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Original Article

Phytochemical analysis and *in vitro* antioxidant activity of *Terminalia catappa*

P. Punniya Kotti [1*] and A. Vijaya Anand [2]

[1]Department of Biochemistry, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kancheepuram-631 551, Tamil Nadu, India
[2]Department of Science and Humanities, M.I.E.T. Engineering College, Gundur, Trichy-620 007, Tamil Nadu, India

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ABSTRACT

The present study evaluates the phytochemical constituents and *in vitro* antioxidant potential of *Terminalia catappa* Linn. A qualitative phytochemical analysis of six solvent extract methanol, ethanol, petroleum ether, chloroform, 1%chloroform and ethyl acetate was performed for the presence of phytochemical constituents. Among six extracts methanol has been found to possess the highest phytochemical constituents when compare others. Further, the antioxidant activity of methanol extracts of *T. catappa* was evaluated by various antioxidant assays which include DPPH assay, nirtic oxide assay, reducing power assay and H_2O_2 assay. In all the tested methods *T. catappa* has been found to possess the antioxidant activity in a dose-dependent manner. The present study revealed that *T. catappa* is very rich in phytochemicals and a good source of natural antioxidants.

Keywords: *Terminalia catappa*; *in vitro*; antioxidant.

INTRODUCTION

Oxidative stress can damage carbohydrate, protein, lipids and DNA in cells and tissue, which consequently leads to several degenerative diseases such as diabetes, cardiovascular disease, cancer etc., [1, 2, 3]. In this situation, the defence against oxidative stress is getting worsen. Additional supply of antioxidants is necessary to meet out the subsequent injurious due to oxidative stress. Supplementation of synthetic antioxidants like butylated hydroxtoluene and BHA are limited due their side effects [4].

Recently, several antioxidants are obtained from naturally occurring plants and have ability to scavenge free radical or active oxygen [5]. *Terminalia catappa* Linn. is usually grown in tropical regions of the world as an ornamental plant. The fruit is edible, tasting slightly acidic and eaten as raw. The leaves contain flavonoids, tannins, saponines and phytosterols. Due to this chemical richness, the leaves are used in different herbal medicines for various purposes. Therefore, the present study is aimed to investigate the phytochemical constituents and the antioxidant potential of *T. catappa* leaves by various *in vitro* methods.

MATERIALS AND METHODS

Plant Material Collection and Extract Preparation: The plant leaves specimen was collected from Kancheepuram, Tamil Nadu, India. The collected plant leaves were air dried under shade which was previously washed and dried, ground into powder using mortar and sieved. The powdered plant material was subjected to successively soxhlet extraction with solvents such as methanol, ethanol, petroleum ether, chloroform, 1% chloroform, ethyl acetate. After concentration and drying the extracts identification of phytocontitutents was carried out using chemical tests. Standard methods were used for phytochemical screening of the different extracts to know the nature of phytoconstitutents present within them [6].

In vitro antioxidants analysis

DPPH radical scavenging assay: The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay was performed by using the method of Brand-Williams *et al.*, [7]. About 1.0 ml of the 0.004% methanol solution of DPPH was added to 1 ml of various concentrations (250-1500 µg/ml) of the extracts. The mixture was vortexed thoroughly and left at room temperature for 30 min in the dark. The

***Corresponding Author Address:** P. Punniya Kotti, Assistant Professor, Department of Biochemistry, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kancheepuram -631 551, Tamil Nadu, India; E-mail: pkbio2005@gmail.com*

absorbance was measured at 517 nm. Radical scavenging activity was calculated as percent inhibition using the formula: % inhibition = [Abs control- Abs sample] / [Abs control] x 100.

Nitric oxide scavenging activity: The method of Fan *et al.,* [8] was adopted to evaluate the scavenging activity of *T. catappa* against nitric oxide radical. A volume of 2 ml sodium nitroprusside (10 mM) prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract at various concentrations (250-1500 µg/ml). The mixture was incubated at 25^{o}C for 2.5 h. After incubation, 0.5 ml of the reaction mixture was removed; 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand at room temperature for 5 min for complete diazotization reaction. 1 ml of naphthyl ethylene diamine dichloride (0.1%*w/v)* was added and the mixture was allowed to stand for 30 min at room temperature. Absorbance was measured at 540 nm. Nitric oxide percent inhibition = [Abs control –Abs sample]/ [Abs control] x100.

Ferric reducing power assay: The reducing power of methanolic extract of *T. catappa* was evaluated according to the method of Wu *et al.,* [9]. Different concentrations (250-1500 µg/ml) of the extracts and standard drugs were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium hexacyanoferrate II. The mixture was incubated at 50^{o}C for 20 min, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $Fecl_3$. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated stronger reducing power.

Hydrogen peroxide scavenging activity: The method of Hemwimol *et al.,* [10] was used to assess the ability of the extracts to scavenge hydrogen peroxide. A volume of 0.6 ml of 4 mM H_2O_2 solution prepared in 0.1M phosphate buffer (pH 7.4) was mixed with different concentrations (200-1000 µg/ml) of the extracts. The absorbance of the solution was measured at 230 nm after 15 min against a blank solution containing phosphate buffer without H_2O_2. The scavenging activity of the plant extract on H_2O_2 was expressed as: % scavenged [H_2O_2] = [Abs control-Abs sample]/ [Abs sample] x100.

RESULTS

The results obtained from the phytochemical screening conducted on the *T. catappa* leaf extracts are presented in Table 1. Table 2 showed the DPPH radical scavenging activity of the methanolic extract of *T. catappa* in a concentration dependent manner. In general however, the highest % scavenging activity (88.40%) was recorded at 1500 µg/ml of the fraction of *T. catappa* leaves while the lowest % scavenging activity 21.03% at 250 µg/ml of the fraction of *T. catappa* leaves. The IC_{50} value for plant extract was 670 µM and it showed that showed the leaves of *T. catappa* is a good source of natural antioxidants.

This study further observed varying degrees of nitric oxide scavenging potential of test extract fractions. Nitric oxide scavenging capacity of *T. catappa* increased remarkably following increase in extract concentration. In general however, the highest % scavenging activity (65.12%) was recorded at 1500 µg/ml of the fraction of *T. catappa* leaves while the lowest % scavenging activity 21.10% at 250 µg/ml of the fraction of *T. catappa* leaves (Table 2). The ability of *T. catappa* extracts to reduce ferric cyanide to its ferrous form is presented in Table 2. At all the concentrations tested, extract showed significantly higher reducing power in a dose dependent manner. In general however, the highest % scavenging activity (90.04%) was recorded at 1500 µg/ml of the fraction of *T. catappa* leaves while the lowest % scavenging activity 52.20% at 250 µg/ml of the fraction of *T. catappa* leaves. The scavenging ability of methanolic extract of *T. catappa* leaves extract on hydrogen peroxide is shown Table 3. The *T. catappa* leaves were capable of scavenging hydrogen peroxide in an amount dependent manner. 1000 µg of methanolic extracts of *T. catappa* leaves exhibited 88% scavenging activity on hydrogen peroxide. The IC_{50} value for plant extract was 650 µM.

DISCUSSION

The present study clearly confirms the presence of various phytoconstituents like alkaloids, tannins, steroidal glycosides, flavonoids and phenol in different extracts. These secondary plant metabolites may be responsible for its numerous medicinal effects. The present results demonstrated strong antioxidant activity of *T. catappa.* One of the direct methods to assess the antioxidant activity of a plant is the scavenging activity on DPPH, a stable free radical and widely used index. In the present study the phenolic compounds present in the *T. catappa* extracts may possibly responsible for the observed DPPH radical scavenging activity, because phenols can readily provide atom to the radical and it has been reported that the quantity of phenolic compounds in an extract is directly proportional to its free radical scavenging ability [11]. Nitric oxide radical is a very high reactive

compound with ability of changing the structural and functional activities of many cellular components [12]. In the present study the inhibitory potentials of the extracts on nitric oxide radical can be recognized to the presence of flavonoids which are able to compete with oxygen and its derivatives because they can easily donate electrons to the radicals. Flavonoids are concerned in scavenging the oxygen derived free radicals [8]. In the present study the percentage reducing power of plant extracts, indicative that some components of the extracts were electron donors that could react with the free radicals and convert them into more stable products. Hydrogen peroxide is a pro-oxidant that can able to crossing the membrane to oxidize a number of compounds. H_2O_2 can give rise to hydroxyl radical thereby interacting with cellular components to cause tissue damage and eventually cell death [13]. In the present findings the inhibition of H_2O_2 radical by the extracts can be attributed to the proton donating abilities of their phenolic contents. The present findings additionally underscored the test plant extracts as good sources of antioxidants [12].

CONCLUSION

It is demonstrated that methanolic extract of *T. catappa* had strong scavenging and reducing potentials. Attempt at further fractionation of these extracts to isolate and identify the most effective bioactive phytoconstituents responsible for the observed activities as well as their interactive mechanisms is highly advocated.

Table 1 Phytochemical characteristics of leaves extracts of *Terminallia catappa*

Characteristics	Methanol	Ethanol	Pet. Ether	Chloroform	1% Chloroform	Ethyl acetate
Carbohydrates	+	+	-	+	+	-
Protein	-	-	-	-	-	-
Steroid	-	-	-	-	-	+
Glycoside	-	+	-	-	-	-
Tannin	+	+	-	-	+	-
Flavonoids	+	+	-	-	+	-
Alkaloids	+	+	-	-	-	-
Saponin	+	+	+	-	-	-
Starch	-	-	-	-	-	-
Terpenoids	+	-	-	+	-	-
Anthocyanin	+	+	+	-	-	+
Coumarin	+	+	-	+	-	+
Emodins	+	+	-	-	-	-
Phytosterol	+	+	-	+	-	+
Phlobatannins	-	-	-	-	-	+
Cardial Glycosides	+	-	-	+	+	-
Chalcones	+	+	-	-	-	+

a) + sign indicates positive test (presence of compound);
b) – sign indicates negative test (absence of compound)

Table 2 *In vitro* antioxidant assay of *T. catappa* leaves extract; (DPPH assay, Nitric oxide assay and Reducing power assay)

Plant Concentration (µg)	DPPH assay (%)	Nitric oxide assay (%)	Reducing power assay (%)
250	21.03	21.10	52.20
500	40.19	32.90	61.15
750	54.05	42.14	74.80
1000	72.12	53.19	82.14
1500	88.40	65.12	90.04
IC_{50}	670	950	250

Table 3: *In vitro* antioxidant assay of *T. catappa* leaves extract (H_2O_2 assay)

Plant Concentration (µg)	H_2O_2 assay (%)
200	22
400	31
600	45
800	64
1000	88
IC_{50}	650

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