IN-VITRO ANTIOXIDANT ACTIVITY OF *TECTONA GRANDIS* LINN.

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Summary

Free radicals are implicated in many diseases like diabetes, inflammation, cancer, which leads to gained more attraction of antioxidant therapy. The present study deals with *in-vitro* antioxidant activity of ethanolic extract of *Tectona grandis* Linn. (TG) by using DPPH assay, FRAP scavenging assay and H_2O_2 radical scavenging assay. The IC_{50} value observed in DPPH and H_2O_2 radical scavenging assay were 37.5 µg/ml, 32.0 µg/ml respectively, and 50 % reduction in ferricynide complex at 190 µg/ml concentration. The results were compared with ascorbic acid as a standard. Hence antioxidant property of TG may be due to presence of tannins and saponins. These results clearly indicate that TG is effective against free radical mediated diseases.

Keywords: Antioxidant, $DPPH^{\cdot}$, H_2O_2, free radicals, *Tectona grandis.*

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Introduction

The necessity of compounds with antioxidant activity is increasing as there is a realization that the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been linked in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer (1,2). Oxygen free radicals disintegrate DNA, destroy cell membranes, and create havoc among cell's basic enzymatic metabolic processes (3,4).

The oxygen molecule might produce a highly reactive oxygen species (ROS) by some exogenous factors and endogenous metabolic processes in human body. ROS include a number of chemically reactive molecules such as hydrogen peroxide (H_2O_2); superoxide (Oz^-); and the hydroxyl radical (·OH). When the generation of ROS induced by various stimuli in the organism exceeds the antioxidant capacity of the organism, it will lead to a variety of pathophysiological processes. The harmful intervention of ROS in normal metabolic processes leading to pathologic changes is a consequence of their interaction with various biological compounds inside and outside cells (5).

The antioxidant compounds act by several mechanisms such as inhibition of generation and scavenging activity against ROS/RNS; reducing power; metal chelation; activity as antioxidative enzyme; inhibition of oxidative enzymes. Oxidative damage caused by ROS and RNS will lead to among others to DNA lesions, loss of functions of enzymes, increased cell permeability, disturbed signaling over the cell and eventually necrotic cell death or apoptosis (3,4).

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The DPPH assay is technically simple, but some disadvantages limit its applications. Besides the mechanistic difference from the HAT reaction that normally occurs between antioxidants and peroxyl radicals, DPPH is a long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH (6).

Hydrogen peroxide is highly diffusible can cross plasma membrane and most importantly it causes activation of nuclear translocation of transcription factors NFkB, which subsequently allows the transcription of genes and leads to the inflammation and Syndrome X. Oxidants which are produced can modulate generation of secondary messengers such as diacyl glycerols or phosphatidic acid which are reported to have mitogenic properties increasing DNA synthesis and cell proliferation in smooth muscle cells and cell proliferation is important in formation of atherosclerotic plaques Thus, by their deleterious effects on macromolecules , oxidants can induce cellular alterations which can lead to the development of various pathologies (6).

Ferric reducing antioxidant power (FRAP) measures the ability of antioxidants to reduce ferric 2, 4, 6-triperidyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium. Hence any compound which is having redox potential lower than that of redox pair Fe (III)/Fe (II), can theoretically reduce Fe (III) to Fe (II) (6).

In-vitro antioxidation methods has two approaches In the competitive scheme, the target species, defined here as a compound that represents a biomolecule which may be attacked in vivo, and the antioxidant compounds compete for the reactive species (radical or non-radicals). The assessment of antioxidant capacity is based on the quantification of a compound that facilitates the analytical measurement, defined here as the probe. In most of the competitive assays, the probe is the target species or its oxidized form (7).

In the non-competitive assays, putative antioxidant compounds interact with reactive species without the presence of any other competing target molecule. In this way, these assays involve two components in the initial reaction mixture: the antioxidant compound(s) and the reactive species, which may also be the probe for reaction monitoring. Otherwise, the remaining reactive species may be measured after addition of
some derivative reagent. (7).

The plant *Tectona grandis* Linn. is widely distributed in Asian countries and is reported to have antimicrobial activity (8), wound healing activity (9) and used traditionaly for treating bronchitis, diabetes, skin disease and cancer. Several studies have been carried out to identify some active constituents, including anti-cancer and lipid peroxidation factors **(**10**)**.

In the present study the free radical scavenging activity of *Tectona grandis* Linn. against 1,1-diphenyl-2-pycrylhydrazyl (DPPH•) radical formation, H_2O_2 scavenging activity, and ferric reducing antioxidant power (FRAP)assay was evaluated.

Materials and methods

Chemicals

All chemicals and solvents used were of analytical grade and obtained from Ranbaxy Fine Chemicals Indor. Ascorbic acid was obtained from Merck Ltd., Mumbai and 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) was obtained from Sigma Aldrich, Co, USA. The other chemicals used were trichloro acetic acid, (TCA), Ferrous sulphate (FeSO4), Ferric Chloride (FeCl3), dimethyl sulfoxide (DMSO) and potassium ferricyanide.

Plant material

Fresh bark of *Tectona grandis* Linn. (Verbenaceae) was collected from Nanded, Maharashtra. The specimen was authenticated at Agharkar Research Institute, Pune with voucher specimen no. 08-12 and catalogued. The bark was washed with distilled water and shed dried and latter powdered. This powder was then defatted with petroleum ether and then macerated with

ethanol for 72 hours with occasional shaking. It was then filtered and the solvent was evaporated under vacuum. The yield of ethanolic extract of bark of *Tectona grandis* Linn. (TG) was 2.7 % w/w.

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH•) free radical scavenging activity

The free radical scavenging activity of TG was measured by DPPH· assay, following the methodology described by Blois, 1958 wherein the bleaching rate of the stable free radical, DPPH· is monitored at a characteristic wavelength in the presence of the sample. In its radical from, DPPH• absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorbance decreases. Briefly, 0.1 mM solution of DPPH• in ethanol was prepared and 1ml of this solution was added to 3 ml of TG solution in water at different concentration (25-250 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (11, 12).

IC_{50} value in the tested compound is concentration required to scavenge 50% DPPH• free radicals. The DPPH• radical scavenging activity was calculated according to the following equation

DPPH radical scavenging activity (%) = [{Ao - A_1/Ao}] ×100.

Where,

Ao is the absorbance of DPPH•,

A_1 is the absorbance of DPPH• solution in presence of the extract.

Hydrogen peroxide (H_2O_2) scavenging capacity assay

The hydrogen peroxide scavenging ability of TG was determined according to the method of Ruch, 1989**.** A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). The different concentration of TG (10-50 µg/ml) in phosphate buffer was added to a H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H_2O_2 (13). The percentage of H_2O_2 scavenging of *Tectona grandis* and standard compound was calculated as

H_2O_2 radical scavenging activity (%) = [{Ao - A_1/Ao}] ×100.

Where,

Ao is the absorbance of H_2O_2,

A_1 is the absorbance of H_2O_2 solution in presence of the extract.

Ferric Reducing Antioxidant power (FRAP) assay

The reducing capability of TG was determined by the method of Oyaizu, 1986. In this assay, the brown color of the TG solution changes to various shades of green and blue, depending upon the reducing power of each antioxidant sample. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substance causes the reduction of the Fe^{3+} / ferricynide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (14, 15).

Different concentrations of TG (25-250 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricynide (2.5 ml, 1%). The mixture was then centrifuged for 10 min at 1000 rpm. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Reducing power assay (%) = $[\{Ao - A_1/Ao\}] \times 100$.

Where,

Ao is the absorbance of $FeCl_3$,

A_1 is the absorbance of $FeCl_3$ solution in presence of the extract.

Results

Several concentrations ranging from 25-250 µg/ml of the ethanolic extract of *Tectona grandis* (TG) was tested for antioxidant activity in different in-vitro models. It was observed that free radicals were scavenged by the TG in a concentration dependent manner in all the assay viz $DPPH^{\cdot}$, H_2O_2 scavenging activity, reducing power activity, were found to be 37.5, 32.0 and 190.0 µg/ml concentration respectively.

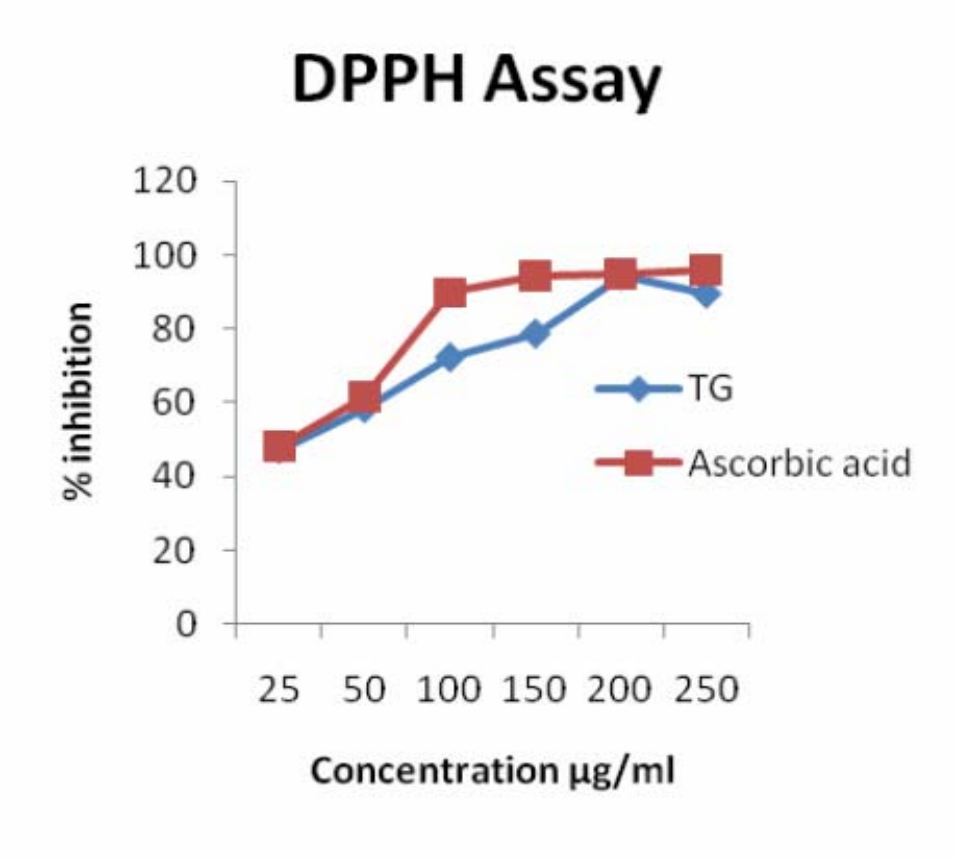
On a comparative basis the extract showed better activity in $DPPH^{\cdot}$ radicals with IC_{50} value of 37.5 µg/ml. However the extract also showed encouraging response in quenching H_2O_2 radicals with IC_{50} value of 32.0 µg/ml, and reducing power assay showed 50 % reduction at 190.0 µg/ml concentration.

Table 1: Free radical scavenging activity of *Tectona grandis* by DPPH radical inhibition.

TG extract (µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
25	47.3±0.69	25	48.1±0.31
50	58.3±0.59	50	61.7±0.61
100	72.1±0.72	100	89.7±0.42
150	78.5±0.46	150	94.1±0.72
200	94.2±0.55	200	94.5±0.53
250	89.3±0.26	250	95.8±0.65
IC_{50} (µg/ml)	37.5		25.0

Values are mean ± SEM of triplicate determinations

Fig. 1. Effect of *Tectona grandis* on DPPH radical inhibition *in-vitro*.



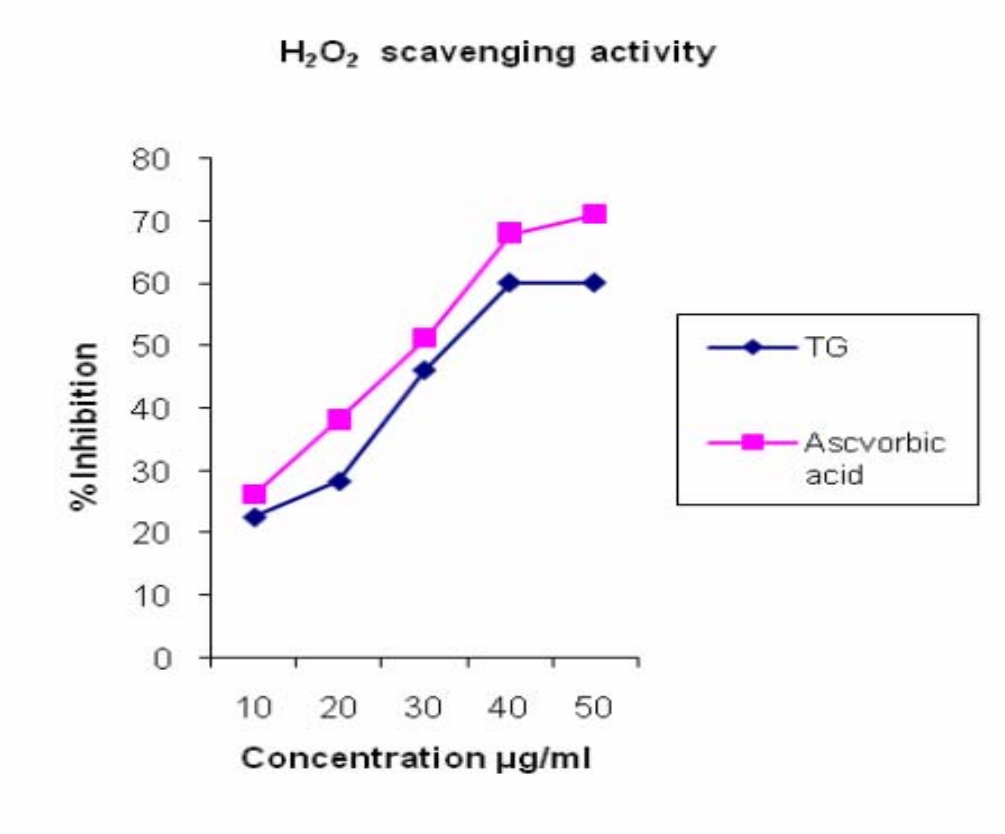
Values are mean ± SEM of triplicate determinations

Table 2: Hydrogen peroxide scavenging activity of *Tectona grandis.*

TG extract (µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
10	22.61±0.21	10	26.25±0.12
20	28.37±0.31	20	38.19±0.26
30	46.19±0.59	30	51.20±0.31
40	60.21±0.33	40	68.07±0.48
50	60.21±0.45	50	71.09±0.61
IC_{50} (µg/ml)	32.0		29.0

Values are mean ± SEM of triplicate determinations

Fig. 2. Effect of *Tectona grandis* on H_2O_2 scavenging activity *in-vitro*.



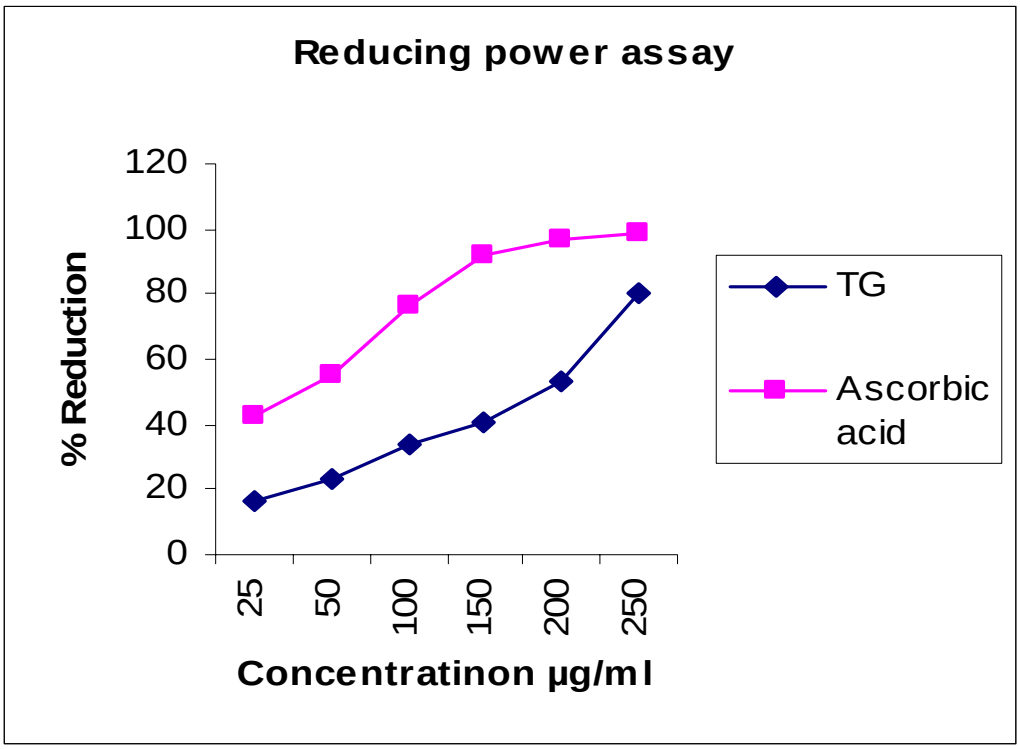
Values are mean ± SEM of triplicate determinations

Table 3: Reducing power activity of *Tectona grandis.*

TG extract (µg/ml)	% Inhibition	Ascorbic acid (µg/ml)	% Inhibition
25	16.58±0.13	25	42.28±0.41
50	23.36±0.21	50	54.68±0.53
100	34.19±0.15	100	76.29±0.65
150	41.07±0.21	150	92.08±0.88
200	53.67±0.31	200	96.32±0.78
250	79.91±0.56	250	98.26±0.74
IC_{50} (µg/ml)	190.0		42.5

Values are mean ± SEM of triplicate determinations

Fig. 3. Effect of *Tectona grandis* on Reducing power assay *in-vitro*.



Values are mean ± SEM of triplicate determinations

Discussion

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing. Antioxidants my offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and by many other mechanisms and thus prevent disease (16).

DPPH• is relatively stable free radical. The assay is based on the measurement of scavenging ability of antioxidants towards the stable radical DPPH•. From the present result it may be postulated that TG reduces the radicals to the corresponding hydrazine when it reacts with the hydrogen donor in the antioxidant principles. DPPH• radicals react with suitable reducing agent, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up (17).

As the H_2O_2 concentration is decreased by scavenger compounds, the absorbance value is also decreased. Nevertheless, it is quite usual that samples also absorb at this wavelength, requiring the performance of a "blank" measurement. TG showed potent scavenging activity against H_2O_2 radicals. However, these peroxidase-based approaches do not allow determining whether the antioxidant is reacting directly with H_2O_2, or reacting with intermediates formed from the enzyme and H_2O_2 (it is possible that the superoxide radical is produced during enzyme activity).

Ferric reducing antioxidant power (FRAP) measures the ability of antioxidants to reduce ferric 2, 4, 6-triperidyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium (6). TG showed antioxidant property may be by reducing ferric complex to ferrous complex.

The results justify the therapeutic application of such herbal plants in the several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer (18).

It is reported that tannins and saponins are natural products which have been shown to possess various biological properties related to antioxidant mechanism (19). Thus in the present study, the antioxidant potential of TG may be attributed to the presence of tannins and the other constituents present therein.

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