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PHYTOCHEMICAL SCREENING AND DEVELOPMENT OF VARIOUS THIN LAYER CHROMATOGRAPHIC METHODS FOR DIFFERENT CEDRELA TOONA ROXB. FRUIT EXTRACTS

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ABSTRACT

Air dried powdered material of the fruits of cedrela toona Roxb. was successively extracted with petroleum ether, hexane, acetone, methanol and water extract by soxhlet extraction and subjected to various qualitative chemical tests to determine presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phtosterols, fixed oils and fats, proteins, amino acids, flavonoids, saponins etc. The various extracts of fruits of cedrela toona Roxb. were than subjected to thin layer chromatographic studies to identify the number and nature of the chemical constituents present. This study helps researchers for developmentof isolation method of active ingredient having vast pharmacological effects.

Keywords: Cedrela toona, Extracts, Phytoconstituents, TLC

INTRODUCTION

Literature survey reveals that *Cedrela toona* Roxb. is medium sized to large deciduous tree with brown to grey scaly bark. Leaves 15 – 45 cm long usually paripinnate but sometimes with a terminal leaflet in juvenile growth, leaflets mostly 8-20, ± ovate, often falcate, 4-15 cm long, 15-50 mm wide, apex acuminate, base strongly asymmetric, margins entire, mostly glabrous, domatia present as small hair – tuffs; petiole 4-11 cm long, petiolules 5-12 mm long. Penicles 20-40 cm long. Petals 5-6 mm long, white. Capsule ellipsoid, 10-20 mm long, 6-8 mm diameter; seeds winged at both ends.[1,2,3,4] Traditionally the bark is astringent, antidysentric, antiperiodic.[5] Flowers are emmenagogue, leaf is spasmolytic, hypoglycaemic and antiprotozoal.[6] Bark and heartwood yielded tetraterpenoids, including toonacillin. Heartwood also gave a coumarin gerarnyl gernalol as its fatty esters. Toonacillin and its 6 – hydroxyl derivatives are antifeedent.[5]

MATERIALS AND METHODS[7,8,9,10,11,12]

Authentication and Collection of Fresh Plant

The fresh parts of *Cedrela toona* Roxb.were collected in March 2010, from botanical garden of Dang, Gujarat, India. Dried Samples of Bark and fruit of Cedrela toona Roxb. were collected from Paritosh Herbals, Dehradun. The plant was identified by comparing its morphological and microscopical with description given in different standard texts, floras and Ayurvedic Pharmacopoeia of India[1]. Besides these, the plant was then identified and authenticated by Dr. M. S. Jangid, Botany Department, Sir P. T. Science College, Modasa, Gujarat, India and a voucher specimen was deposited. For further confirmation, the microscopic characters of this plant was studied and compared with available literature as mentioned above. The leaves were dried in shade and stored at 27°C. It was powdered, passed through 40# and stored in air tight containers.

Phytochemical Studies

Preliminary phytochemical screening [258,259]

Successive solvent extraction:

100g of each of air-dried powdered material of leaves, stems and fruits of *Cedrela toona* Roxb. was successively extracted with the following solvents of increasing polarity in a soxhlet apparatus.

- petroleum ether (60° - 80°c)
- hexane
- chloroform/acetone
- ethanol/methanol
- distilled water

All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 50°c. Each time before extracting with the next solvent, the marc was dried in an air oven below at

50^0c.The marc was finally macerated with water for 24 hours to obtain the aqueous extract. The completion of the extraction was confirmed by evaporating a few drops of extract from the thimble on watch glass to observe that no residue remained after evaporation of the solvent. The liquid extracts obtained with different solvents were collected. The consistency, odour, colour, appearance of the extracts and their percentage yield were noted.

The extracts were then subjected to various qualitative tests using reported methods, to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, sterols and terpenoids, anthraquinone glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils, fats etc.

Qualitative chemical identification of*Cedrela toona* Roxb.[7-19]

The extracts were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, phytosterols, fixed oils and fats, proteins amino acids, flavonoids, saponins, etc. using reported methods.

Alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully & treated with alkaloid reagents.

- **Mayer's Test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.
- **Wagner's Test:** Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown or reddish brown precipitate indicated the presence of alkaloids.
- **Dragendorff's Test:** Filtrates were treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicated the presence of alkaloids.
- **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of yellow colored precipitate indicated the presence of alkaloids.

Proteins and Amino acids:

- **Millon's Test:** The extracts were treated with 2 ml of Millon's reagent. The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.
- **Biuret Test:** The extracts were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet color indicated the presence of proteins.
- **Ninhydrin Test:** To the extracts, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated presence of amino acid.

Carbohydrates: Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

- **Benedict's test:** Filtrates were treated with Benedict's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.
- **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.
- **Fehling's Test:** Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with Fehling's A and B solutions. A red precipitate was formed which indicated the presence of carbohydrates.
- **Barfoed's Test:** Filtrates were treated with Barfoed's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Flavonoids:

- **Shinoda test:** 1-5mg of dried extract was extracted with 10ml of ethanol (95%v/v) for 15 min on a boiling water bath and filtered. To the filtrate, added a small piece of magnesium ribbon and 3 to 4 drops of concentrated hydrochloric acid. Formation of red colour was observed.
- **Fluorescence test:** 1-2 mg of dried extract was extracted with 15 ml methanol for 2 min., on a boiling water bath, filtered while hot and evaporated to dryness. To the residue 0.3 ml boric acid solution (3% w/v) and 1 ml oxalic acid solution (10% w/v) were added. The mixture was evaporated to dryness and the residue was dissolved in 10 ml ether. The ethereal layer was observed under U.V. light.
- **$FeCl_3$ test:** To the test solution, added $FeCl_3$ solution. A change of colour from green to black was observed.
- **Lead acetate solution test*:*** To the test solution, added 10% lead acetate solution. A yellow precipitate was observed.

Phenols: A drop of ethanolic extract was spotted on a filter paper and a drop of phosphomolybdic acid reagent was added on it. The spot was thenexposed to ammonia vapor. Blue coloration of the spot indicated the presence of phenols.

Glycosides: Extracts were hydrolyzed with dilute hydrochloric acid and the hydrolysate was subjected to glycosides tests.

- **Modified Borntrager's Test:** The extracts were treated with ferric chloride solution and heated on boiling water bath for about 5 mins. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. The formation of rose pink or cherry red color in the ammonical layer indicated the presence of anthranol glycoside.
- **Legal's Test:** The extracts were treated with sodium nitroprusside in pyridine and methanolic alkali. The formation of pink to red color indicated the presence of cardiac glycosides.
- **Balget Test:** The extract of drug was treated with sodium picrate and the formation of a yellowish orange color confirmed the presence of cardiac glycosides.
- **Killer killani Test:** Take 0.5g of dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solutions. This was then under laid with 1 ml of concentrated H_2SO_4. A brown ring obtained at the presence of a cardenolides.

Saponins:

- **Froth's Test:** The extracts (alcoholic and aqueous) were diluted with 20 ml of distilled water separately and further shaken for 15 mins in a graduated cylinder. A layer of foam measuring about 1 cm was formed which indicated the presence of saponins.
- **Tannins (Phenolic compounds):**
- **Ferric chloride Test:** The extract was treated with few drops of neutral ferric chloride solution (5%). The formation of bluish black color indicated the presence of phenolic nucleus.
- **Lead acetate Test:** The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids.
- **Alkaline reagent Test:** The extracts were treated with few drops of sodium hydroxide separately. Formation of intense yellow color, which turned colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.
- **Shinoda Test:** The extracts were treated with few fragments of magnesium metal separately, followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta color indicated the presence of flavonoid.
- **Vanillin hydrochloric Test:** The extracts were treated with few drops of vanillin hydrochloride reagent. The formation of pinkish red color indicated the presence of tannins.

Steroids and Triterpenoid:

- **Libermann-Burchard's test:** To one ml of ethanolic extract of drug, one ml of chloroform and 2 to 3 ml of acetic anhydride was added. To the above mixture, 1 to 2 drops of concentrated Sulphuric acid was added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of triterpenoids.
- **Salkowski test:** Treat extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow standing for sometime, redcolor appears in the lower layer indicates the presence of sterols and formation of yellow colored lower layer indicating the presence of triterpenoids.
- **Fixed Oils and Fats:**
- **Stain test:** Small quantity of extracts was pressed between two filter papers separately. An oily stain on filter paper indicated the presence of fixed oil.
- **Saponification test:** The extracts were heated on water bath with 0.5 N alcoholic potassium hydroxide solutions. Formation of soap indicated the presence of fixed oils and fats.
- **Coumarins:**
- **WithAmmonia *:***
- Took a drop of ammonia on a filter paper; to this added a drop of aqueous extract. Fluorescence was observed.
- **With Hydroxylamine hydrochloride :**

Took ethereal extract; treated it with one drop of saturated alcoholic hydroxylamine hydrochloride and a drop of alcoholic KOH. Heated it, cooled and acidified with 0.5 N hydrochloric acid and added a drop of 1 %w/v $FeCl_3$. Violet color was observed.

Anthraquinone glycosides:

- **Borntrager's test:**
- Boiled the test solution with dilute sulphuric acid, filtered and added chloroform to the filtrate. Shook well and collected the organic layer . A few drops of strong ammonia solution was added , and shaken slightly and the test tube kept aside for a few minutes. The colour of lower ammonical layer was observed.
- **Modified borntrager's test:**

To the test solution of drug ferric chloride and dilute HCl were added; heated it, cooled it and filtered. Filtrate was shaken with ether or any other organic solvent. The organic layer was shaken with strong ammonia solution and the test tube kept aside. The colour of lower ammonical layer was observed.

Chromatographic Examination of Various Extracts [20,21,22]

The various extracts of fruits of *Cedrela toona* Roxb prepared by successive solvent extraction procedure were subjected to thin layer chromatographic studies to identify the number and nature of chemical constituents present. The R_f values of different phytoconstituents present in various extracts of leaves and fruits of *Cedrela toona* Roxbwere recorded.

Preparation of TLC plate:

Absorbent used : Silica gel G.

Vehicle used for preparation of slurry: Distilled water

Method of preparation: Pour plate method

Activation of plate: In oven at 110˚c for 30 minutes

Application of sample: About 10 to 15 µl of sample was applied with thehelp of glass capillary.

Mobile phase: Take required quantity of solvents in TLC chamber, shake well and utilized for chamber saturation.

Chamber saturation: 30 minutes

Parameters observed: Color of the spot, $R_f$value

RESULT AND DISCUSSION:

Preliminary phytoprofiles

The presence of different chemical constituents in the crude drug can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity. The extracts obtained were then dried completely and kept in vacuum desiccator. They were then subjected to qualitative chemical tests in order to detect the various chemical constituents present in them. The colour, consistency and percentage yield of extracts were determined which are shown in (Table 1)

Table 1: Preliminary phytoprofiles of fruits of *Cedrela toona* Roxb.

Sr. No.	*Solvent*	*Color and consistency after drying*	*Average value of extractive (% w/w)*
1	Petroleum ether (60 – 80 ºc)	Dark green sticky mass	1.52
2	Hexane	Green sticky mass	2.93
3	Acetone	Greenish yellow sticky mass	1.78
4	Methanol	Greenish brown sticky mass	7.21
5	Distilled Water	Reddish brown sticky mass	9.24

Qualitative chemical analysis of various extracts of *Cedrela toona* Roxb

Qualitative chemical examination of various extracts of powder of fruits of *Cedrela toona* Roxb. indicated the presence of carbohydrates, flavonoids, phytosterols, cardiac glycosides, phenolic compounds, triterpenoids, tannins and saponins. The results obtained by chemical examination of various extracts are shown in (Table 2).

Table 2: Qualitative chemical analysis of various extracts of fruits of *Cedrela toona* Roxb.

Sr. No.	*Tests of phytoconstituents*	*P. ether extract*	*Hexane extract*	*Actone extract*	*Methanol extract*	*Water extract*
1	Tests for alkaloids					
	a) Mayer's reagent	-ve	-ve	-ve	-ve	-ve
	b) Dragendorff's reagent	-ve	-ve	-ve	-ve	-ve
	c) Hager's reagent	-ve	-ve	-ve	-ve	-ve
	d) Wagner's reagent	-ve	-ve	-ve	-ve	-ve
2	Tests for flavonoids					
	a) Shinoda test	-ve	-ve	+ve	+ve	+ve
	b) Fluorescence test	-ve	-ve	+ve	+ve	+ve
	c) FeCl3 test	-ve	-ve	+ve	+ve	+ve
	d) Lead acetate test	-ve	-ve	+ve	+ve	+ve
3	Tests for saponins					
	a) Froth test	-ve	-ve	-ve	+ve	+ve
	b) Haemolytic zone	-ve	-ve	-ve	+ve	+ve

4	Tests for carbohydrates					
	a) Molisch's test	-ve	-ve	-ve	+ve	+ve
	b) Fehling's solution test	-ve	-ve	-ve	+ve	+ve
	c) Benedict's test	-ve	-ve	-ve	+ve	+ve
5	Tests for cardiac glycoside					
	a) Legal's test	-ve	-ve	+ve	+ve	+ve
	b) Keller Killiani's test	-ve	-ve	+ve	+ve	+ve
	c) Baljet test	-ve	-ve	+ve	+ve	+ve
6	Tests for fixed oil and fat					
	a) Spot test	+ve	+ve	-ve	-ve	-ve
	b) Saponification test	+ve	+ve	-ve	-ve	-ve
7	Tests for sterols and triterpenoids					
	a) Libermann-burchard`s test	+ve	+ve	+ve	+ve	+ve
	b) Salkowski reaction	+ve	+ve	+ve	+ve	+ve
8	Tests for anthraquinone glycosides					
	a) Borntrager's test	-ve	-ve	-ve	-ve	-ve
	b) Modifying borntrager's test	-ve	-ve	-ve	-ve	-ve
9	Tests for phenolic compounds					
	a) Test with $Fecl_3$	-ve	-ve	+ve	+ve	+ve
	b) Test with folin-ciocalteu reagent	-ve	-ve	+ve	+ve	+ve
10	Tests for coumarins					
	a) With ammonia	-ve	-ve	-ve	-ve	-ve
	b) With hydroxylamine hydrochloride	-ve	-ve	-ve	-ve	-ve
11	Tests for tannins					
	a) Test with gelatin	-ve	-ve	-ve	+ve	+ve
	b) Reaction with lead acetate	-ve	-ve	-ve	+ve	+ve

+ve: **Present,** ***-ve:*** **Absent**

Result showed that methanolic extract of *Cedrela toona* fruits contained carbohydrates, steroids & triterpenoids, flavonoids, saponin glycosides, carbohydrates, tannins and phenolics. Petroleum ether extract of *Cedrela toona* fruits contained sterols and triterpenoids, fixed oil and fat. Acetone extract of *Cedrela toona* fruits contained Flavonoids, steroids, triterpenoids, phenolics, tannins and glycosides. Hexane extract of *Cedrela toona* fruitscontained sterols and triterpenoids, fixed oil and fat. Aqueous extract of *Cedrela toona* fruits contains flavonoids, sterols and triterpenoids, carbohydrates, saponins, glycosides, tanins and phenolics.

Table 3: Thin layer chromatography of fruits extracts of *Cedrela toona* Roxb.

Solvent System	***P. ether Extract (Colour of spot)***	***Hexane Extract (R_f and color of spot)***	***Acetone Extract (R_f and color of spot)***	***Methanol Extract (R_f and color of spot)***	***Water Extract (R_f and color of spot)***	***Spraying reagent***	***Phytochemical constituents***
Chloroform:		R_{f-} 0.68	R_{f-} 0.63	R_{f-}	0.74	Vanillin-sulphuric	Saponins

Glacial acetic acid: methanol: water (64:32:12:8)	---	---	(blue) R_{f-} 0.57 (blue violet)	(blue) R_{f-} 0.53 (yellow brown)	(yellow) R_{f-} 0.67 (brown)	acid reagent	may be present.
Ethyl acetate: Formic acid:--- Glacial acetic acid: Water (80:10:10:20)		---	R_{f-} 0.85 (blue) R_{f-} 0.78 (green) R_{f-} 0.64 (blue)	R_{f-} 0.79 (orange yellow) R_{f-} 0.68 (green) R_{f-} 0.53 (brown) R_{f-} 0.51 (green) R_{f-} 0.48 (blue)	R_{f-} 0.82 (yellow) R_{f-} 0.73 (orange) R_{f-} 0.65 (blue)	NP-PEG reagent	Flavonoids (UV-365nm) Flavone/Flavonol glycosides may be present.
Toluene: Ethyl acetate: --- Acetone: Glacial acetic acid (20: 40: 40: 10)		---	R_{f-} 0.85 (yellow) R_{f-} 0.76 (orange) R_{f-} 0.70 (green)	R_{f-} 0.75 (blue) R_{f-} 0.68 (brown) R_{f-} 0.56 (green) R_{f-} 0.47 (blue)	R_{f-} 0.69 (blue) R_{f-} 0.58 (brown) R_{f-} 0.45 (yellow)	NP-PEG reagent	Flavone/Flavonol aglycone may be present.
Ethyl acetate: Glacial acetic acid: Formic acid: Water (10:11:11:26)	R_{f-} 0.65 (reddish violet) R_{f-} 0.58 (violet)	R_{f-} 0.47 (purple) R_{f-} 0.35 (blue violet)	R_{f-} 0.55 (yellow) R_{f-} 0.42 (red) R_{f-} 0.30 (violet)	R_{f-} 0.78 (green) R_{f-} 0.69 (violet blue) R_{f-} 0.47 (violet)	R_{f-} 0.64 (green) R_{f-} 0.56 (purple) R_{f-} 0.43 (yellow)	Anisaldehyde sulphuric acid reagent	Tri-terpenoids may be present.
Hexane: Ethyl acetate (65 : 35)	R_{f-} 0.78 (blue) R_{f-} 0.74 (violet) R_{f-} 0.63 (yellow)	R_{f-} 0.68 (purple) R_{f-} 0.54 (blue) R_{f-} 0.42 (blue) R_{f-} 0.40 (yellow)	R_{f-} 0.86 (blue) R_{f-} 0.72 (blue) R_{f-} 0.68 (purple)	R_{f-} 0.65 (violet) R_{f-} 0.53 (blue) R_{f-} 0.43 (blue)	R_{f-} 0.79 (purple) R_{f-} 0.62 (blue)	Anisaldehyde sulphuric acid reagent	Phytosterols may be present
Ethyl acetate: methanol: water (100:13.5:10)			R_{f-} 0.68 (grey) R_{f-} 0.65 (violet)	R_{f-} 0.89 (brown) R_{f-} 0.78 (violet grey) R_{f-} 0.72 (grey) R_{f-} 0.67 (violet) R_{f-} 0.62 (purple)	R_{f-} 0.74 (grey) R_{f7-} 0.67 (violet grey)	Antimony-III-chloride reagent	Cardenolides may be present.
n-butanol : acetic acid : water (5:1:4)	---	---	---	R_{f-} 0.52 (reddish brown) R_{f-} 0.45 (brown)	R_{f-} 0.52 (sandy brown) R_{f-} 0.38 (yellow)	Ninhydrin reagent	Sugars may be present.

CONCLUSION:

The TLC study revealed presence of phytoconstituents like flavonoids, carbohydrates, saponins, phytosterols, phenolic compounds, triterpenoids, cardiac glycosides, fixed oils and tannins in fruits of *Cedrelatoona*Roxb.

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