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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *TERMINALIA CATAPPA* LEAVES

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ABSTRACT

Objectives: Free radicals are the important role in causing various kinds of diseases. Free radicals neutralized with the help of antioxidants, so the aim of this study is to find out the antioxidant potential of ethanolic extract of *Terminalia catappa* Linn. leaves by *in vitro* models.

Methods: The plant material is collected, authenticated, dried, and grind into powder. The powdered plant material is subjected to successively Soxhlet extraction with a solvent of ethanol. Ethanol extract of leaves were taken and the extract is subjected to reducing power assay, H_2O_2 assay, nitric oxide assay, and 2,2-diphenylpicrylhydrazyl (DPPH) assay by using standardized methods.

Results: The results of all the assays proved that the ethanolic extract of *T. catappa* has an antioxidant potential on a dose-dependent manner. The highest percentage of reducing power (78.04%) is recorded at 500 µg/ml, 500 µg/ml have a (56.4%) of scavenging activity, (74.68%) activity of nitrous oxide production and DPPH percentage of inhibition is (99.6%). The results were compared with the antioxidant activity of standard ascorbic acid.

Conclusion: The study proves that the ethanolic extract of *T. catappa* leaves is a good source of antioxidants, and future medicated world choose this phytochemical constituent rich plant for their drug preparation after the deepness of research. In all the methods the plant leaves has been found to possess the antioxidant activity. which may be the responsible for various therapeutic properties. The current study showing that *T.catappa* is having high quantity of phytochemicals and a worthy source of natural antioxidants. Using this kind of herbal medicine we can lead the life with harmless drug for harmful illness.

Keywords: *Terminalia catappa,* Reducing power assay, Hydrogen peroxide assay, Nitric oxide assay and 2-2-diphenyl-1-picrylhydrazylassay.

INTRODUCTION

Free radicals are atoms or molecules with an unpaired electron, which makes them extremely reactive. The free radicals such as hydrogen peroxide, nitric oxide, and superoxide anions, which is responsible for oxidative stress, it in turn damages the macromolecules and it causes the various illnesses in humans such as arthritis, anemia, diabetes mellitus, aging, and cancer [1]. The human body has a natural protective mechanism to prevent the production of free radicals [2]. The protective mechanisms are disturbed at several pathophysiological conditions which enforce our body needs external supplements with antioxidant potentials to prevent the formation of free radicals. Although herbs had been rated for their therapeutic, aromatic potentials for periods, the artificial products of the up-to-date age exceeded their prominence. However, the dependence on synthetics is over, and people are recurring to the natural resources with the faith of protection related with health.

Several synthetic agents such as butylated hydroxylanisole, butylated hydroxyltoluene are commercially available, but these are causing various side-effects in human and animals [3]. Natural products of plants which have a compound such as flavonoids, tannins, proanthrocyanidins, and phenols. These are natural occurring compounds have a strong antioxidant activity. So, the researchers are tried to find the antioxidant drug from natural sources, without causing harmful effects.

Terminalia catappa Linn. is an important plant in Combretaceae family. It mainly presents in the warmer place of the world. It is otherwise called as almonds of Indian, tropical and Malabar [4]. The various extracts of the leaves and bark of the *T. catappa* have been reported to antimicrobial [5], anti-inflammatory [6], nephroprotective [7], hepatoprotective [8], anticancer, etc. The present study aims to evaluate the *in vitro* antioxidant activity of ethanolic extract of *T. catappa* leaves.

METHODS

Plant material collection and extract preparation

The plant leaves specimen is collected from Trichy and authenticated at St. Joseph's College, Trichy, Tamil Nadu. The collected plant leaves are air dried under shade and ground into powder using mortar and sieved. The powdered plant material is subjected to successively Soxhlet extraction with a solvent of ethanol.

Reducing power assay

1 ml of varying concentrations (100, 200, 300, 400, 500 µg/ml) of plant extract is mixed with 2.5 ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture is incubated at 50°C for 20 minutes. Aliquots of 2.5 ml of trichloroacetic acid are added to the mixture, which is then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) is mixed with equal volume of distilled water, to this 0.5 ml of freshly prepared ferric chloride solution is added and the absorbance is measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power,

$$\% \text{ Increase in reducing power} = \frac{A_{test}}{A_{blank}} \times 100$$

A_{test} is the absorbance of test solution: A_{blank} is absorbance of blank.

H_2O_2 radical scavenging activity

H_2O_2 scavenging ability of ethanolic extract of *T. catappa* leaves is determined according to the method (100, 200, 300, 400, 500 µg/ml) is taken in different test tubes to which 1 ml of H_2O_2 is added. The tubes

are incubated for 5 minutes at room temperature. After 5 minutes, 2 ml of potassium dichromate: Acetic acid reagent is added and the tubes are incubating for 10 minutes at room temperature. The absorbance value of the reaction mixture is recorded at 700 nm. Blank containing the phosphate buffer without the plant extract and a standard is also calculated as,

$$\% \text{ Scavenging } (H_2O_2) = (A_{control}:A_{test}/A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample.

Nitric oxide scavenging activity

Sodium nitroprusside (5 mM) in standard phosphate buffer solution is incubated with different concentrations (100-500 µg/ml) of the ethanolic plant extract dissolved in phosphate buffer (0.025 M, pH 7.4) and tubes are incubated at 25°C for 5 hrs. Control tube without the plant extract, but with an equivalent amount of buffer is maintained in an identical manner. After 5 hrs, 0.5 ml of the incubated solution is removed and diluted with 0.5 ml of Griess reagent (1% sulfanilic acid, 5 % phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite ions with sufanilic acid and its subsequent coupling with napthylethylenediamine is read at 546 nm. The experiment is repeated in triplicate.

$$\text{NO scavenging activity } (\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging capacity of the ethanolic extract of *T. catappa* is determined using DPPH. DPPH (200 µM) solution is prepared in 95% ethanol. From the stock plant extract solution 100, 200, 300, 400, and 500 µg/ml are taken in five test tubes. 0.5 ml of freshly prepared DPPH solution is incubated with test drug and after 10 minutes, absorbance is taken as 517 nm using a spectrophotometer. Standard ascorbic acid is used as a reference.

% Scavenging of the DPPH free radical is measured using following equation

$$\text{DPPH scavenging activity } (\%) = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

RESULTS

In vitro* antioxidant activity of ethanolic extract of *T. catappa

Table 1 shows the reducing power of the plant extract. The highest percentage of reducing power (78.04%) is recorded at 500 µg/ml and the lower percentage of increasing power (37.8%) is recorded at the lower concentration of 100 µg/ml. Table 2 shows that the H_2O_2 scavenging activity of ethanolic extract of *T. catappa.* The results proved that the scavenging activity is clearly a dose-dependent manner. The concentration of 100 µg/ml have 2.56% and the higher concentration of 500 µg/ml have a 56.4% of scavenging activity. Table 3 shows the percentage of inhibition of NO production of the *T. catappa.* The results shows the higher concentration (500 µg/ml) of the plant extract have a higher inhibition (74.68%) activity of nitrous oxide production and lower concentration (100 µg/ml) have a lower inhibition activity (20.7%). Table 4 shows that the DPPH scavenging activity of *T. catappa*. The results clearly show the increasing concentration of the extract has a higher percentage of inhibition (99.6%) at the concentration of 500 µg/ml and the lower inhibition (63.6%) is noted at the concentration of 100 µg/ml.

Table 1: Reducing power of ethanolic extract of *T. catappa*

S. No.	Concentrations (in µg)	% Reducing power	% Inhibition of standard
1.	100	37.8	40.4
2.	200	51.0	53.2
3.	300	62.9	65.4
4.	400	69.6	72.3
5.	500	78.04	80.02

T. catappa: Terminalia catappa

Table 2: H_2O_2 scavenging activity of ethanolic extract of *T. catappa*

S. No.	Concentrations (in µg)	% of H_2O_2 scavenging activity	% Inhibition of standard
1.	100	2.56	4.23
2.	200	10.2	12.4
3.	300	20.5	23.6
4.	400	33.3	35.8
5.	500	56.4	58.7

T. catappa: Terminalia catappa

Table 3: Inhibition of NO_2 production activity of ethanolic extract of *T. catappa*

S. No.	Concentrations (in µg)	% of inhibition of NO_2 production	% Inhibition of standard
1	100	20.7	22.6
2	200	30.9	33.8
3	300	50.13	52.7
4	400	62.15	65.9
5	500	74.68	76.7

T. catappa: Terminalia catappa

Table 4: DPPH scavenging activity of ethanolic extract of *T. catappa*

S. No.	Concentrations (in µg)	% of DPPH inhibition	% Inhibition of standard
1.	100	63.6	65.7
2.	200	76.2	79.9
3.	300	85.7	89.4
4.	400	91.8	96.7
5.	500	99.6	102.5

T. catappa: Terminalia catappa, DPPH: 2,2-diphenylpicrylhydrazyl

DISCUSSION

In this study, the ethanolic extract of *T. catappa* has been evaluated related to antioxidant potentials. Various secondary metabolites are responsible for their therapeutic values which include phenol, flavonoids, sterols, alkaloids, and tannins. Based on this, the reducing power of the extracts determined by the electron transferring ability. The activity is depending on the transfer of Fe^{3+} ion into Fe^{2+} ion. The result obtained showed that the extract possessed antioxidant activity in a concentration dependent manner. The results clearly show the ethanol extract of *T. catappa* have the ability of transferring the Fe^{3+} into Fe^{2+}, and it diminishes the oxidative hurt in the tissues.

Hydrogen peroxide has a capability to penetrate membranes present in the cells. If the hydrogen peroxide molecule is converted to hydroxyl radicals, it may damage the cells [9]. The phenolic compounds give the electrons and thus convert the hydroxyl ions into water [10]. The presence of the phenolic compounds of the ethanolic extract of *T. catappa* may be the reason for its scavenging activity.

Nitric oxide is a free radical they have the potential to change the structural and functional activity of the many cellular membranes [11]. Flavonoid is a compound which has the scavenging activity to the oxygen derived free radicals [12]. The results prove that the extract

have inhibition activity of nitric oxide production. The inhibitory effect of the ethanolic extract of *T. catappa* is may be due to the presence of flavonoids.

DPPH is an important free radical, which damages the cell membrane. The free radical scavenging activity is directly proportional to the amount of phenolic compound present in the extract [13]. In phytochemical studies of *T. catappa* shows that phenolic compounds [14]. The phenol compounds are readily giving the atom to the radical and it's neutralize the oxidative stress, Due to this the presence of phenolic compounds one of the reason for the radical scavenging activity. The present study results prove the inhibition of DPPH is increased in the increasing concentration of the ethanolic extract of *T. catappa.* The present findings further more highlighted the plant extracts as a worthy source of antioxidants [11].

CONCLUSION

In the present study, we evaluated the *in vitro* antioxidant activity of crude ethanolic extract of *T. catappa* leaves. The percentage of inhibition values clearly suggested that the therapeutic antioxidant potentials and also having significant scavenging and reducing power activities. So, further the studies are needed to the compound isolation, and to find that which compound is responsible for its scavenging activity. For this depth of research is needed and it will help to find new therapeutic drug related to oxidative hurt.

REFERENCES

1. Polterat O. Anti-oxidants and free radical scavengers of natural origin. Curr Org Chem 1997;1:415-40.
2. Chandra J, Samali A, Orrenius S. Triggering and modulation of apoptosis by oxidative stress. Free Radic Biol Med 2000;29(3-4):323-33.
3. Madhavi DL, Salunkhe DK. Toxicological Aspects of Food Antioxidants. New York: Dekker; 1995. p. 267.
4. Nadkarni KM. Indian Materia Medica. 4th ed., Vol. I. Bombay: Popular Prakashan; 1976. p. 1205.
5. Neelavathi P, Venkatalakshmi P, Brindha P. Antibacterial activities of aqueous and ethanolic extracts of *Terminalia catappa* leaves and bark against some pathogenic bacteria. Int J Pharm Sci 2012;5(1):114-20.
6. Pawar SP, Pal SC, Kasture VS. Anti-inflammatory activity of *Terminalia catappa* Linn. Pharmacy Focus in the New Millennium Abstracts; 1997. p. 110.
7. Vijayaprakash S, Langeswaran K, Jagadeesan AJ, Revathy R, Balasubramanian MP. Protective efficacy of *Terminalia catappa* leaves against lead induced nephrotoxicity in experimental rats. Int J Pharm Pharm Sci 2012;4 Suppl 3:454-8.
8. Lin CC, Chen YL, Lin JM, Ujiie T. Evaluation of the antioxidant and hepatoprotective activity of *Terminalia catappa*. Am J Chin Med 1997;25(2):153-61.
9. Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI. Screening of antioxidant and antimicrobial activities of anise (*Pimpinellaanisum* L) seed extracts. Food Chem 2003;83:371-82.
10. Mathew S, Abraham TE. *In vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food Chem Toxicol 2006;44(2):198-206.
11. Nagappa AN, Thakurdesai PA, Venkat Rao N, Singh J. Antidiabetic activity of *Terminalia catappa* Linn fruits. J Ethnopharmacol 2003;88(1):45-50.
12. Fan YM, Xu LZ, Gao J, Wang Y, Tang XH, Zhao XN, *et al.* Phytochemical and antiinflammatory studies on *Terminalia catappa*. Fitoterapia 2004;75(3-4):253-60.
13. Kalia K, Sharma K, Singh HP, Singh B. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of *Potentilla atrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC. J Agric Food Chem 2008;56(21):10129-34.
14. Evans WC, Evans T. Pharmacognosy. 5th ed. London: Cambridge University Press; 2003. p. 336-93.