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Navya Gurram
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India

Manish Kamble
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India

Mahendra Gunde
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India

Ashwini Ingole
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India

Disha Dhabarde
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India

Dr. Jagdish Baheti
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India

Correspondence:
Navya Gurram
Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra-441108, India
Email: navya.gurram15[at]gmail.com

Pharmacognostic, Physicochemical investigation and evaluation of anti-oxidant potential of fruit flesh extract of *Terminalia catappa*. Linn.

Navya Gurram*, Manish Kamble, Mahendra Gunde, Ashwini Ingole, Disha Dhabarde, Jagdish Baheti

ABSTRACT

Terminalia catappa Linn. also known as Indian almond or tropical almond is a native throughout the tropical Asia belongs to family Combretaceae. Traditionally, the parts of this plant of are used for the treatment of dermatitis, hepatitis, diarrhea and pyresis, caribbeans used the decoction for gastritis and urinary tract infection. The present study aimed to evaluate the pharmacognostic and phytochemical properties along with the antioxidant activity of *Teminalia catappa* Linn. The fresh fruits of *Teminalia catappa* were collected and preliminary microscopic screening was done by evaluating the transverse section. The phytochemical screening of the fruit revealed the presence of flavonoids, tannins, saponins, and alkaloids. The phycochemical analysis also showed the total ash content 7.913% and was slightly higher than acid insoluble and water soluble ash. The fruit flesh was subjected to hydroalcoholic extraction to obtain a semisolid extract. The antioxidant activity was carried out by using DPPH radical scavenging assay spectrophotometrically at 517nm. The various concentration of fruit flesh extract (62.5, 125, 250, 500, 1000 ug/ml) were used and increasing antioxidant activity was observed with increasing concentration. About 41% scavenging activity for DPPH radical was observed at 1000 ug/ml.

Thus, *Terminalia catappa* was found to be a rich folk medicine with prominent radical scavenging activity and can be a good future herbal antioxidant.

Keywords: DPPH radical scavenging assay, Anti-oxidant, Pharmacognostic properties.

INTRODUCTION

The secondary metabolite with medicinal potential have been identified and used as medicinal components from ancient or traditional medicinal system to current advanced herbal medicinal system. Plants have been used for treating various acute or chronic ailments and diseases. The part of the plants to be used is depends upon the type of plants, and generally one part includes (flowers, leaves, stem, and roots) or whole sector (Ariel parts of roots). *Termianalia catappa* Linn. (Combretaceae) is also called as tropical almond is found in warmer parts of India. The tree is often characterized by horizontal branches that are divided repeatedly into tiered (3-6 feet apart) whorls generally in single erect trunk. Usually the height of tree found to be 50-80 feet. Leaves are alternate and crowned together near the end of the twigs. Petioles are short and stout. Leaf blades are thick and big, with smother margins. New leaves have soft covering of hairs while matured have shiny above and pubescent below. They are leathery and dark green. They turn shades of bright yellow, red and purple before falling in the winter. Flowers are arranged on long slender racemes up to 6 inches long, creamed colored, five lobed. Normally they appear in early summer and fruits follow quite late in the year. Plants usually commence flowering and fruiting within 2 to 3 years after transplanting, but this can vary with site and genotype. Fruits are drupes about the size and shape of an almond fruit but with a slight wing. They are 2 inches or more long and 1 inch across. Full-sized fruits are at first green and turn red, brown, or yellow at maturity. When young, the fleshy fibrous pulp surrounding the large seed is edible and sweet and slightly sour thereafter.

The leaves, bark, frits have been used in traditional medicine for various purpose. *Terminalia catappa* have ability to reduce blood glucose level, also used as nutritional supplements in diabetes treatment as source of Vit. C and E, dilatory minerals [1].

Traditionally, the parts of this plant of are used for the treatment of dermatitis, hepatitis, diarrhea and

pyresis, caribbeans used the decoction for gastritis and urinary tract infection. Polar extract from different parts (leaves, fruit, and bark) of *Terminalia catappa* shown antimicrobial, antifungal, antioxidant, anti-metastatic, anti-inflammatory, mutagenic, aphrodisiac and antidiabetic [2]. This work therefore evaluates the phytochemical screening and anti-oxidant potential of *Terminalia catappa* usually have active ingredients which are used to cure ailments or diseases. The ingredients may be in the form of secondary metabolites like alkaloids, saponins, steroids glycosides flavonoids phenols etc.

The reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen are capable of damaging DNA and Proteins at cellular level. Free radicals contribute more than one hundred disorders in human beings including atherosclerosis, hypertension, arthritis, ischemia, gastritis, central nervous system injury, reperfusion injury of many tissues, cancer, Alzheimer's disease, parkinsonism, diabetes mellitus and AIDS [3,4]. These adverse effects of ROS are controlled by a system of enzymic and non-enzymic antioxidants in biological system. These antioxidants eliminate pro-oxidants and scavenge free radicals [5]. There are considerable evidences that antioxidants could prevent these diseases because they have the capacity to quench free radicals [6]. Apart from endogenous source, external antioxidants have also been reported to prevent the oxidative damage caused by the ROS and protect from oxidative-stress mediated diseases. Although some synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), exhibit potent free radical scavenging effects, they have been demonstrated to exert toxicological effects as compared to natural antioxidants [7,8]. Hence, there is a great emerging interest on natural source of antioxidants.

MATERIALS AND METHODS

Collection of Plant Materials

The fruits of *Terminalia cataapa* were collected from college campus of Kamla Nehru College of Pharmacy, Butibori, Nagpur Maharastra. Plant material was identified and authenticated in the department of Botany, RTM Nagpur University Nagpur, Maharashtra. The collected materials were cleaned and flesh was removed for further processes of extraction.

Preparation of the Extract

Fruit Flesh was taken (100 g) and subjected to hydroalcoholic extraction (Soxhlet Extraction) Then, it was filtered and the filtrate was allowed to evaporate under vacuums evaporator until semi solid consistency is obtained. Then the aqueous extract was then re-dissolved in water at 1 mg/ml ratio and used for evaluating the study.

RESULTS AND DISCUSSION

Fruit microscopic characters

The transverse section of fruit (Figure 1) shows single layered epidermis. Epidermal cells are rectangular in shape and distinct. Beneath the epidermal cells on both the sides the layers of collenchymatous cells is wider towards the ventral side which is 3-4 layers. Followed by collenchyma cells is the 6-7 layers of parenchymatous cells with angular thickening. Some of the parenchyma cells contain dark reddish brown matter in cells and rosette of calcium oxalate crystal next to parenchymatous layer is 1-2 layer thick walled sclerenchyma cells and groups of stone cells scattered in between. Air cavity is present in parenchymatous cells. Few thick wall cells are also scattered.

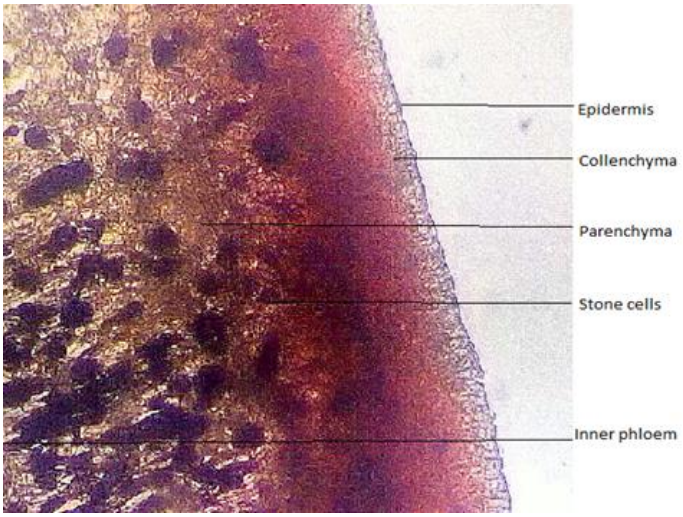

Figure 1: Transverse section of ripped fruit flesh of *Terminalia catappa*

Phytochemical screening

The phytochemical screening of fruit flesh extract of *Terminalia catappa* was found to contain several flavoinoids, tannins, saponons, and alkaloids. Due to richness in chemical entities, the *Terminalia catappa* is used in different traditional medicine for various purposes.

Table 1: Preliminary Phytochemical Screening of fruit flesh extract of *Terminalia catappa* Linn

Phytoconstituents	Test reagent	inference
Alkaloids	Dragandroff's	+
	Hager's	++
	Wagner's	++
Flavonoids	Shinoda	+++
Tannins	Ferric chloride test	+++
	Lead Acetate test	+++
	Potassium dichromate test	+
Steroids	Salkowaski test	++
Saponins	Foam test	+++

Physicochemical Parameters

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 1) of the powdered *Terminalia catappa* Linn. Fruit flesh. The total ash value was higher than that of the acid insoluble and water soluble ash value and a decrease in the acid insoluble ash value may be due to presence of smaller quantity of siliceous matters. The extractive values are primarily useful for the determination of exhausted or adulterated drug.

Table 2: The average percentage of physicochemical analysis of fruit flesh of *Terminalia catappa* Linn

Parameters	Average percentage (% w/w)
Total Ash	7.913
Acid insoluble Ash	1.047
Water soluble Ash	0.38
Alcohol Soluble Extractive	23.66
Water Soluble Extractive	30.14

Antioxidant Activity

DPPH Radical Scavenging Activity

Antioxidant activity can be measured using DPPH radical scavenging assay [9]. This test provides information on the ability of compounds present in the extract to donate a hydrogen atom which results in antioxidant ability. Antioxidants react with DPPH, a stable free radical and reduce it to DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compound. The extracts were dissolved in ethanol and 5% DMSO and various concentrations (62.5, 125, 250, 500 and 1000 µg/ml) of the extracts were used for the study. BHT (3.6 mg/ml) was used as a positive control. Assay mixture contained 500 µl of the extract, 125 µl of DPPH (100 mM) and 375 µl solvent (5% DMSO). This mixture was incubated for 30 min at 25^0 C in dark condition. The decrease in absorbance was measured at 517 nm spectrophotometrically and the percentage of radical scavenging activity was calculated using the formula (Abs. control - Abs. extract) / Abs. control x 100.

In the present study, the radical scavenging activity *Terminalia catappa* extracts was tested using methanolic solution of a stable free radical, DPPH (2, 2-diphenyl-1-picrylhydrazyl). Unlike laboratory generated free radical such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple color, which get fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecule can quench DPPH free radicals. (Conceivably by providing hydrogen atom or by electron transfer.)

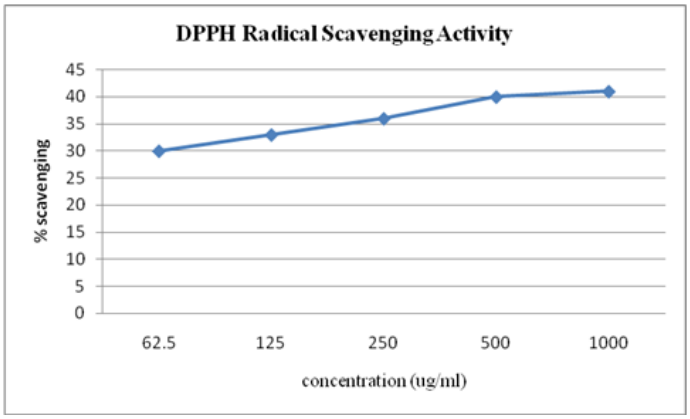

Figure 2: DPPH radical scavenging activity of *Terminalia catappa* fruit flesh extract.

CONCLUSION

Pharmacognostical studies can serve as a basis for proper identification, collection and investigation of the plant. The present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations. These parameters, which are being reported, could be useful in the preparation of the herbal monograph for its proper identification. The study deals with antioxidant potentials of *Terminalia catappa* fruits flesh. Results obtained from the present study provide scientific evidences for the use of this plant in folk medicine. Further, the present study suggests that parts of *Terminalia catappa* could serve as a lead in the development of a novel herbal antioxidant agent.

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