

**ANTIOXIDANT ACTIVITY OF DIFFERENT VARIETIES OF *TERMINALIA CATAPPA***Farzana Tasneem M. I.*¹ and Narsegowda P. N.²Research Scholar Tumkur University, Tumkur-572101 Karnataka India Associate Professor, Dept of Biotechnology, V
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ABSTRACT

Terminalia catappa, an ornamental tropical tree belonging from the family Combretaceae which is native to Southeast Asia, commonly known as Tropical almond. The bark of the tree is used to cure fever and has diaphoretic, anti-indigestion, hepatoprotective, antiperoxidation, antisickling, anti-dysentery, antibacterial, antifungal, analgesic, anticolic, antihyperanalgesic and anti-inflammatory properties. The leaves are used in the preparation of ointments to cure for scabies, leprosy and cutaneous diseases. The two varieties of plants were collected from Bangalore. The methanol extract of leaf was evaluated for qualitative phytochemical analysis, which showed the presence of phenol, flavonoid, tannin, steroid and alkaloid. The secondary metabolites such as phenol and flavonoids were analyzed quantitatively. The radical scavenging potential of methanol leaf extract of 2 different varieties *Terminalia catappa* of showing remarkable effect with the IC₅₀ value ranged from 3.54-5.52µg/ml which was comparable with standard. The result suggest that the tree has potential for the excellent source to formulate the herbal medicine.

KEYWORDS: Antioxidant, *Terminalia catappa*, DPPH, Phenol, Flavonoid.**1. INTRODUCTION**

Nature has provided a source of medicinal agents for thousands of years and modern drugs are isolated from natural resources they are traditional medicines use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research Use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research.^[1] Phytochemistry play important role in treatment of different diseases and disorders are still used in both traditional and modern medicine.^[2] An Antioxidant are natural substances that prevent or delay damage, they protect the body from damage caused by harmful molecules called free radical ,oxidative stress can damage carbohydrates ,protein, lipids and DNA in cells and tissue which consequently leads to several degenerative diseases and plays important role in the development of chronic and degenerative ailments such as cancer ,arthritis ,aging, autoimmune disorders ,cardiovascular and neurodegenerative diseases, exogenous and endogenous antioxidants act as a free radical scavengers by preventing and repairing damages.^[3,4,5] Recent developments have shown that antioxidants from natural phytochemical products have ability to scavenge free radical or active oxygen.^[6,7] The increasing in the search for natural alternative of synthetic antioxidants has led to the evaluation of antioxidants in a number of plant sources.^[8]

Terminalia catappa is an ornamental tropical tree belonging to the family Combretaceae is native to Southeast Asia in the tropical regions of Asia, Africa, and Australia .It is known by the common names *Bengal almond*, *country almond*, *false kamani*, *Indian almond*, *Malabar almond*, *sea almond*, and *tropical almond*. It is a perennial tree reaching a height of between 15-25 m and about 9 m in width of its symmetrical canopy).^[9] It is large deciduous stately tree with a characteristic pagoda shape.^[10] It is cultivated as a shade tree and for its fruits and seeds which are eaten as a fruit as well as for medicinal uses.^[11] Two common varieties of *Terminalia catappa* (Indian almond)like yellow variety and red variety.^[12] The fruits which consist of the epicarp, fleshy mesocarp, stony mesocarp and kernel are ovoid in shape, laterally compressed with various sizes and colours at maturity.^[13] The fruit of *Terminalia catappa* is a rich source of allelicacid, gallicacid and many more unidentified flavonoid compound Juices from the leaves were used as an ointment for scabies ,leprosy and other skin diseases.^[14] The bark is excellent source of tannins.^[15] With the study conducted by various scientists have worked on the antioxidant properties of aqueous extracts from three different leaves of *Terminalia catappa* might be potential antioxidant supplement for application in food product or as a drink.^[16] An invitro Antioxidant activity of ethanol extract from *Terminalia catappa* leaves and fruit effect of fruit ripening showed that leaf extract showed highest

level of total phenol and flavonoid content ,DPPH free radical scavenging activity and Reducing power potential compared to unripe fruit this may be related to that increased vitamin C through Ripening process.^[17] Phytochemical analysis and invitro antioxidant activity of *Terminalia catappa* had strong potential attempt at the extract to isolate and bioactive phytoconstituents observed activities as mechanisms is highly active,^[18] Study recommended that the three varieties are good for human consumption like other fruit,^[19] nutritious nut containing secondary metabolites used as herbal drug preparation after clinical.^[20] Methanol extract of *Terminalia catappa* was selected for further study of synergistic activity with standard antibiotics.^[21] Medicinal uses of *Terminalia catappa* reported from various countries.^[22]

Therefore, the present study was undertaken to evaluate phenol and flavonoid content in the methanol extract of leaves and their correlation with antioxidant activity and their enriching bioactive compound as well as fruit .

2. MATERIALS AND METHOD

2.1 Plant material collection and Extract Preparation

The leaves of different varieties like yellow (T1, T2) and red (T3 ,T4) of *Terminalia catappa* were collected from Bangalore. The leaves were dried at room temperature crushed into fine powdered by mixer grinder and stored in polythene bag until use.

Solvent extraction: Twenty five grams of powdered sample were filled in the thimble and extracted successfully with methanol solvent in a Soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5° C in airtight bottle until further use. The crude extract of methanol leaf extracts of *Terminalia catappa* varieties was subjected to phytochemical screening tests(Qualitative analysis) for the detection of major secondary metabolites by using standard procedure, the qualitative results as expressed as (+) and (-) for the absence of phytochemical. The presence of alkaloids, tannins, flavonoids and proteins, carbohydrates, and phenols were performed according to the method described by.^[23,24]

Table 1: Qualitative analysis of leaf extract.

| Qualitative Test | Methanol | | | |
|------------------|----------|----|----|----|
| | T1 | T2 | T3 | T4 |
| Protein | + | + | + | + |
| Alkaloids | + | + | + | + |
| Saponins | ++ | ++ | ++ | ++ |
| Carbohydrates | + | + | ++ | + |
| Glycosides | + | + | ++ | + |
| Tannin | ++ | ++ | ++ | ++ |
| Flavonoid | ++ | ++ | ++ | ++ |
| Amino acid | + | + | ++ | + |
| Phenol | ++ | ++ | ++ | ++ |

Note: T1-yellow variety 1,T2-yellow variety 2,T3-Red variety 1,T4-Red variety 2

2.2 Quantification of Total phenols and Total Flavonoid

The total phenol content in methanol extracts of leaves of *Terminalia catappa* variety were determined according to the method employed by using Catechol as standard.^[25] one milliliter of plant extracts was measured with 1ml of FC reagent and 3ml of 20 % of Na₂CO₃ solution .the mixture was incubated for 40min at room temperature and absorbance was measured at 760nm .An alluminium chloride colorimetric method was used to determine the flavonoid content.

The plant extracts was mixed with 0.5ml of aluminium chloride (1.2%) and 0.5 ml of 120mM potassium acetate. The mixture was allowed to stand for 30 min at room temperature. The absorbance was measured at 415nm flavonoid content was expressed in terms of Rutin equivalent.

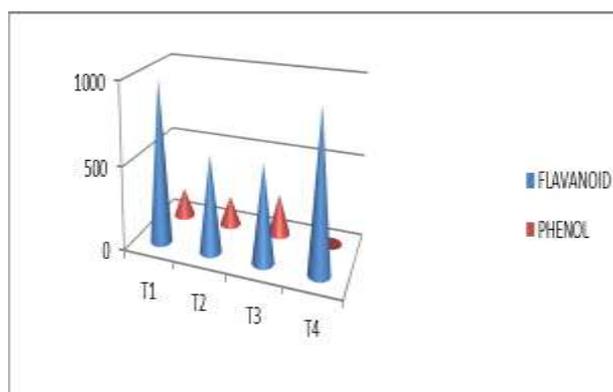


Figure 2: Quantification of Total phenols and Total Flavonoid.

3. Antioxidant Assay

3.1. DPPH radical scavenging assay

The free radical scavenging activity was measured by using DPPH radical (1,1 Diphenyl 1-2-picryl – Hydrazyl).^[26,27] The scavenging activity of the methanol leaf extracts of *Terminalia catappa* variety was estimated using a 2ml of DPPH solution in ethanol and acetone was mixed with increasing phenol concentration of extract. The ascorbic acid is a standard reference to antioxidant. The reaction mixture was incubated for 15 min and thereafter the optical density of 517nm against blank using UV-VIS spectrophotometer. For The control DPPH solution in ethanol or acetone was taken without plant extracts and optical density was recorded after 15min. The assay was carried out in triplicate. The decreasing optical density of DPPH on addition to test samples in relation to the control was used to calculate the antioxidant activity as percentage inhibition of DPPH radical scavenging calculated using the following equation.

3.2 The Percentage of scavenging activity was calculated by using the following formula

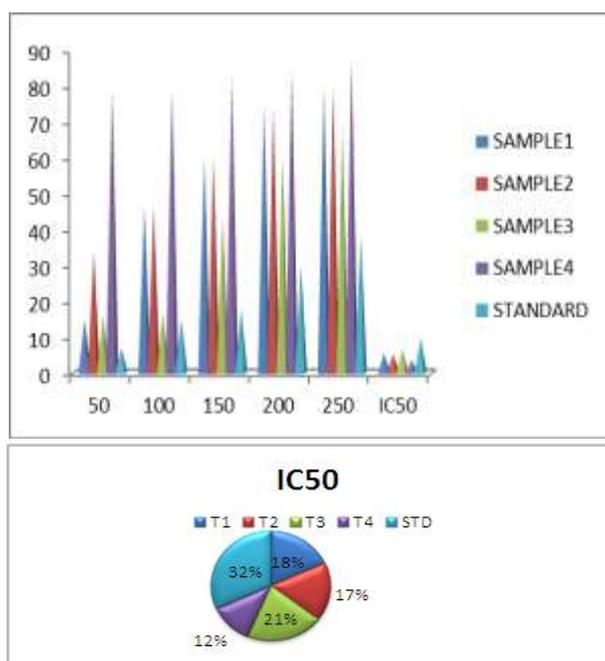
Effect of scavenging O/D= {1 – A sample (517nm)/A control (517nm) } x 100.

Table 2: Representing quantitative estimation of *Terminalia catappa* leaves.

| Sample | Amount of flavanoid | Amount of phenol |
|--------|---------------------|------------------|
| T1 | 990µg/ml | 178 µg/ml |
| T2 | 585 µg/ml | 180 µg/ml |
| T3 | 587 µg/ml | 195 µg/ml |
| T4 | 950 µg/ml | 185 µg/ml |

Table 3: Representing IC50 VALUE.

| Sl. No. | Conc | T1 | % | T2 | % | T3 | % | T4 | Std | % |
|---------|----------|-----------|----|-----------|----|-----------|------|-----------|-----------|----|
| 1 | 50 µl | 15 | 5 | 33.9 | 11 | 16.2 | 8 | 79.3 | 07 | 6 |
| 2 | 100 µl | 50 | 17 | 46.6 | 16 | 16.9 | 8.25 | 79.4 | 15 | 8 |
| 3 | 150 µl | 66 | 23 | 60.1 | 21 | 43.3 | 21 | 82.3 | 18 | 11 |
| 4 | 200 µl | 74.5 | 26 | 74.6 | 26 | 60.3 | 30 | 84.5 | 30 | 29 |
| 5 | 250 µl | 81.7 | 28 | 80.1 | 28 | 68.2 | 33 | 87.5 | 38 | 37 |
| | IC Value | IC50=5.52 | | IC50=5.24 | | IC50=6.38 | | IC50=3.54 | IC50=9.68 | |

**Fig. 2: Representing the difference of IC50 value with reference to the *Terminalia catappa* varieties**

RESULT AND DISCUSSION

The Summarised result of the qualitative , qauntitative analysis of chemical constitutes of *Terminalia catappa* with a focus on flavanoids and phenol from methanol extract of different varieties of *Terminalia catappa* in Table 1and Table 2 .The phenol content was determined by folinciocalteau reagent. The maximum concentration of phenol was observed T3- 195 µg/ml ,T4-185 µg/ml T2- 180 µg/ml minimum concentration was observed in T1-178 µg/ml The flavonoid content was evaluated by alumunium chloride method highest amount of flavonoid content was obtained in sample T1-0.89 µg/ml T4-0.86 µg/ml T3-0.75 µg/ml and low in sampleT2-0.72 µg/ml shown in figure3 The free radical scavenging of methanol leaf extract was measured by DPPH radical which is stable organic free radical with absorption maxima at 517nm it loses the optimal absorption when accepting an electron resulting in colour variation from purple to yellow this discoloration indicates the

scavenging potential of antioxidant activity of methanol extract of different varieties of *Terminalia catappa* compared to standard .The standard sample should be dependent activity and the concentration used was 50,100,150,200,250m g/ml, shown in figure1 the percentage of free radical scavenging capacity of the sample T1,T2 were ranged from T1-15 to 81.7, T2- 33.9 to 80.1 ,T3-16.2 to 60.2 ,T4-82.7 to 87.5 respectively shown in Table 3,the methanol extract of sample exhibited strong antioxidant activity followed by the sample showed considerably low in which was reported as T1-15to T3-16.2 with the different concentration the radical scavenging activity in likely to be related to the value of phytochemical and their hydrogen donating ability to reach from radical converting the in to more stable non reacting species of previously reported.^[28] Phytochemical are widely distributed in plant and have gained much ability due to their role in the ability to scavenge the free radical and have beneficial effects on human health the present study clearly indicates that methanol extract of different variety of *Terminalia catappa* leaves possess significant antioxidant activity which may be developed as a functional ingredients in pharmaceutical product.

CONCLUSION

The present study evaluated the significant antioxidant activity in DPPH assay, leaf of T3 variety showed highest antioxidant activity compare to variety of T1,T2,T4.An Antioxidant activity is due to the highest phenol compounds in T2 variety than variety T1 showed less activity .This finding of this study suggest that the plant is a potential source of natural antioxidant that could have therapeutical agent in preventing or slowing progress of oxidative stress related to degenerative diseases .Further investigation of the isolation and characterization of the antioxidant constituent ,so *Terminalia catappa* varieties are wonder drug as the species have cure for many diseases and leaves of *Terminalia catappa* as a good source of natural antioxidants.^[29]

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