

**Estimation of total tannins, total phenolics and total flavonoids of different extracts of *Cedrela toona* Roxb. Fruits.****Dr. Shah Kinjal H^{1*} and Dr. Patel Piyush M.²**¹ Professor, B. Pharmacy College, Rampura, Gujarat, India.² Controller of Examination, Gujarat University, India.**Article Info:** Received 15 December 2020; Accepted 04 January, 2021DOI: <https://doi.org/10.32553/jbpr.v10i1.830>**Address for Correspondence:** Dr. Shah Kinjal H**Conflict of interest statement:** No conflict of interest**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, phosterols, fixed oils and fats, proteins, amino acids, flavonoids, saponins etc. Total flavonoids content was measured with the 'Aluminium Chloride Colorimetric Assay Method'. Total Phenolic content in the aqueous and methanol extracts of fruits of *Cedrela toona* Roxb. were determined using the Folin Denis Method and expressed as mg of tannic acid equivalents per gram dry weight of extract. This study helps researchers for development of isolation method of active ingredient having vast pharmacological effects.

Keywords: *Cedrela toona*, total flavonoids, total tannins and phenolics, Tannic acid**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 – tufts; petiole 4-11 cm long, petiolules 5-12 mm long. Penicels 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, antidyseric, antiperiodic.^[5] Flowers are emmenagogue, leaf is spasmolytic, hypoglycaemic and antiprotozoal.^[6] Bark and heartwood yielded tetraterpenoids, including toonacillin. Heartwood also gave a coumarin geranyl gernalol as its fatty esters. Toonacillin and its 6 – hydroxyl derivatives are antifeedent.^[5]

Materials and Methods^[7-22]**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 India¹. 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.

Preliminary phytochemical screening**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 50⁰c. Each time before extracting with the next solvent, the marc was dried in an air oven below at 50⁰c. 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.

Determination of total flavonoids content of leaves of *Cedrela toona* Roxb.

Aluminum chloride colorimetric assay method:^{23,24}

Total flavonoids content was measured with the 'Aluminum chloride colorimetric assay method'. Aqueous and methanol extracts of leaves of *Cedrela toona* Roxb. that has been adjusted to come under the linearity range i.e. (400 µg/ml) and different dilution of standard solution of Quercetin and Rutin (10-100 µg/ml) were added to 10ml volumetric flask containing 4ml of water. To the above mixture, 0.3ml of 5% NaNO₂ was added. After 5 minutes, 0.3ml of 10% AlCl₃ was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as percentage of Quercetin and Rutin equivalent per 100 g dry weight of sample.

Determination of total phenolic content of leaves of *Cedrela toona* Roxb.^{25,26}

Folin – Denis Method:

Total Phenolic content in the aqueous and methanol extracts of leaves of *Cedrela toona* Roxb. were determined using the Folin-Denis method and expressed as mg of tannic acid equivalents per gm dry weight of extract. The absorbance values of the test extracts after subtraction of control (y) were translated into total phenolic content (mg/gm of TAEs) using the tannic acid calibration plot with the following formula:

$$[\text{Total phenolic content (mg/gm of TAEs)} = y - 0.0004 / 0.0012]$$

Principle: The polyphenolic compounds are estimated by spectrophotometric method known as Folin-Denis method. The method is based on the oxidation of the molecules containing a phenolic hydroxyl group. The tannins and tannin like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly blue coloured solution, the intensity of which is proportional to the amount of tannins and phenolic compounds and can be estimated against standard tannic acid solution.

Preparation of Reagents:

1) Folin-Denis Reagent: Sodium tungstate (10 gm) and phosphomolybdic acid (2 gm) were dissolved in distilled water (75 ml) along with phosphoric acid (5 ml). The mixture was refluxed for 2 hours and volume was made with water up to 100 ml.

2) Sodium carbonate solution: sodium carbonate (35 gm) was dissolved at 70-80°C in distilled water and volume was made up to 100 ml. It was filtered through glass wool after allowing it to stand over night.

3) Working standard solution of Tannic acid: Accurately weighed standard Tannic acid (100 mg) was dissolved in distilled water in volumetric flask. 5 ml of this solution was diluted with water to 100 ml in another volumetric flask to give 50 µg/ml Tannic acid solutions.

4) Preparation of solution: A series of calibrated 10 ml volumetric flasks were taken and appropriate aliquots of standard Tannic acid solution ranging from 1.6, 2, 2.4, 2.8, 3.4, 4, 5, 6 ml were added. To these solutions Folin-Denis reagent (0.5 ml) and Sodium Carbonate solution (1 ml) and Distilled water (up to 10 ml) were added. And the absorbance was measured at 700 nm within 30 min of the reaction. The calibration curve was prepared and concentration of total phenolic compound was found out from methanol and water extract by taking their absorbance respectively.

5) Preparation of test solution:

a) Methanol Extract: Take 2 gm dried powder obtained from methanolic extract. Solubilize it into 50 ml methanol and take 0.5 ml of this solution and dilute up to 10 ml with methanol. Take 1 ml of this solution and dilute up to 10 ml with methanol and take this solution as a test solution.

b) Aqueous Extract: Take 5 gm dried powder obtained from Water extract. Solubilize it into 20 ml water and take 0.1 ml of this solution and dilute up to 10 ml with methanol. Take 1 ml of this solution and dilute up to 10 ml with methanol and take this solution as a test solution.

Determination of total tannin content of fruit extract of *Cedrela toona* Roxb.^{27,28}

Take 0.1 gm of both extracts (aqueous and methanol) of *Cedrela toona* Roxb., dissolved in 10 ml of distilled water separately, add 10ml of indigo carmine dye, add 300 ml of distilled water, heat it at 60-70°C and titrate with 0.1 M KMnO₄. Carry out same experiment by omitting substance.

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 °c)	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

Total flavonoids content of *Cedrela toona* Roxb. Fruits

Total flavonoids content in the methanol and aqueous extracts of fruits *Cedrela toona* Roxb. were determined according to the 'Aluminum chloride colorimetric assay method'. The total flavonoids content of methanol and aqueous extracts of *Cedrela toona* Roxb. are shown in (Table 2 & 3).

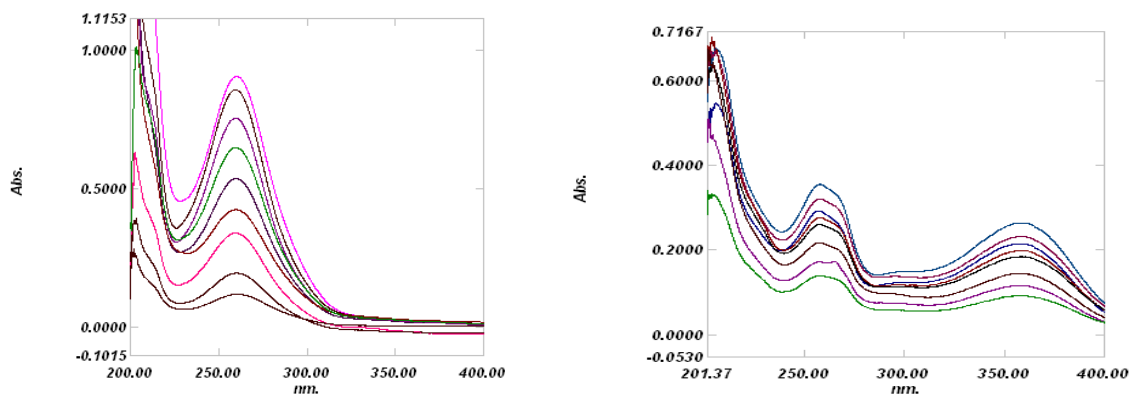


Figure 1: Overlay spectra of Quercetin and Rutin in *Cedrela toona* Roxb. extracts

Table 2: Quercetin content in *Cedrela toona* Roxb. extracts

<i>Cedrela toona</i> Roxb. extracts	Concentration µg/ml	Absorbance	Total Flavonoid %w/w
Quercetin	20	0.26	-
	30	0.313	-
	40	0.371	-
Quercetin	60	0.456	-
	80	0.561	-
	100	0.668	-
Aqueous	50	0.185	0.91
Ethanol	50	0.201	1.56

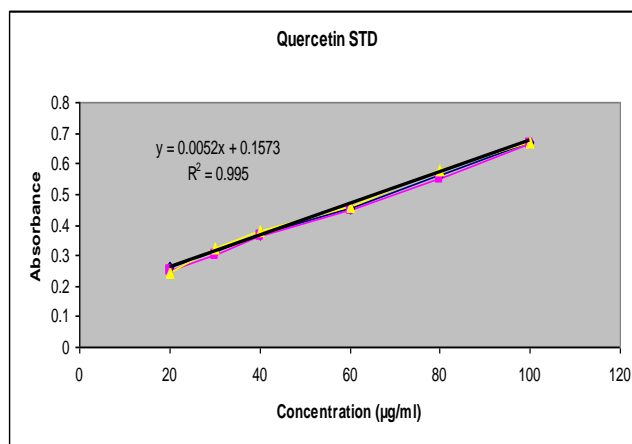


Figure 3: Calibration curve of Quercetin

Result indicated that Quercetin gave maximum absorbance at λ_{\max} 257 and linear relationship with concentration and absorbance between the concentration applied. ($Y= 0.081X+0.1573$; $R^2 =0.995$). Aqueous extract of *Cedrela toona* Roxb. was found to contain total Flavonoid 0.91%w/w equivalent to Quercetin. Ethanol extract of *Cedrela toona* Roxb. contained total Flavonoid 1.56%w/w equivalent to Quercetin. Flavonoid content was higher in alcoholic extract of *Cedrela toona* Roxb. fruits extract than water extract.

Table 3: Rutin content in *Cedrela toona* Roxb.

<i>Cedrela toona</i> Roxb.	Concentration ($\mu\text{g/ml}$)	Absorbance	Total Flavonoid %w/w
Rutin	20	0.256	-
	30	0.342	-
	40	0.431	-
	60	0.578	-
Rutin	80	0.789	-
	100	0.898	-
Aqueous	50	0.189	1.85
Ethanol	50	0.207	2.54

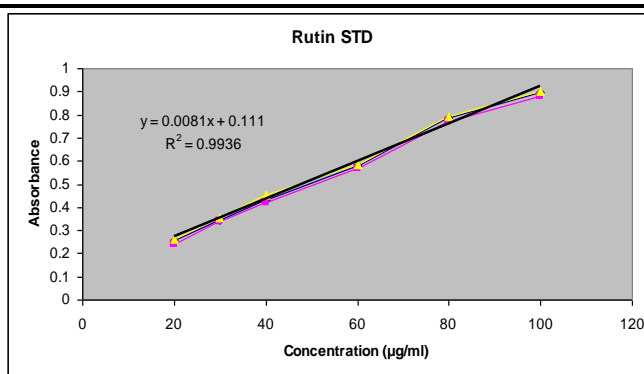


Figure 4: Calibration curve of Rutin

Result indicated that Rutin gave maximum absorbance at λ_{\max} 254 and linear relationship with concentration and absorbance between the concentration applied. ($Y= 0.081X+0.1573$; $R^2 =0.995$). Aqueous extract of *Cedrela toona* Roxb. was found to contain total Flavonoid 1.85%w/w equivalent to Rutin. Ethanol extract of *Cedrela toona* Roxb. contained total Flavonoid 2.54%w/w equivalent to Rutin. Flavonoid content was higher in alcoholic extract of *Cedrela toona* Roxb. leaves extract than water extract.

Total phenolics content of *Cedrela toona* Roxb. fruit extracts

Total phenolics: Total Phenolic content in the aqueous and ethanol extracts of fruits of *Cedrela toona* Roxb. were determined using the 'Folin-Denis method' and expressed as mg of tannic acid equivalents per gm dry weight of extract (Table 5.12). The absorbance values of the test extracts after subtraction of control (y) were translated into total phenolic content (mg/gm of TAEs) using the tannic acid calibration plot with the following formula:

Total phenolic content (mg/gm of TAEs) = $y - 0.0004 / 0.0012$

Table 5: Total phenolics content of *Cedrela toona* Roxb. fruit extracts

<i>Cedrela toona</i> Roxb. extract	Concentration $\mu\text{g/ml}$	Absorbance	Total Phenolic content %w/w
Tannic acid	5	0.22	-
	10	0.313	-
	15	0.412	-
	20	0.472	-
	25	0.588	-
	30	0.645	-
	Water	50	0.236
Ethanol	50	0.31	18.44

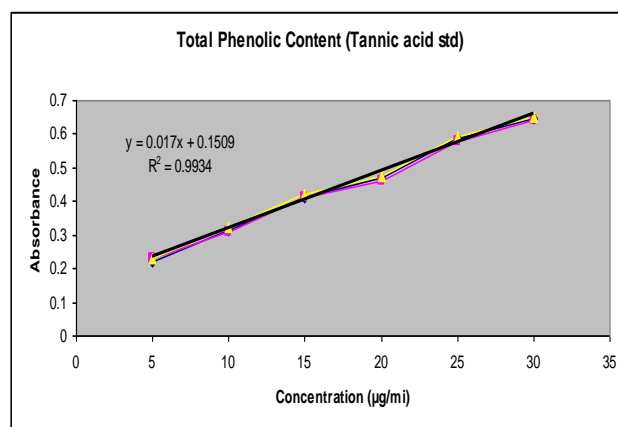


Figure 5.9: Calibration curve of tannic acid

Total phenolic content calculated in terms of tannic acid in the aqueous and methanol extracts of fruits of *Cedrela toona* Roxb. were found to be 9.44 %w/w and 18.44 % w/w respectively.

Total tannin content in *Cedrela toona* Roxb. fruits extracts

Total tannin content was determined by titrimetric method. Results indicated that total tannin content in aqueous and ethanol extracts of leaves of *Cedrela toona* Roxb. were 24.55 %w/w and 28.31 % w/w respectively equivalent to tannic acid.

Conclusion:

Total flavonoids content in the methanol and aqueous extracts of fruits of *Cedrela toona* Roxb. were determined according to the 'Aluminum chloride colorimetric assay method'. Aqueous extract of *Cedrela toona* Roxb contains total flavonoids 0.91%w/w equivalent to Quercetin. Methanol extract of *Cedrela toona* Roxb contains total flavonoids 1.56%w/w equivalent to quercetin. Flavonoid content was higher in alcoholic extract of fruits of *Cedrela toona* Roxb than water extract.

Total phenolics content in the aqueous and methanol extracts of fruits of *Cedrela toona* Roxb. were determined using the 'Folin-Denis method' and expressed as mg of tannic acid equivalents per gm dry weight of extract. Total phenolics content in the aqueous and methanol extracts of fruits of *Cedrela toona* Roxb were found to be 9.44 %w/w and 18.44 % w/w respectively.

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