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Research Article

Phytochemical And Biological Screening of Tectona Grandis Leaves

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ABSTRACT

The present study was aimed to evaluate the antioxidants and antibacterial activities of Tectona grandis (teak) leaves extracts. The extracts of this plant also found applications in many of the traditional medicines. In-vitro antioxidant activity of ethanolic extract of Tectona grandis Linn. by using phosphomolybednum, DPPH assay and H2O2 radical scavenging assay. The results were compared with ascorbic acid as a standard. The antibacterial activities were assay of ethanolic extract (Tectona grandis Linn.) by using the agar well nutrient broth method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated using standard microbiological techniques. They showed a high significant antibacterial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lacto bacillus and Klebsiella pneumoniae. Phytochemical studies of the extracts were determined. Appreciable quantities of phytochemical such as saponins, tannins, flavonoids, glycosides, anthraquinone and alkaloids were present in both extracts of the plants with flavonoids having a higher percentage.

INTRODUCTION

Tectona grandis Linn. (TG) is commonly known as “Teak” belongs to *Lamiaceae* family. Teak has been widely used in India for more than 2,000 years. It is a large deciduous tree 30-35 metre tall with light brown bark, leaves simple, opposite, broadly elliptical or acute or acuminate, with minute glandular dots, flowers are white in colour, small and have a pleasant smell [1]. The plant Tectona grandis is probably the most widely cultivated high value hardwood (HVH) in the world and is native to India and Myanmar and South- East Asian countries [2, 3]. It is now one of the most important species of tropical plantation forestry. The whole plant is medicinally important and many reports claim to cure several diseases according to Indian traditional system of medicines. The survey reveals that the plant is used in the treatment of urinary discharge, bronchitis, cold and headache, in-scabies. Also used as a laxative, sedative, diuretic, anti-diabetic, analgesic and anti-inflammatory [4-7].The various

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phytoconstituents isolated from Tectona grandis are Juglone, which has been reported to antimicrobial activity [8], botulin aldehyde shows antitumor activity [9], lapachol shows anti-ulcerogenic activity [10].

Plant Profile:

Table 1: Taxonomical Classification

Kingdom	Plantae	Plants
Subkingdom	Tracheobionta	Vascular Plant
Superdivision	Spermatophyta	Seed Plants
Division	Magnoliophyta	Flowering Plants
Class	Magnoliopsida	Dicotyledons
Subclass	Astridae	-
Order	Lamiales	-
Family	Lamiaceae	Lamiaceae
Genus	Tectona L.f.	Tectona
Species	Tectona grandis L. f.	Teak

Table 2: Other names of the plant (Tectona)

Language	Name of the plant
English	Indian Teak, Teak
Hindi	Sagwan, Sagauna, Sagu, Sagun, Sakhu
Bengali	Segunngachh, Segun
Gujarati	Sagwan, Sag, Saga, Sagach
Kannada	Tegu, Sagawani, Thega, Jadi, Tega, Tyagadamara, Tekka-maram
Malyalam	Thekku, Tekka-maram, Tekku, Tekka
Marwadi	Sagwan
Punjabi	Sagwan, Sagun
Tamil	Tekku, Tekkumaram, Tek, Kalindi
Telugu	Teku, Pedda, Tek, Peddateku, teku-manu, Adaviteku, Teechekka
Assam	Chingjagu sagun
Oriya	Saguana, Sagan, Sagun, Singuru
Sanskrit	Anila, Arjunopama, Arna, Balasara, Dvarada, Gandhasara, Halimaka
Urdu	Sagwan

Table 3: Traditional Uses of Tectona

S. No	Part of Plant	Uses
1	Leaves	• Cooling, haemostatic, depurative, anti-inflammatory, vulnerary, leprosy, pruritus, stomatitis, indolent ulcers, haemorrhages, haemoptysis and skin diseases,
2	Bark	• Astringent, constipation, anthelmintic, depurative, bronchitis, hyperacidity, dysentery, burning sensation, diabetes, leprosy and skin diseases.
3	Wood	• Acrid, cooling, laxative, sedative to gravid uterus, • Useful in treatment of piles, leucoderma and dysentery. • Oil extracted from the wood is best for headache, biliousness, burning pains particularly over a region of liver
4	Roots	• Anuria and retention of urine

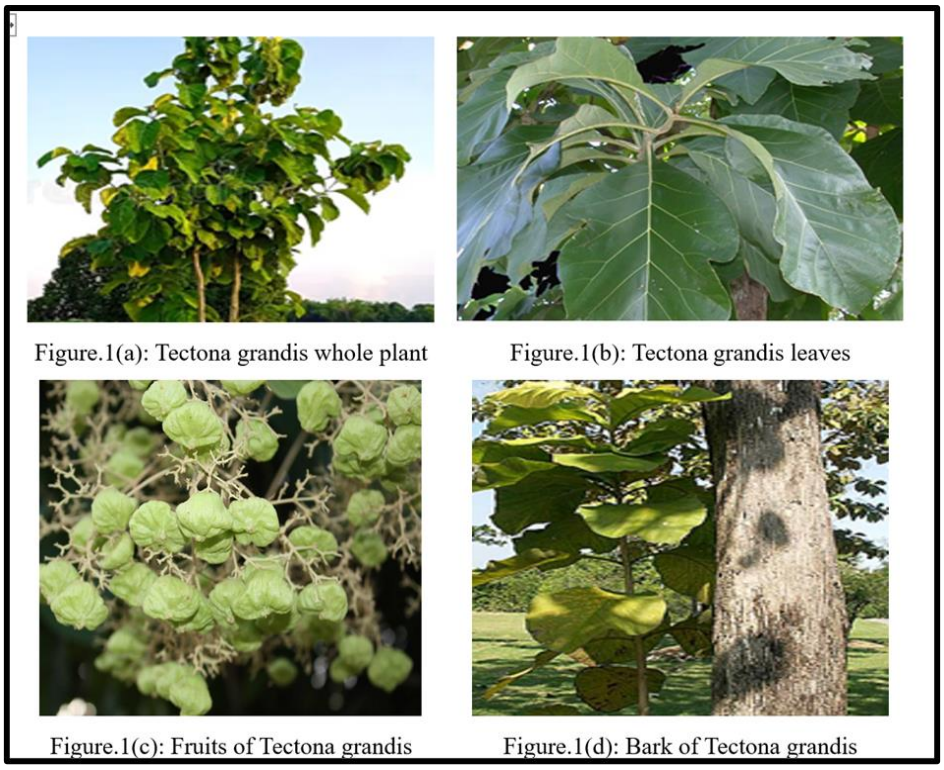

Figure. 1 (a, b, c & d): Tectona grandis plant

MATERIALS AND METHODS:

Collection and identification of plant material:

Tectona grandis tree consists root, flowers, fruits, long and short leaves, bark etc. The long and short leaves were procured from the backyard of Sri Venkateswara College of Pharmacy, etcherla Srikakulam, Andhra Pradesh, India.

Preparation of Tectona grandis leaf extract:

The plant leaves were collected and dried for seven days and leaves were crushed and midribs were separated then soaked in ethanol. The crushed leaves were macerated in alcohol for three days. Reflux process is conducted for one and a half hour. Then solution is filtered and the filtrate is collected and subjected to steam distillation. The solution thus obtained is concentrated till it get converted into semisolid mass and it is kept in desiccator.

Preparation of the Tectona grandis leaf extract by using Solvents (Ethanol, Benzene, Chloroform & Hexane):

- 5gm of leaf extract is dissolved in 100ml of ethanol [95%] in conical flask.
- 5gm of leaf extract is dissolved in 100ml of benzene in conical flask.
- 5gm of leaf extract is dissolved in 100ml of chloroform solution in conical flask.
- 5gm of leaf extract is dissolved in 100ml of hexane solution in conical flask.

Phytochemical Screening [11]:

Following chemical tests were carried out for different extracts of Tectonagrandis to identify the presence of various phytochemical constituents. Chemical tests for detection of organic constituents:

Table 4: Test and procedures for Phytochemical Screening

S. No	Test for Phytochemicals		Procedure
1	Alkaloids	Dragendroff's Test	1g of the formulation was extracted with 20 ml alcohol by refluxing for 15 min and filtered and the filtrate was evaporated to dryness. The residue was dissolved in 15 ml of 2N H2SO4 and filtered. Filtrate was made alkaline and extracted with chloroform. The chloroform extract was evaporated to dryness on the water bath. The residue left after

			evaporation was tested for the presence of alkaloids with dragendroff's reagent. Formation of orange-coloured precipitates indicates the presence of alkaloids.
2	Triterpenes	Liberman-Burchard's Test	Extract was treated with few drops of acetic anhydride, boiled and cooled. Few drops of sulphuric acid were added through sides of test tube. Formation of reddish colour ring at the interface indicates the presence of steroids and triterpenes.
		Salkowski's Test	Extract was treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid shaken and allowed to stand. Appearance of red or violet colour at the interface indicates the presence of triterpenes.
3	Steroids	Liberman-Burchard's Test	Extract was treated with few drops of acetic anhydride, boiled and cooled. Few drops of sulphuric acid were added through sides of test tube. Formation of reddish colour ring at the interface indicates the presence of steroids and triterpenes.
		Sulphur Test	To the chloroform solution of the sample, powder sulphur was sprinkled on the surface, if sulphur shrinks down; it indicates the presence of cholesterol.
4	Carbohydrates	Molisch's Test	To the ethanolic extracts of formulations, α-napthol and concentrated H2SO4 were added. Development of purple colored ring indicates the presence of carbohydrates.
		Fehling's Test	To 1 ml of ethanolic extracts of formulations, 1 ml of the Fehling solution (Fehling A + Fchling B) was added. The mixture was heated on boiling water bath for 5-10 min. Development of yellow precipitates, changing to brick red precipitates indicates the presence of reducing sugars.
5	Glycosides	Chrysarobin Test	0.1gm of powder was dissolved in H2SO4, deep red solution is produced indicates the presence of glycosides.
6	Flavonoids	FeCl3 Test	Extract was treated with FeCl3 solution. Formation of green to black color indicates presence of flavonoids.
		Shinoda Test	Extract was treated with the mixture containing piece of magnesium ribbonand HCl. Formation of red colour indicates presence of flavonoids.
		NaOH Test	To the extract add 10% NaOH was added, Formation of yellow colour indicates presence of flavonoids.
		Lead acetate Test	To the extract add 10% Lead acetate solution was added. Formation of yellow colour ppt. indicates presence of flavonoids.
		Mineral acid Test	To the drug add conc. H2SO4 was added. Formation of orange colour indicates presence of flavonoids.

		Zn/HCL Test	Pinch of zinc dust was added to extract and conc. HCL was added. Formation of red colour indicates presence of flavonoids.
7	Tannins	Lead acetate Test	To 2-3 ml of aqueous extracts of the formulations, 2 ml of 10 %w/w solution of lead acetate was added. Formation of heavy dull yellowish precipitates indicates the presence of tannins.
8	Proteins	Biuret Test	To 3 ml of extracts of the formulations, add 4% NaOH and few drops of 1% CuSO4, solution. Violet or pink colour appears.
		Millon's Test	To 3 ml of extracts of the formulations, add 5ml Millon's reagent. White precipitate is formed. Upon warming, the precipitate turns brick red or the precipitate dissolves giving red coloured solution.
9	Fixed Oils		a) The methanolic extracts of the formulations permanently stains the filter paper indicating the presence of fixed oils. b) Place a sample of the formulations on glass slide. Add a drop of Sudan Red III reagent. After 2 minutes, wash with 50% alcohol. Mount in glycerin. Observe under microscope. Oil globules appear red.

Evaluation Of Antioxidant Activity

Phospho-molybdenum assay:[12]

Hydroalcoholic extract of plant in different concentration ranging from 100 to 500 µg/ml were added to each test tube individually containing 3ml of distilled water and 1ml of molybdenum reagent. These test tubes were kept in incubator at 95°c for 90 minutes After incubation these test tubes were normalised to room temperature for 20-30 minutes and absorbance of reaction mixture was measured at 695nm. Mean value from three independent sample were calculated for each extract. Ascorbic acid was used as reference standard.

DPPH free Radical Scavenging Activity:[13]

The free radical scavenging activity of Tectona grandis was measured by DPPH assay following the methodology described by Blowis in 1958 where in the bleaching of the stable free radical, DPPH is monitored at characteristic wavelength in the presence of sample. In its radical form DPPH absorbs at 517nm, but upon reduction by antioxidant or radical species its absorbance decreases. Briefly, 0.1mM solution of DPPH in ethanol was prepared and 1ml of solution was added to 3ml of Tectona grandis solution in water at different concentration [25-250µg/ml] 30mins later, the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The free radical scavenging activity was calculated according to the following equation

DPPH radical scavenging activity [%] = [{Ao-A1/Ao}] ×100

Where, Ao is absorbance of DPPH

A1 is absorbance of DPPH solution in presence of extract.

Hydrogen peroxide scavenging capacity assay:[14]

The hydrogen peroxide scavenging ability of Tectona grandis was determined according to the method of Ruch,1989. Solution of hydrogen peroxide [40mM] was prepared in phosphate buffer [pH7.4]. The different concentration of Tectona grandis[10-50µg/ml] in phosphate buffer was added to hydrogen peroxide solution [0.6ml, 40mM]. The absorbance value of reaction mixture was recorded at 330nm. Blank solution was containing phosphate buffer without hydrogen peroxide. The % of hydrogen peroxide scavenging of Tectona grandis and standard compound was calculated as

Hydrogen radical scavenging activity [%] = [{Ao-A1/Ao}] ×100

Where, Ao is absorbance of hydrogen peroxide
A1 is absorbance of hydrogen peroxide solution in presence of extract.

Evaluation Of Antimicrobial Activity [15, 16]:

Antimicrobial activity using nutrient broth method Firstly, the prepared nutrient broth with the standard procedure is dividing into different concentration like 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml of Tectona grandis leaves drug extract. Now few drops of the specific microorganism [*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Lacto bacillus* and *Klebsiella pneumoniae*] is added to each concentration test tubes and kept for incubation for 24 hours. Then growth of microorganism is not observed.

RESULTS AND DISCUSSION:

In the present work, leaves extracts of different solvents like ethanolic, benzene, chloroform, hexane of Tectona grandis was used for phytochemical screening and antioxidant and antimicrobial activities. Phytochemical screening of extracts of Tectona grandis leaves was carried out by employing standard conventional protocols (i.e., alkaloids, glycosides, carbohydrates, tannins, steroids)

Table 5: Phytochemical Screening for Tectona Grands Leaves Extract

S.no	Test	Solvents			
		Ethanolic	Benzene	Chloroform	Hexane
1	Alkaloids	Positive	Positive	Positive	Positive
2	Carbohydrate	Positive	Positive	Positive	Positive
3	Glycosides	Positive	Positive	Positive	Positive
4	Tannins	Positive	Positive	Positive	Positive
5	Steroids	Positive	Positive	Positive	Positive

According to this report, Tectona grandis showed the presence of alkaloids, carbohydrates, glycosides, tannins and steroids.

Evaluation Of Antioxidants Activity:

Table 6: Phosphomolybdate Method

S. No	Concentration (µg/ml)	Absorbance
1	50µg/ml	0.06
2	100µg/ml	0.08
3	300µg/ml	0.30
4	500 µg/ml	0.34

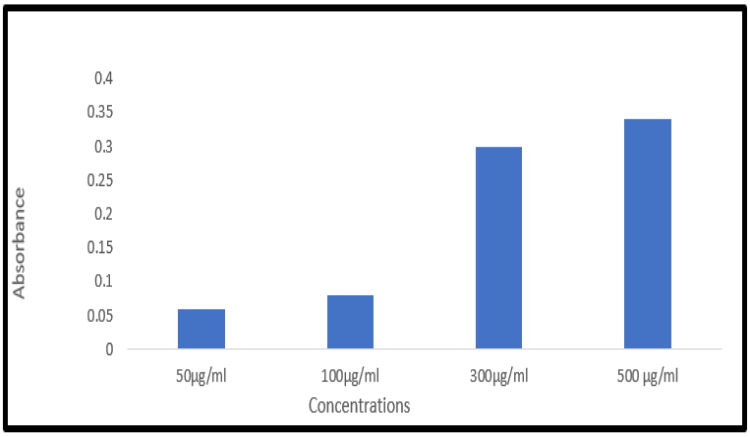

Figure. 2: Graphical Represent of Phosphomolybdate Method

Table 7: DPPH Free Radical Scavenging Activity

S. No	Leaf extract[µg/ml]	% Inhibition	Ascorbic acid [µg/ml]	% Inhibition
1	25	46.8±0.65	25	48.1±0.31
2	50	57.3 ±0.52	50	61.7±0.61
3	100	73.8±0.72	100	89.7±0.42
4	150	77.1±0.41	250	94.1±0.72
5	200	95.1±0.53	200	94.5±0.53
6	250	88.4±0.28	250	95.8±0.65

Values are mean ± SEM o triplicate determinations

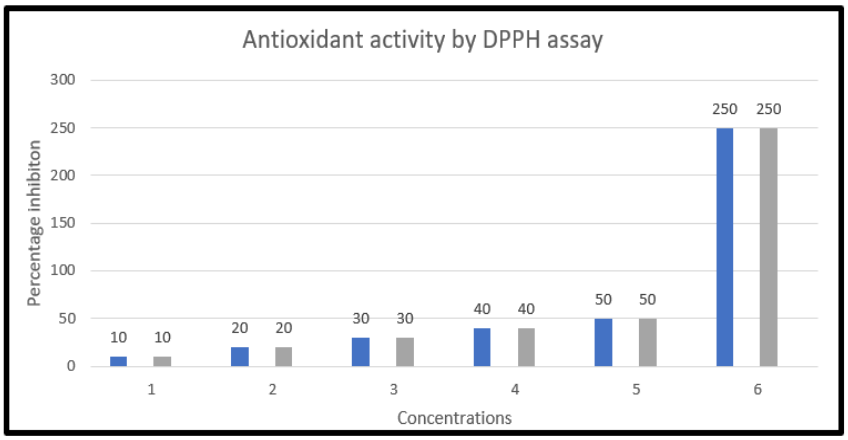

Figure. 3: Graphical Represent of DPPH Free Radical Scavenging Activity

Table 8: Hydrogen Peroxide Scavenging Activity

S. No	Leaf extract[µg/ml]	% Inhibition	Ascorbic acid [µg/ml]	% Inhibition
1	10	21.22±0.12	10	26.25±0.12
2	20	27.56±0.28	20	38.19±0.26
3	30	48.57±0.68	30	51.20±0.31
4	40	62.01±0.39	40	68.07±0.48
5	50	62.08±0.40	50	71.09±0.61

Values are mean ± SEM o triplicate determinations

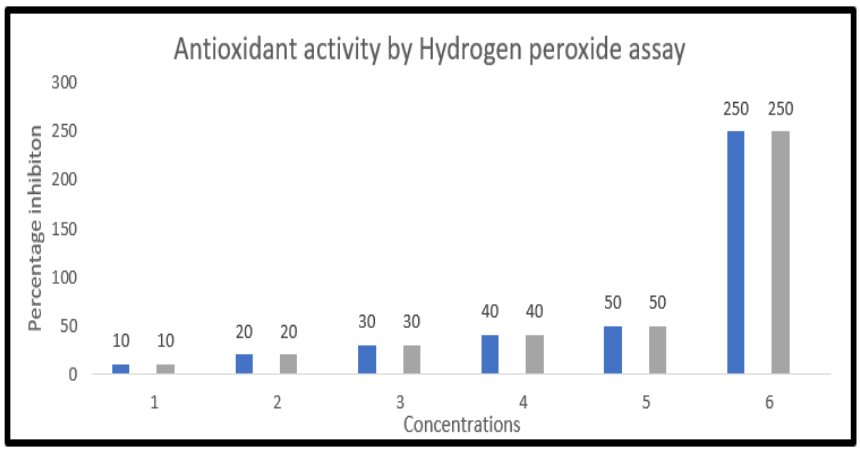

Figure. 4: Graphical Represent of Hydrogen Peroxide Scavenging Activity

Evaluation Of Antimicrobial Activity:

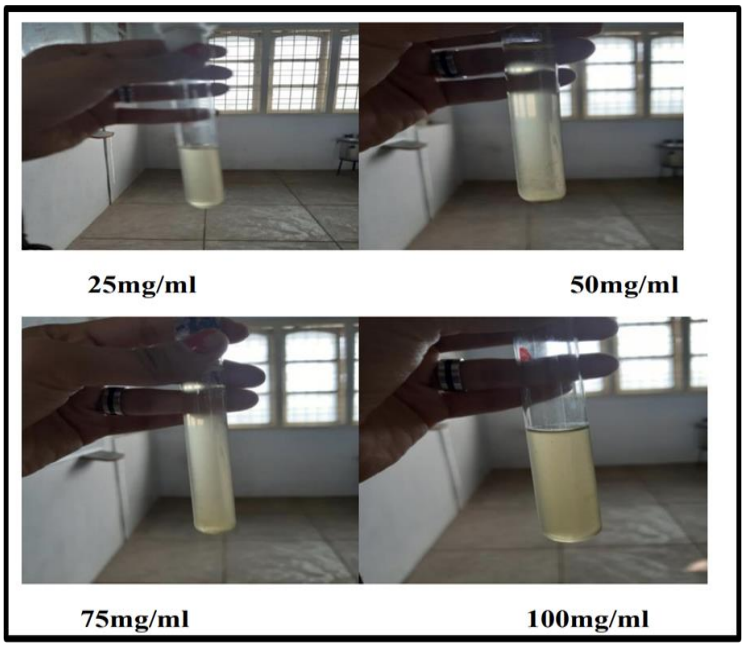

Figure. 5: Antimicrobial Activity of Tectona Grandis Leaves

25mg/ml 50mg/ml 75mg/ml 100mg/ml from the above observation it shows that as the concentration increases, the antimicrobial activity of Tectona grandis leaf extract increases.

CONCLUSION:

The present work was carried out to investigate the antioxidant and antimicrobial of Tectona grandis leaves along with its phytoconstituents. First the Tectona grandis leaf extract was prepared as per the standard procedure, by using solvents like ethanol, chloroform, benzene and n-hexane. Result demonstrated that, the extract of Tectona grandis leaves shows the presence of carbohydrates, steroids, glycosides, tannis and alkaloids. The antioxidant activity is tested by using phosphomolybdum, DPPH and hydrogen peroxide scavenging methods. The results are then compared with the standard ascorbic acid. The result shows that Tectona grandis leaf extract exhibits high antioxidant property. The antimicrobial property of Tectona grandis leaf extract was revealed. The antimicrobial activity tested against bacterial pathogens using nutrients broth medium. The result shows the test sample exhibits potent antimicrobial activity. It was found that all the sample possess good antimicrobial activity at their highest concentration. Finally, it is concluded that the ethanolic leaf extract of Tectona grandis exhibited encouraging and significant antioxidant and antimicrobial activities.

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