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Investigation of phytoconstituents along with total phenolic and flavonoids in methanolic and aqueous extract of bark of *Prosopis cineraria* (L.) Druce

Anirudh Khatri, Anita Rathore and Chandra Prakash Jain

Abstract
Prosopis cineraria (L.) Druce is an important medicinal plant of Thar Desert of Rajasthan. The present study deals with the phytochemical analysis of constituents of methanolic and aqueous extract of the bark and quantitative analysis of total phenol by Folin- Ciocalteu reagent method and flavonoids determination by using Aluminum chloride colorimetric method. Alkaloids, glycosides, steroids, carbohydrates, phenolic compounds, saponins, terpenoid, resins, flavonoids, tannins, protein, and amino acid were present in phytochemical analysis. Total phenolic content in methanolic and aqueous extract of the bark of *Prosopis cineraria* was found to be 370 mg/g, 280 mg/g and total flavonoid content was found to be 145.6 mg/g, 122.8 mg/g respectively.

Keywords: phytochemical analysis, quantitative analysis, total phenol, total flavonoid.

Introduction
Prosopis cineraria (L.) Druce fam: Leguminosae, known locally as khejri or janti, is a state tree of Rajasthan and Telangana. It is a deep-rooted, nitrogen-fixing and multipurpose tree endemic to the hot deserts of India; it is contributing to the ecological stability of the region and providing extensive support to human beings, livestock and the nutrient deficient soils. Prosopis has been found to contain 5-hydroxytryptamine, prosogerin A, prosogerin B, prosogerin C, prosogerin D and prosogerin E, apigenin, isorhamnetin-3-diglucoside, l-arabinose, patuletin, patulitrin, luteolin, rutin, quercetin, sitosterol, spicigerine, tannin, and tryptamine. Leaves are nutritional and highly palatable source of fodder for major desert livestock, such as camel and cattle. Pods increase milk production in milch animals. Leaf paste of khejri is applied on boils and blisters, in livestock and leaf infusion is used to treat open sores on the skin. The bark of the tree is dry, acrid, and bitter with a sharp taste, anthelmintic, tonic, cures leprosy, dysentery, bronchitis, asthma, leucoderma, piles and tremors of the muscles. Its pollen serves as a dietary source for mice [1-5].

Collection and identification of plant material
The sample of *Prosopis cineraria* was collected in the month of February 2016 from Desert area of Shekhawati region (Rajasthan). Authentication (Identification) of plant materials was done by Dr. Vinod Maina, Botanical Survey of India, Jodhpur (Rajasthan). The voucher number is BSI/AZRC/ I.12015/Tech./2015-16- (PI. Id.)/790, Dated 01/03/2016.

Preparation of plant extract
The bark of Prosopis was dried initially under shade at room temperature after washed with natural water twice and preserved in polyethylene bag. The dry bark sample (480 g) was ground using grinder (Lords Mixer Grinder, Model no. Hummer-1200, New Delhi) and packed in sealed plastic bottle until extraction. Dried and powered bark defatted firstly in Soxhlet apparatus with petroleum ether to remove fatty material from the extract and defatted plant material was subjected to Soxhlet extraction with methanol. Extraction was confirmed by pouring a drop of extract from the thimble on a filter paper, which does not show the presence of any oil spot on that. Dried and powdered bark was macerated with distilled water (5 days) to obtain the aqueous extract. After complete extraction the solvent was evaporated by using

rotary vacuum evaporator (Macro Scientific Works Pvt. Ltd., Model no. MSW-192, New Delhi) to give amorphous solid masses.

Preliminary phytochemical investigation

The methanolic and aqueous extract of the bark of *Prosopis cineraria* subjected to qualitative phytochemical analysis. Any change of colours or the precipitate formation was used as indicative of positive response to these tests. [6-8].

Determination of total phenol by Folin- Ciocalteu reagent method

The amount of total phenolics in extract was determined with the Folin- Ciocalteu reagent [9]. Gallic acid was used for the calibration curve (Figure 1) as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). For this purpose, gallic acid (3 mg) was dissolved in 10 ml of methanol which make 300 mg/L concentration. Serial dilution of concentration 200, 100, 50 and 25 mg/L was prepared by diluting the stock solution with methanol. The absorbance was measured for all standard solutions by using UV-spectrophotometer at constant wavelength of 750 nm.

Extracts of the bark of *Prosopis cineraria* (1 g) were dissolved in methanol (1 ml). Folin- Ciocalteu reagent (10 ml) was dissolved in water (90 ml) to make 10% solution. Then sodium carbonate (3 g) was dissolved in water (50 ml) to make 5% solution. Sample (200 µl) was taken in a test tube and 10% Folin- Ciocalteu reagent (1.5 ml) was added. After that test tube was kept for 5 minutes in a dark place. Finally, 5% Na_2CO_3 (1.5 ml) was added to the solution, mixed well and kept in the dark for 2 hours. The absorbance was measured by using UV-spectrophotometer at constant wavelength of 750 nm.

Table 1: Absorbance of Standard Compound (Gallic Acid)

Concentration (mg/L)	Absorbance (Mean) λmax =750 nm
25	0.03
50	0.062
100	0.156
200	0.239
300	0.363

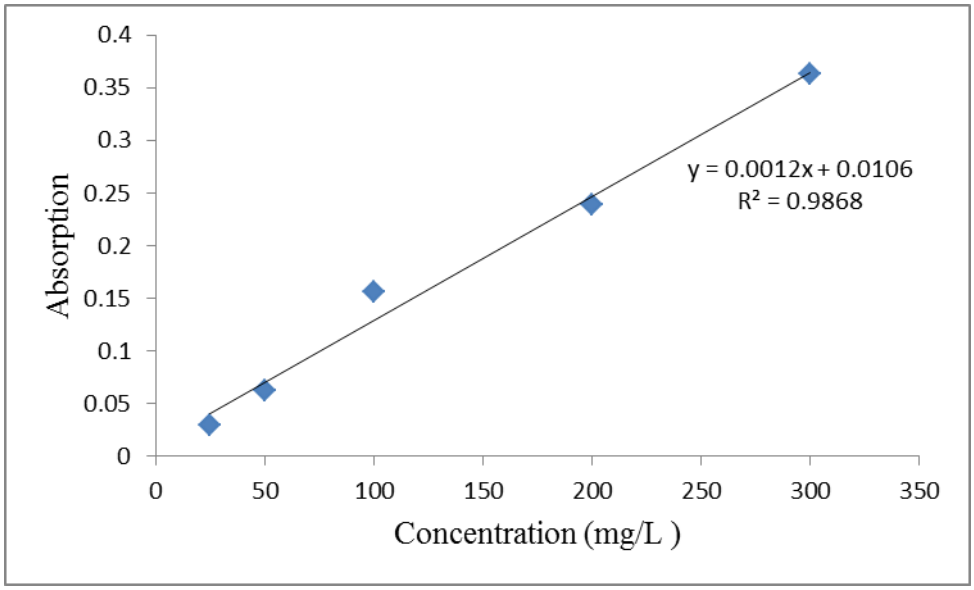

Fig 1: Gallic acid standard curve.

Determination of total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination [10]. 5% sodium nitrate was prepared by dissolving sodium nitrate (2.5 g) in water (50 ml) in a volumetric flask. 5% sodium hydroxide was prepared by dissolving sodium hydroxide (2.5 g) in water (50 ml) in a volumetric flask. Then 10% aluminium chloride solution was prepared with the same procedure. Plant extracts (0.25 mg) were taken in a test tube and dissolved in water (1.25 ml) then 5% sodium nitrate (0.75 µl) was added and mixed together. After that test tube was kept for 6 minutes in a dark place. Then 10% aluminium chloride solution (0.150 µl) was added into the test tube and wait for 5 minutes in dark for complete reaction. Finally, 5% sodium hydroxide (0.5 ml) and water (0.275 ml) were added to the test tube. The absorbance was measured by using UV-spectrophotometer at a constant wavelength of 510 nm. Quercetin was used for the calibration curve (Figure 2) as a standard and flavonoid contents were measured as quercetin equivalent. The estimation of total flavonoids contents in the crude extracts was carried out in triplicate and the results were averaged.

Table 2: Absorbance of Standard Compound (Quercetin)

Concentration (mg/L)	Absorbance (Mean) λmax =510 nm
25	0.675
50	1.263
100	2.395
200	4.721
300	7.662

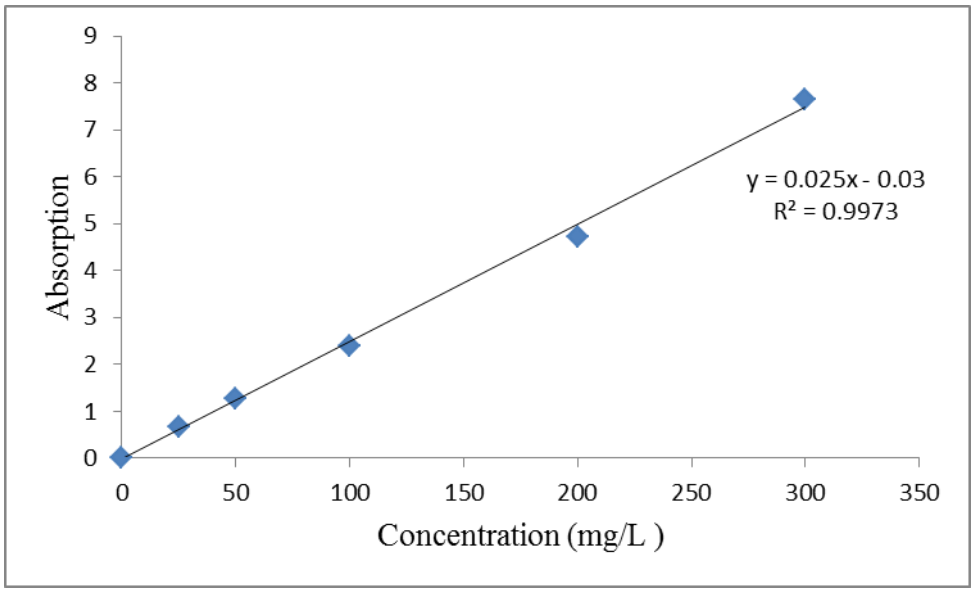

Fig 2: Calibration curve of quercetin standard.

Results

Phytochemical analysis indicated that the plant contains alkaloids, glycosides, steroids, carbohydrates, phenolic compounds, saponins, terpenoid, resins, flavonoids, tannins, protein, and amino acid. The result obtained from phytochemical investigations by different identification test was enlisted in table 3.

Table 3: Phytochemical investigation of *Prosopis cineraria* bark

S. No.	Phyto-constituent	Identification test	Methanolic extract of Prosopis	Aqueous extract of Prosopis
1	Alkaloid	Mayer's Test	+ (Yellow- white ppt.)	+ (Yellow- white ppt.)
		Wagner Test	+ (Reddish-brown ppt.)	+ (Reddish-brown ppt.)
		Hager Test	+ (Yellow ppt.)	+ (Yellow ppt.)
		Dragendroff's Test	+ (Orange ppt.)	+ (Orange ppt.)
2	Glycoside	Keller Killani Test	-	+ (Reddish-brown layer at junction of two liquid and upper layer becomes bluish-green on standing)
		Borntrager's Test	+ (Red colour)	+ (Red colour)
		Legal Test	+ (Pinkish-red colour)	+ (Pinkish-red colour)
3	Protein and Amino Acid	Biuret Test (for protein)	+ (Purple colour)	+ (Purple- violet colour)
		Ninhydrin Test (for amino-acids)	+ (Blue colour)	+ (Blue colour)
		Millon's Test (for soluble proteins)	+ (Yellow ppt.)	+ (Yellow ppt.)
		Xanthoprotein Test (for aromatic amino acid)	+ (Yellowish-orange colour)	+ (Orange colour)
4	Steroids	Salkowaski Test	+ (Bluish-red colour in $CHCl_3$ layer and green fluorescence in acid layer)	+ (Bluish-red colour in $CHCl_3$ layer and green fluorescence in acid layer)
5	Carbohydrates	Molisch's Test	+ (Purple ring)	+ (Purple ring)
		Benedict's Test	+ (Red ppt.)	+ (Red ppt.)
6	Phenolic compound and Tannins	Ferric chloride Test	+ (Dark blue)	+ (Greenish-black)
		Lead acetate Test	+ (White ppt.)	+ (White ppt.)
		Gelatin Test	+ (White ppt.)	+ (White ppt.)
7	Flavonoids	Aq. NaOH Test	+ (Light yellow colour)	+ (Yellow to orange colour)
		Conc. H_2SO_4 Test	+ (Crimson colour)	+ (Crimson- orange colour)
		Shinoda Test	+ (Orange-red colour)	+ (Cherry-red colour)
8	Saponins	Foam Test	-	+
		Hemolytic Test	-	+
9	Terpenoid	Chloroform-Conc.H_2SO_4 Test	+ (Reddish-brown colour)	+ (Reddish-brown colour)
10	Resins	Acetone-water Test	+ (Slight turbid)	+ (Slight turbid)
11	Fat and Fixed oil	Spot Test	-	-
		Saponification Test	-	-

The total phenolic content in extract was determined by Folin-Ciocalteu method which were reported as mg/g gallic acid equivalents (Figure 1) using the standard curve equation: y = 0.001x + 0.010, R^2 = 0.986, where y is absorbance at 750 nm and x is total phenolic content in methanolic extract of the bark of *Prosopis cineraria*. Total phenolic content in methanolic and aqueous extract of the bark of *Prosopis cineraria* was found to be 370 mg/g and 280 mg/g respectively.

The amount of total flavonoids was determined by Aluminum chloride colorimetric method. Quercetin was used as a standard compound and the total flavonoids were expressed as mg/g Quercetin equivalent (Figure 2) using the standard curve equation: $y = 0.025x - 0.03$, $R^2 = 0.997$, where y is absorbance at 510 nm and x is total flavonoid content in methanolic extract of the bark of *Prosopis cineraria*. Total flavonoid content in methanolic and aqueous extract of the bark of *Prosopis cineraria* was found to be 145.6 mg/g and122.8 mg/g respectively.

Discussion

Prosopis cineraria containing a diverse group of secondary metabolites which has unique and multifactorial medicinal properties. Research from the previous study suggests that 5-hydroxytryptamine have antidepressant activity, apigenin have antibacterial, anti-dermatitic, anti-inflammatory and anti-viral activity, isorhamnetin-3-diglucoside have hepatoprotective activity, quercetin have analgesic, anti-allergenic, anti-bacterial, anti-diabetic, anti-inflammatory and anti-viral activity, tryptamine has anti-amebic activity, tannin has antibacterial, anti-diarrheic and antiviral activity and flavonoids have anti-inflammatory and anti-oxidant activity [11]. In the present study, it was concluded that bark of *Prosopis cineraria* have the potential to act as a source of useful drugs because of the presence of various phytochemical constituents such as alkaloids, glycosides, steroids, carbohydrates, phenolic compounds, saponins, terpenoid, resins, flavonoids, tannins, protein, and amino acid.

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