with minimum maintenance in suitable environment. The leaves are arranged in close spirals, often crowded towards the ends of the inturned branchlets. The leaf blade is simple, broadly, obovate with pair of secondary veins. They have small petiole, 15-28 cm long, 10.5-16.5 cm broad, glabrous, shining above and tomentose below with two glandular depression near the base of the mid rib on the underside. Leaves turn pink – red yellow before falling. Pigments responsible for the change of the colour of the leaves include violaxanthin, cutein & Zeaxanthin. Flowers are regular, polygamous in simple solitary, axillary, rusty, pilose spike , 16-18 cm long, upper flower male and lower flower bisexual.

Medicinal plants contain some organic compounds which provide definite physiological action on human body and their bioactive substances include tannins, alkaloids, carbohydrates, steroids and flavonoids.[6,11,24,25] Secondary metabolites are chemically and taxonomically extremely diverse compounds

with obscure functions. They are widely used in the human therapy, Veterinary, agriculture and Scientific research.[23]

The leaves of this plant were widely used as medicine in southeast Asia for dermatosis and hepatitis. A lot of pharmacological studies have reported that the extract of leaves & fruits have anticancer, antioxidant, anti HIV, anti inflammatory, anti diabetic and hepatoprotective activities. This plant was popularly known as Deshi-badam in Ayurvedic medicine. Juice of young leaves is employed externally as ointment for leprosy and scabies, internally for colic and headache. The seeds also have aphrodisiac activity.[8,19,22]

Knowledge of the chemical constituent of plant is desirable for the discovery of therapeutic agents and to find the actual value of folklore remedies. Hence, this work was carried out to find out the phytochemical constituents of the leaf.

The leaves from the tree were collected in August-2012 from the college campus. The plant material was taxonomically identified by Botanical Survey of India, Allahabad, U.P. and authenticated by Prof. S.S.Khan of Bhopal.

The leaves of *Terminalia catappa* were shade dried and subjected to soxhalation. Solvent used for extraction where ethanol, methanol, petroleum ether and acetone. The extracts were kept in hot air oven at 30-40°C. Dried crude extract was kept in refrigerator at 4°C for future use in phytochemical analysis.

A. Qualitative phytochemical analysis.

The phytochemical tests were carried out using different solvent extracts using standard procedures to identify the consitutents as described by Harbone J.U. (1973).

Test For Carbohydrate

1. **Molish test:-** To 2.3 ml aqueous extract, added few drops of α napthol solution in alcohol. Shake and add conc. H_2SO_4 from sides of the test tube. Violet ring formed at the junction of two liquids shows the presence of carbohydrate in the extract.

2. **Test for non- reducing polysaccharides (Starch)**
   - i. ***Iodine test :*** Mixed 3 ml test solution and few drop of dilute iodine solution. Blue colour appears.
   - ii. **Tannic acid test for Starch :** Add 20% tannic acid in the test solution. Precipitate appears.

Test for Protein

- i. **Biuret test:** To 3 ml test solution added 4% NaOH and few drop of 1% $CuSO_4$ solution violet colour appears.
- ii. **Millon's test:** Mixed 2 ml. test solution and Millon's reagent. White precipitate appears.

Test for Amino Acid

- i. **Test for cysteine –** To 5 ml. test solution added few drop of 40% NaOH and 10% lead acetate and solution was boiled. Black ppt of lead sulphate was formed.

Test for Steroid

- i. **Salkowski reactions –** To 2 ml. of extract added 2 ml. chloroform and 2 ml conc.

H_2SO_4 and shaked well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Test for glycosides

i. Test for deoxysugar (Keller Killiani test) To 2ml extract added glacial acetic acid, one drop of 5 % $FeCl_3$ and conc. H_2SO_4. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green.

Test for Flavonoids :

i. To small quantity of residue add lead acetate solution (0.1%). Yellow coloured ppt. is formed.
ii. Addition of increasing amount of sodium hydroxide to the residue shows decoloration.

Test for alkaloids :

i. Mayer's test:- 2.3 ml. filtrate with few drops Mayer's reagent precipitate appears.

ii. Wagner's test:- 2-3 ml. filtrate with few drops Wagner's reagent reddish brown precipitate appears.

Test for tannic and phenolic compounds:-

To 2-3 ml. of aqueous or alcoholic extract, added few drops of following reagent.

a. 5% $FeCl_3$ Solution---deep blue black colour.
b. Lead acetate solution --- White ppt.
c. Dilute Iodine solution --- red colour.

Test for Saponin:- To 2 ml extract add 5 ml. distilled water and heat to boil. Frothing appears shows the presence of Saponin.

Behavior of the Leaf powder of *Terminalia catappa* with different chemical reagents is shown in table-1.

Table 1. Showing Preliminary phytochemical analysis and various colour changes when treated with different chemical reagents

S.No.	Reagent	Colour of the powder
1.	Powder as such	Light Brown
2.	Powder + 2% $FeCl_3$	Greenish black
3.	Powder+ 10% NaOH	Yellowish Brown
4	Powder+5% KOH	Yellowish Brown
5.	Powder+ Water	Light Brown
6	Powder+Iodine	Yellow
6	Powder+NaOH + H_2O	Yellow
7	Powder+C_2H_5OH	Green
8	Powder+HNO_3	Orange red
9.	Powder+H_2SO_4	Yellowish Red

Table 2. Phytochemical charecteristics of leaf extracts of *Terminallia catappa* in four different solvents tested.

TESTS	METHANOL	ETHANOL	PET. ETHER	ACETONE
I. Carbohydrates				
a. Molish Test	-	-	-	-
II. Protein				
a. Biuret Test	+	-	-	-
b. Millon's Test	+	-	-	-
III. Steroid	+	+	-	-
IV. Glycoside	+	+	-	-
V. Tannic & Phenolic				
a. 5% ferric chloride	+	+	-	+
b. Lead acetate	+	+	-	+
c. Iodine solution	+	-	-	+
VI. Flavonoid	+	+	-	+
VII. Alkaloid				
a. Mayer's Test	-	-	-	+
b. Wagner's Test	+	-	-	+
VIII. Saponin	+	+	-	+
IX. Amino Acid	+	-	-	+
X. Starch	-	-	-	-

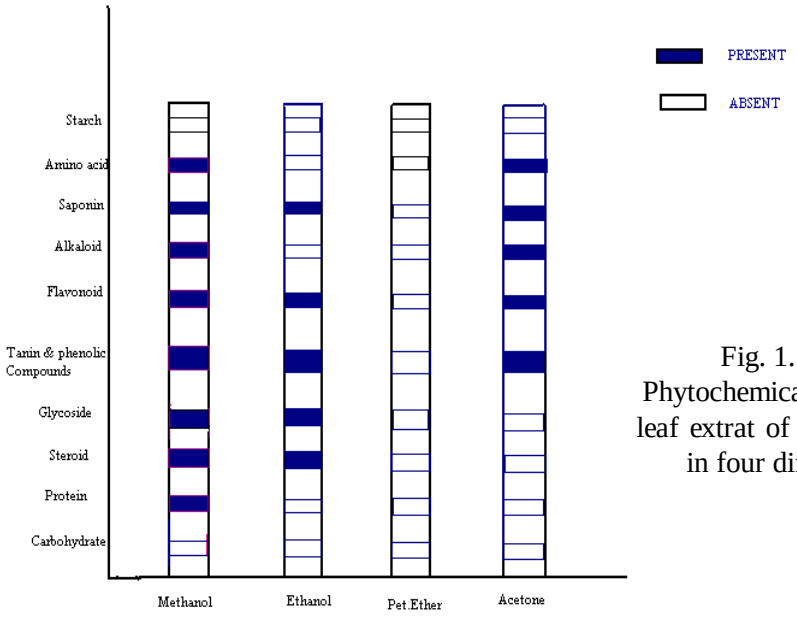

Fig. 1. Summary of Phytochemical characteristics of leaf extrat of *Terminalia catappa* in four different solvents.

Phytochemical analysis of all the four solvent extracts were screened for the presence of various, bioactive phytochemical compounds. The analysis revealed the presence of proteins, steroids, glycosides, tannin and phenolic compounds, flavonoids, saponin ,amino acids in methanol extracts. Alkaloids are present in less amount. Starch and carbohydrates were absent.

In ethanol extract, steroids, glycosides, flavonoid and saponin were found present. Tannin and phenolic compound were present in small amount. Carbohydrates, amino acids, protein, starch and alkaloids were absent.

In petroleum ether extract only saponin were found to be present.

In acetone extract tannin and phenolic compounds, flavonoid, alkaloids and saponin were present. Carbohydrates, protein, glycosides, amino acid and starch were absent.

Phytochemical analysis conducted on the leaf extract revealed the presence of compounds which are known to exhibit medicinal as well as physiological activities[21] such as phenols, tannins, flavonoids, saponins, glycosides, steroids and alkaloids.

The phenolic compounds possess biological properties such as antiaging, anticarcinogenic, antiinflammatory, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities[7]. Several studies have described the antitoxidant properties of medicinal plants which are rich in phenolic compounds.[4,10] Natural antitoxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, etc.[1] Tannins bind to proline rich protein and interfere with protein synthesis. Flavanoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microo-rganisms *in vitro*. Their activities is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.[12] They are also effective antioxidant and show strong anticancer activities.[5,17,20] The leaf extract also revealed to contain saponins which are known to produce inhibitory effect on inflammation.[9] Saponins have the property of precipitating and coagulating red blood cells. Some of the characterstics of saponins include formation of foams in aqueous solution, haemolytic activity and cholesterol binding properties[16]. Steroids have been reported to have antibacterial properties[18] and they are very important compounds especially due to their relationship with compounds such as sex harmones.[15] Alkaloids have been associated with medicinal uses for centuries and one of its common biological properties is their cytotoxicity.[13] Several workers have reported the analgesic,[2] antioxidant,[3] antispasmodic and antibacterial properties[16] of alkaloids. Glycosides are known to lower the blood pressure according to many reports.[14] The result obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive compounds. Therefore this plant is proving to be an increa-singly valuable reservoir of bioactive compounds and substantial medicinal merits. The phyto-chemical analysis of leaf extract shows positive results which render the presence of bioactive compounds in it. Based on the above findings it may be concluded that methanol served as the best solvent for extraction of bioactive compounds. Several studies have confirmed the presence

of the phytochemical content and medicinal as well as physiological properties in the plant for the treatment of different ailments. Therefore leaf extract of this plant could be seen as a good source of useful drugs. It is suggested that further work should be carried out to locate, purify and characterise the active compounds responsible for the medicinal value of this plant.

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