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In-Vitro antioxidant potential and phytochemical screening of *Tectona grandis* Linn. leaves

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ABSTRACT:

Tectona grandis Linn. belongs to family Verbenaceae commonly known as Teak and is found in India and South-East Asian countries. It is known for its medicinal value traditionally to cure diseases such as diabetes, anti-inflammatory and infectious diseases and has its timber value too. *In-vitro* antioxidant activity and phytochemical investigation was carried out for successive solvent extracts such as petroleum ether, chloroform, ethyl acetate, methanol and water. The reducing power assay and nitric oxide inhibition assay and DPPH assay demonstrate that the methanolic extract have higher antioxidant activity than others when compared with ascorbic acid as standard. The Phytochemical investigation of methanolic extract also reveals that it has tannins, flavonoids and phenolic contents present in it which are responsible for its antioxidant activity. Thus methanolic extract of the plant will be suggested for futher investigation to determine in-vivo antioxidant activity for diseases in which oxidative stress play a major role.

1. Introduction

The plant *Tectona grandis* (TG) belongs to family "Verbenaceae" native to India, Burma, Thailand, Indochina and Myanmar and is found in the monsoon vegetation forest[1]. It is commonly known as sagwan (Hindi), saka (Sanskrit) and teak tree (English). It is a large deciduous tree with a height up to 35 m leaves simple, opposite, broadly elliptical or acute or acuminate, with minute glandular dots; the flowers are white in colour and small with a pleasant smell[2].

Phytoconstituents of *Tectona grandis* Linn. Present in various parts are Triterpenic hemiterpenic compound, Lignins, Quinones, Steroidal compounds, Phenolic acids and Flavonoids[3,4,5]. Traditional and ethnopharmacological uses of *Tectona grandis* Linn. are in treatment of various disorders like anthelmintic, anti-inflammatory, antioxidant and lipid disorders hypoglycaemic, anti- diabetic, bronchitis, constipation and diuretic[1,6,7]. Reactive oxygen species (ROS) such as superoxide, OH radicals, H_2O_2 and O^- species are generated as by product of biological reactions or from exogenous source. These ROS may attack cell membrane lipids causing lipid peroxidation or DNA mutation leading to cancer by inducing oxidation[8]. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a battery of detoxifying proteins or preventing the generation of reactive oxygen species[9]. Synthetic antioxidants have adverse side effects. Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants.

2. Material and methods

2.1. Collection of plant material

The leaves of *Tectona grandis* Linn. were collected from Patiala, Punjab region, India. The plant specimen has been submitted to the herbarium of Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab (India). Authentication was done by Dr. Adarsh Pal Vig (Head Dept. of Botanical & environment Sciences), with a voucher specimen no. 1458/Bot. & Env. Sci. The leaves of *Tectona grandis* Linn. were washed with running water (to remove any dust impurities) and dried under shade. They were powdered as coarse particles.

2.2. Extraction and determination of extractive value

They were powdered as coarse particles with grinder and are extracted by successive solvent method by pet. ether, chloroform, ethyl acetate, methanol and water using soxhlet

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apparatus. The extracts were concentrated in rotary evaporator under reduced pressure. Powdered drug was packed into soxhlet assembly and successive solvent extraction was done. After the marc was obtained the powder was dried and again used for next solvent extraction. The refluxed samples were dried in rotary evaporator, the extractive value were calculated on dry weight basis. Each extract was subjected to phytochemical screening to identify the chemical constituents, phenolic and flavonoid content and the extract having best antioxidant activity.

2.3 Phytochemical screening

The extract of petroleum ether, chloroform, ethyl acetate, methanol and aqueous should be subjected to preliminary phytochemical investigation for the detection of following compounds; alkaloids, carbohydrates, glycoside, phenolic compounds, flavonoids, protein and amino acids, saponins, sterols, acidic compounds, mucilage resins, lipids/ fats etc [10,11,12].

2.4 *In vitro* antioxidant activity

Determination of total phenolic contents

Total contents of phenols in all extracts of the leaves of *T. grandis* Linn. were determined by the Folin-Ciocalteu assay[13] with some modifications. Briefly, 0.5 ml of plant extracts (0.1 mg/ml) were mixed with 2.5 ml of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to stand for 5 min at room temperature. Then, 2 ml of 1M Na_2CO_3 were added and the mixture was incubated at room temperature for 2 h. Finally, total phenols were estimated at 765 nm using a spectrophotometer (Shimadzu, 1700, Singapore). A standard curve was prepared using gallic acid (10–50 µg/ml). Total phenol values were expressed as gallic acid equivalents (GAE) mg/g of plant extracts.

Determination of total flavonoid contents

Total flavonoid content in all extracts of the leaves of *T. grandis* Linn. were estimated using the Dowd method as adapted by [14]. Briefly, 2.5 ml of 2% $AlCl_3$ in methanol were mixed with 2.5 ml of plant extract (0.1 mg/ml). The mixture was allowed to stand for 10 min at room temperature and the total flavonoid content was determined by UV spectrophotometer (Shimadzu, 1700, Singapore) at 415nm using a quercetin (10, 20, 30, 40, 50 µg/ml) standard curve. Total flavonoid contents are expressed in terms of mg/g quercetin equivalent of dry mass of *T. grandis* extract.

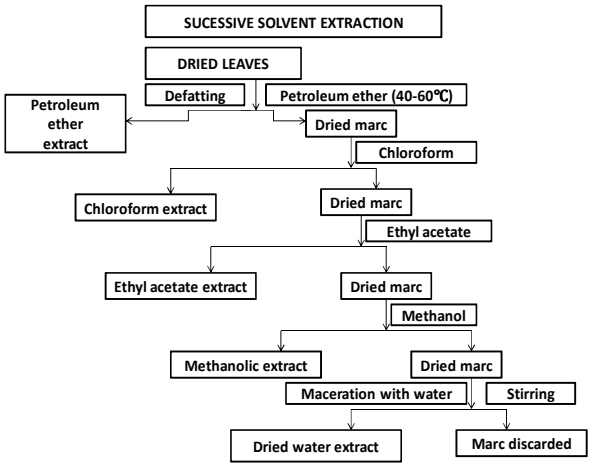

Figure 1. Schematic diagrams for extraction of leaves of TG

Reducing power assay

The reducing power of extract was determined according to the method described by Oyaizu 1986[15]. The different concentrations of extract (50-250 µg/mL) in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of 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 (Shimadzu, 1700, Singapore). Higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as standard antioxidant compound.

DPPH free radical scavenging activity

The free radical scavenging activity was followed by the DPPH method described by Blois 1958[16], 0.1mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (100-500 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm using spectrophotometer (Shimadzu, 1700, Singapore). A blank was prepared without adding extract. Lower

the absorbance of the reaction mixture indicates higher free radical scavenging activity. BHT was used as a standard.

3. Results

3.1 Pharmacognostic study

Phytochemical investigation revealed the presence of alkaloids, flavonoids, glycosides, steroids , tannins and sterols. The total Phenolic and Flavonoid content of methanolic extract was found to be higher than other extracts.

Table 1. Result of preliminary phytochemical screening

Constituents	Test	TGPE	TGCE	TGEE	TGME	TGAE
Alkaloids	Mayer's reagent	-	-	-	+	-
	Dragendroff's reagent	-	-	-	-	-
	Hager's reagent	-	-	+	+	+
Carbohydrates	Molish's test	-	-	-	-	-
	Fehling's test	-	+	-	-	-
	Benedict's test	-	-	-	-	-
Proteins and amino acids	Million's test	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-
	Biuret test	-	-	-	-	-
	Proteins containing sulphur	-	+	-	-	-
	5 % Lead acetate	+	+	+	-	-
	$CuSO_4$	+	+	+	-	-
Glycosides	Keller killiani test	+	-	-	+	+
	Legal's test	-	-	-	-	-
	Bontrager's test	-	-	-	-	-
	Modified Bontrager's test	+	+	+	+	+
Saponins	Froth test	-	-	-	-	+
Flavonoids	NaOH test	-	-	-	+	+
	Lead acetate	-	+	+	+	+
	Shinoda test	-	-	-	-	-
Tannins	Lead acetate solution	-	+	+	+	-
	$FeCl_3$	-	+	+	+	-
Sterols	Salkowaski test	+	+	+	+	-
	Libermann's reagent	+	+	+	-	-
	Libermann's buchard reaction	+	-	+	-	-

Table 2. The total phenolic and flavanoid content of various fractions of *Tectona grandis* Linn. leaves

Extract	Total phenolic content (µg gallic acid equivalent/gm of extract)	Total flavonoid content (µg quercetin equivalent /g of extract)
Pet. ether	7.792±0.015	1.114±0.035
Chloroform	18.890±0.026	6.795±0.046
Ethyl acetate	29.743±0.018	14.295±0.028
Methanolic	54.256±0.022	29.977±0.032
Aqueous	39.621±0.027	3.386±0.027

3.2 DPPH radical-scavenging activity

The results of the free radical scavenging activity of *T. grandis* were assessed by DPPH assay was summarized by IC50 using method of linear regression. Comparing these results with those expressed by BHT showed that *T. grandis* exhibited an equivalent antioxidant effect as compared to BHT (Standard). The lower the value of IC50 the higher is the antioxidant property.

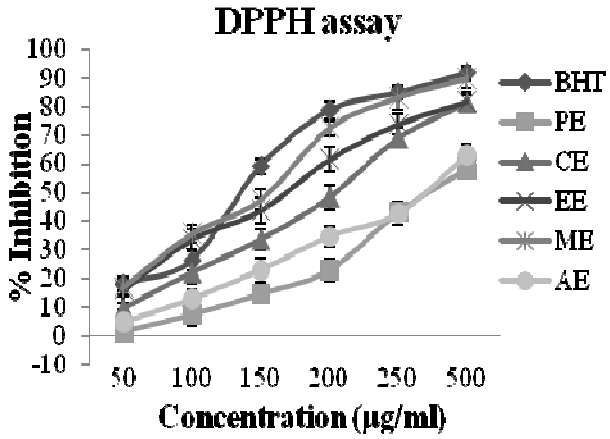

Figure 2. Graph of DPPH assay (% inhibition) for *Tectona grandis* Linn.

Table 3. The IC50 values of different extracts of *Tectona grandis* Linn. leaves

EXTRACT	IC_{50} VALUE
BHT	2.908±2.13
PE	5.721±2.17
CE	3.913±1.75
EE	3.378±3.21
ME	3.013±2.78
AE	5.282±3.87

3.3 Reducing power assay

In this assay, the yellow colour of the test solution changes to various shades of green depending on the reducing power of each compound. The ability to reduce Fe^{3+} to Fe^{2+} may be attributed to donation of hydrogen atom [17], which is related to the presence of reductant. Increased absorbance indicated that increase in reducing ability.

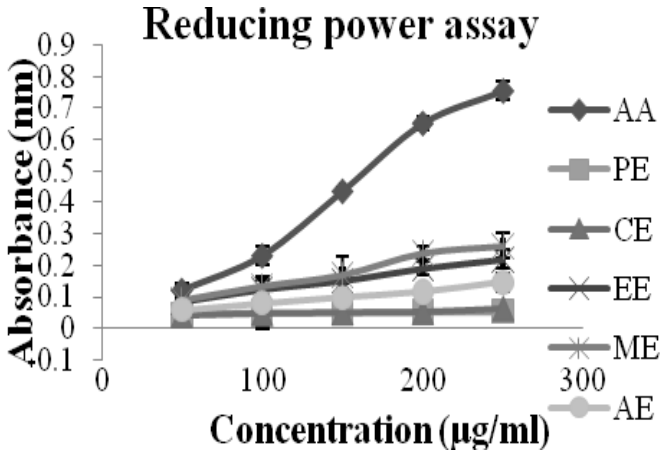

Figure 3. The reducing power assay graph of different fractions of *Tectona grandis* Linn.

4. Discussion

The pharmacognostic study supported that the methanolic extract of leaves of TG resulted in presence of flavonoid and tannin content in during the phytochemical screening. The estimation of total phenolic and flavonoid content resulted in highest content in the methanolic extract suggesting that antioxidant activity of the plant. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities[18]. Flavonoids act as scavengers of various oxidizing species i.e. super oxide anion ($O_2^{\cdot-}$), hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen[19]. The extract also exhibited good in vitro antioxidant activity by DPPH and reducing power assay. The antioxidant activity of the plant is attributed to their redox properties, which allow them to act as free radical scavengers[20].

The literature review of the plant revealed the presence of various phyto-constituents which are responsible for the different activities. The negligible work has been done on the leaves of this plant, whereas a number of studies show that ethanolic extracts of bark[21, 22] and methanolic extract of root[23] was used for the treatment of diabetes mellitus and produced a gradual but significant reduction in blood glucose level and leaves known to have tectone, a hypoglycemic constituent. Inspite of the above activities leaves of the plant also possess analgesic and anti-oxidant activity[5] was therefore thought to work on leaves of this plant to explore its potential as potent drug to reverse or decelerate diabetic neuropathy Based on the above data it is

concluded that methanolic extract of the plant *Tectona grandis* possesses higher antioxidant activity and phytochemical screening also reveals that methanolic extract have constituents which are responsible for its antioxidant activity. It is therefore concluded that this plant can be a good source of natural antioxidants. The results of this study are supportive of the usefulness of this plant in Indian system of medicine and will be able to cure diseases caused by oxidative stress.

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