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Research Article

Quantitative Determination of Phytoconstituents Present in Bark Extract of Teak Wood and its Radical Scavenging Evaluation

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Abstract

Plants are utilized as therapeutic agents in organized form like Ayurveda and unorganized form used by tribal people. In this project Teak wood has been chosen in powder form and its different compounds like Alkaloid, Flavonoid and antioxidants have been analyzed using Thin Layer Chromatography technique. The components were separated with the use of different solvents such as benzene, petroleum ether and chloroform along with Thin Layer Chromatography technique and nitric oxide radical scavenging properties were revealed to a satisfactory level in Teak wood. This study may be extended on clinical trial basis for its therapeutic actions with animals and subsequently be extended to selective diseased human populations provided the experimental animal studies yields satisfactory results.

Keywords: Thin Layer Chromatography; Teak Wood; Radical Scavenging; Alkaloid; Flavonoid

Introduction

Teak (Tectona grandis) [1-18] is a tropical hardwood tree species placed in the flowering plant family Lamiaceae. Some forms of teak are known as Burmese teak, Central Province teak (CP teak), as well as Nagpur teak. The aim of this research work is to prepare powder of Teakwood available in local area. They have exceptional mechanical properties, highly chemical stability and large specific surface area so the powder has attracted researchers' interest to identify the phytochemicals which are responsible for the foresaid properties. In the present work, dried Teak wood was collected, and powdered. The powder was soaked in water for a week and subjected to Sox let extraction. The extract was collected and the number of components present in the extract was identified by thin layer chromatography. The fraction which gave only one spot on TLC is investigated to identify the nature of the compound. Then the estimation of the components were carried out. The antioxidant property of the extracted compound was also evaluated.

Materials and Methods

Collection of plant material

Fresh plants were collected from a place named Thiruvallur, Tamilnadu, India. The bark was washed under running tap water and shade dried at room temperature. The dried bark was ground to fine powder using a blender. The powder was preserved in an airtight bottle for further use. The bark was identified by the botanist of Sri Sairam Siddha Medical College, Tambaram, Chennai and a specimen was kept in their laboratory.

Phytochemical screening

The presence of different extracted phytochemicals was confirmed by the following tests.

Test for carbohydrates

- **Molishs test:** Two milliliter of Molish's reagent was added to2 mL of extract and shaken well. Then 2 mL of concentrated H_2SO_4 was added on the sides of the tubes. A reddish violet ring appeared immediately at the junction of two layers indicating the presence of carbohydrates.
- **Fehling's test:** To the extract added equal amount of Fehling's reagent and mixed well. Heated gently. Formation of brick red precipitate indicated the presence of reducing sugars.

Test for tannins

Five milliliter of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins.

Test for steroids

Leaf extract was mixed with 1 ml chloroform and later 2-3 drops of conc. H_2SO_4 was added. Pink colour formation indicated the presence of steroids.

Test for terpenoids

- **Libbermann-Burchard reaction:** To 200 mg of plant material 10 mL of chloroform was added and filtered using whattman filter paper no: 1 then 2mL acetic anhydride and a 3drops of concentrated H_2SO_4 were added along the sides of the tubes. Blue- green ring indicates the presence of steroids.
- **Triterpenoids detection:** In a test tube 2 (or) 3 granules of tin is added and dissolved in 2ml of thionide solution. Added test solution to it. Pink colour produced indicates the presence of Triterpenoids.

Test for alkaloids

- **Mayers Test:** To the extract added 1% Hydrochloric acid and 6 drops of Mayer's reagent (1.36g of mercury chloride, 5g of potassium iodide in100ml.The formation if cream coloured precipitate showed the presence of alkaloids.

Extraction

The collected material was allowed to dry in sunshade for a week and after that it was crushed and powdered. 30 gm. of powder was weighed and soaked for 3 days. Then it was poured in to Soxlet apparatus and allowed to boil for one hour. This extract is used for further study.

Chromatographic separation

A simple straight glass tube tapered at the bottom was used as column in our study. The adsorbent was supported on a plug of cotton. The cotton piece was kept in position in the tube. The tube was then clamped vertically and adsorbent (silica gel) was added in portions, so that the tube was packed uniformly with the adsorbent. The adsorbent added was pressed from above with the flattened glass rod, before the next portion was added. This was continued till nearly two third of it was filled.

The extract was poured on the top and the column was covered by a plug of cotton wood. Some solvent was put over it through funnel. The developed chromatogram was treated with succession of solvents having increasingly powerful eluent action. The various portions of the column were thus washed out one by one and collected in different receivers. Gradual elution using 500 ml of each solvent and solvent mixture was carried out for the collection of various fractions. The name of the solvents used and the series of fractions collected in this method are given table 1.

Phytochemical screening

The prepared extracts were subjected to phytochemical screening.

The following tests were carried out.

Tests for alkaloids (Morquies test)

A small quantity of the extract was placed in a glass plate and allowed to evaporate to dryness. A drop of Morquies reagent ($HgCl_2$ + KCN) was added and the colour was observed. Appearance of Reddish colour which turns blue indicates the presence of alkaloids.

Test for quinine (Bromine - ammonia test)

To about 10 ml of extract was added with 0.25 ml of Br_2/H_2O was added and shaken well. Then about 2 ml of dil.NH_3 solution was added. No bright coloration shows the presence of quinine.

Test for morphine (Iodic acid test)

Morphine liberates iodine from iodic acid which gives blue coloration. 2 ml of extract acidified with sulphuric acid was added to a solution of KIO_3 containing starch. Appearance of deep blue colour indicates the presence of morphine.

Test for Terpenoids (Leibermann Buchard test)

2 ml of extract was dissolved in chloroform and to these 2 drops of acetic anhydride was added and concentrated sulphuric acid was added along the sides of the test tube and the colour was observed. Appearance of red colour indicates the presence of terpenoids.

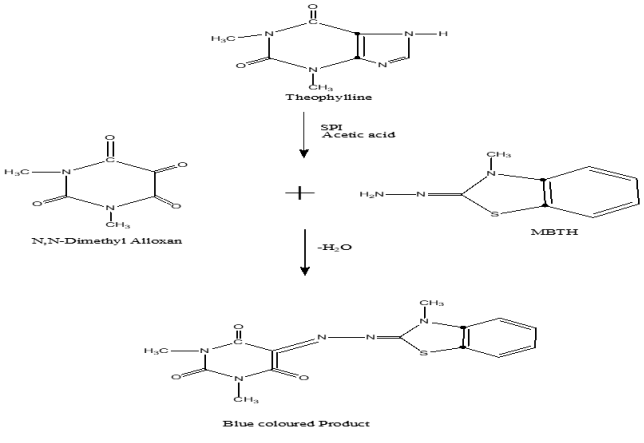
Test for flavonoids (Shendo's test)

2 ml of extract was warmed and to the warmed solution a piece of Magnesium ribbon was added followed by 2 drops of concentrated HCl drop by drop. Absence of orange or yellow colour indicates the presence of flavonoids.

Estimation of phyto constituents

Estimation of alkaloid by Singh MBTH method

Principle: Alkaloids are oxidized by sodium meta per iodate under mild acidic conditions to form N, N' -Dimethyl alloxan, which then reacted with 3- Methyl - 2- Benzo Thiazollinone Hydrazine (MBTH)hydrochloric acid to yield a blue colored product which can be measured spectrophotometrically at 630 nm.



Reagents

- Standard Theophylline

10 mg of theophylline was dissolved in 20 ml of methanol and diluted to 100 ml with distilled water (1ml = 1 mg). 10 ml of this solution was diluted to 100 ml with distilled water. (1ml = 1 mg)

- Acetic acid 0.1 M
- Sodium metaperiodate (SPI) 0.01 M
- 3- Methyl - 2- BenzoThiazollinone Hydrazine (MBTH) (0.1 M)

Estimation of flavanoids by Zishan $ALCL_3$ method

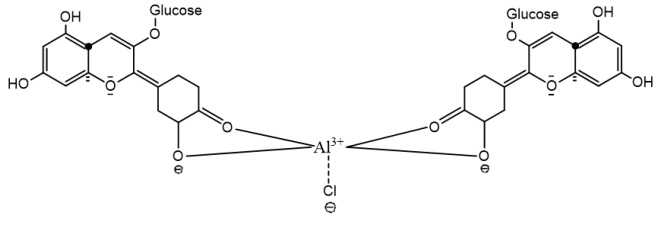
Principle: Flavonoids form pink colored complexwith aluminum chloride in the presence of sodium nitrite and sodium hydroxide which can be measured by spectrophotometrically at 510 nm.

Reagents:

- **Standard Rut:** 10 mg of rut in was dissolved in 10ml of ethanol(1ml = 1 mg) and 10 ml of this was made up into 100 ml using same ethanol. (1ml = 1 mg)
- **Sodium nitrite:** 0.5 gm. of sodium nitrite was dissolved in 10 ml of distilled water.
- **Aluminum chloride:** 0.1 gm. of $AlCl_3$. $6H_2O$ wasdissolved in 10 ml of distilled water.
- **Sodium hydroxide:** 0.4 gm of NaOH was dissolved in 10 ml of distilled water.

Procedure

- 0.5, 1.0, 1.5, 2.0, 2.5 ml of standard solution was pipetted out in to a series of 50 ml standard flasks.
- 2 ml of the sample was pipetted out in a separate 50 ml standard flask.
- To all the flask including the blank 1 ml of 5% $NaNO_2$ was added and incubated at room temperature for 5 minutes.
- 2 ml of 10% AlCl3 solution was added into all the flasks and incubated at room temperature for 6minutes.
- Then 0.5 ml of 1MNaOH solution was added in all flasks and mixed well.
- The Optical Density of the pink colour formed was measured at 510 nm.
- A graph was plotted by taking concentration of Rut in along X-axis and optical density along Y- axis.
- From the standard curve on the graph, the concentration of unknown sample was calculated.

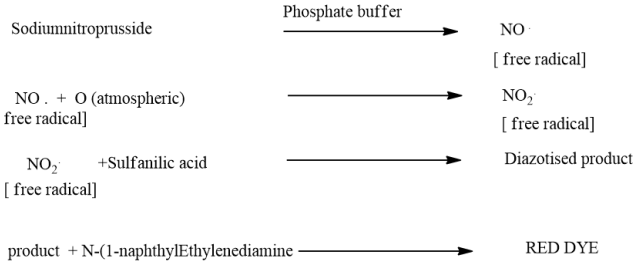


Pharmocological evaluation

Evaluation of antioxidant nitric oxide radical scavenging assay

The principle behind various *in-vitro* methods of evaluating the antioxidant characteristics of drug is discussed below The procedure is based on the method where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, this undergo diazotisation with sulphanilinic acid the diazotized product when added with N-napthyl ethylene gives red dye.

Chemical reactions in nitric oxide scavenging assay



Measured at 540 nm

Preparation of Reagents

- Phosphate buffer saline - 100 Mm, pH 7One gram of buffer tablet is dissolved in 100 ml of distilled water.
- Sodium nitroprusside - 10 m 0.5 gm. of sodium nitroprusside was dissolved in 100 ml of water
- Sulphanilic acid - 0.5% in 20% Glacial acetic aci0.5 g of sulphanilic acid was in 100 ml of 20% glacial acetic acid.
- Napthyl Ethylene DiamineDihydrochloride (NEDD) - 0.1% in 0.1 M Hcl.One gram of NEDD was dissolved in 0.1 M hydrochloric acid.

Results and Discussion

Extraction

Powdered teak wood bark is subjected to Soxhlet extraction with water. The extract was used for the analysis.

Chromatograpic separation for chloroform extract

The extract was subjected to column chromatographic separation using Petroleum ether,10%Benzene in Petroleum ether, 50%Benzene in Petroleum ether, 100%Benzene,10% chloroform in Benzene,50% chloroform in Benzene, and 100%chloroform as eluent. Each fraction was collected separately and a TLC was run with silica gel coated TLC plate and the weight of residue obtained is tabulated in table 1.

S. No	Solvent system	Extract	
		TLC spot	Weight of Residue (gm.)
1	100 %Petroleum ether	1	0.0164
2	10%BenzeneinPetroleumether	1	0.0197
3	50%BenzeneinPetroleumether	2	0.0342
4	100 %Benzene	1	0.0061
5	10% chloroform in Benzene	2	0.0342
6	50% chloroform in Benzene	1	0.0164
7	100 % chloroform	1	0.0197

Table 1: Number TLC spots for various solvent systems and the weight of residue.

Phytochemical screening

5 ml of extracts were dissolved in 100 ml of ethanol and tested for phytochemical components like alkaloids, Terpenoids, and Flavonoids. The results obtained are shown in table 2.

S. No	Name of the tests	Result	Inference
1	Morquies test	+	Presence of Alkaloids
2	Bromine - ammonia test test test)	-	Absence of quinine
3.	Iodic acid test	-	Absence of morphine
4.	Leibermann Buchard test	-	Absence of Terpenoids
5	Shinoda's test	+	Presence of Flavonoids

Table 2: (Phytochemical screening results), The above table shows that the aqueous extract of Lemon Grass contains alkaloids, and flavonoids.

Estimation of phytoconstituents

Estimation of alkaloid by Singh MBTH method

The quantitative determination of alkaloid was carried out by SINGH MBTH method. In this method, a series of theophylline solution were prepared and after adding the required reagents the OD was measured for each concentration. The concentration of theophylline and corresponding optical density values are tabulated in table 3.

Concentration of Theophyline (ppm)	OD
0.2	119
0.3	108
0.4	97
0.5	88
0.6	78
Aqueous extract	64

Table 3: Standard: Theophline.

Graph 1: A calibration graph is drawn by taking concentration of theophylline along x-axis and optical density along the Y-axis.

Calibration graph

The above calibration graph is best fitted to the equation y=102x+138.8 Using this equation

Percentage of alkaloid in aqueous extract =0.733 ppm.

Estimation of flavanoids by Zishan $ALCL_3$ method

The quantitative determination of Flavonoids was carried out. The concentration of Rut in and corresponding optical density values are tabulated in table 4method and a calibration graph is drawn using Concentration of Rut in and corresponding optical density values which are tabulated in table 4.

Volume of rut in (ml)	OD
0.5	99
1.0	86
1.5	75
2.0	61
2.5	55
Aqueous extract	52

Table 4: Standard: Rut in.

Graph 2: A calibration graph is drawn.
By taking- Concentration of Rut in was taken along x-axis and optical density was taken along the Y-axis.

Calibration graph

The above calibration graph is best fitter to the equation y=22.6*x*+ 109.1 from this equation Using this equation Percentage of Flavonoids in aqueous extract = 2.5265 ppm

Concentration of extract (ml)	Optical density	
	STANDARD (ASCORBIC ACID)	EXTRACT
1	25	22
2	28	24
3	32	28
4	34	31
5	38	35

Table 5: Initial OD (A_0) = 20.

The above table shows the optical density values for standard ascorbic acid, and aqueous extract. The OD Values are plotted against concentration of extracts to find out the concentration dependency of scavenging activity of the extract

Concentration of extract (ml)	percentage inhibition × 100
22	10
24	20
28	40
31	55
35	75

Table 6: Calculation of Percentage Inhibition.
Initial OD A_0 = 8.

Conclusion

In the present study teak wood was collected, dried and powdered. The extract concentrated and constituents separated bycolumn chromatography. The different fractions have been collected and run TLC.

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