https://doi.org/10.48047/AFJBS.7.2.2025.842-849

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Isolation and Characterization of flavonoid from Leaves of *Prosopis cineraria*

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Volume 7, Issue 02, Jan 2025

Received: 15 Dec 2024

Accepted: 05 Jan 2025

Published: 29 Jan 2025

doi:10.48047/AFJBS.7.2.2025.842-849

ABSTRACT
Prosopis cineraria are small tree generally found in dry arid regions of Arabia and India mainly in Rajasthan. Its state tree of Rajasthan. The flavonoids contained in *Prosopis cineraria* barks were, identified and characterized. The extraction of bark of *prosopis cineraria* by using sequential soxhelation, various solvents is used according to polarity Petroleum ether, Chloroform, Ethyl-aceatate, Methanol and water. The metabolic extract was subjected to phytochemical screening. TLC Thin layer chromatography and HPLC for isolation of compound and characterization of isolated compound was done by UV, HPLC and IR. onthe basis of comparisons of spectral data of isolated compound with respects to spectral data of standard rutin. The result show maximum yield of the flavonoids (17.6 gm) was obtained from methanolic extract; phytochemical screenings were done by various specific tests of flavonoids. The Rf value (0.62) of isolated compound has been compared with Rf value of standard rutin. Rf value of isolated compound 4.41 in mobile phase ACN: Water with 0.5 formic acid at lamda max 272. Characterization of isolated compound was done by UV, HPLC,IR ,on the basis spectral analysisstructure was elucidated as 2-(3,4 di hydroxyl phenyl)-5,7 di hydroxyl-3-[α-L-rhmnosyl-(1-6)-β-Dglucopyranoyloxy]-4H hydroxyl chromen-4-one, rutin was isolated for the first time from this plant (Prosopis cineraria).

KeywordProsopiscineraria, flavonoids,rutin,chromatography

1. INTRODUCTION

1.1 Plant Description:

Prosopis cineraria (Mimosaceae) is a small sized tree found in the regions of Arabia and various parts of India such as Rajasthan, Gujarat, Haryana, Uttar Pradesh and Tamilnadu*Prosopis cineraria* commonly known as "Khejari" in Rajasthan [1]. It is the State tree of Rajasthan [2] . India. Khejariis the golden tree of Indian deserts, plays a vital role in preserving the ecosystem of arid and semi-arid areas. Since all the parts of the tree are useful, it is called kalptaru. It is also known as the 'wonder tree and the king of desert [3].

Prosopis cineraria are a small tree, ranging in height from 3-5 m (9.8-16.4 ft). Leaves are bipinnate, with seven. to four teen leaflets on

each of one to three pinnate. Branches are thorned along the internodes [4]. Flowers are small and creamy-yellow, and the tree is found in extremely arid conditions, with rainfall as low as 15 cm annually. As with some other *Prosopis spp.Prosopis cineraria* has demonstrated a tolerance of highly alkaline and salineenvironment [6].

1.2 Phytochemical constituents

Various types of chemical constituents are isolated from different part of *prosopis cineraria*. Flower of *P.cineraria* contain Patuletin and Patulitrin[7], leaves containdifferentsteroids such as sitosterol and cholesterol and spicigerin alkaloids [8] and seed of *prosopsopis cineraria* having large no. of constituents like ProsogenineCand Prosogenine D [9]. andluteolin. Seed of *P.cineraria* having maximum partition of unsaturated lipid. Pods of P. cineraria contain lenoleic acid and 7-glycoside [10] .The whole plant of *prosopis cineraria* having multiple phenolic compound and Flavonoides[11].

2. MATERIAL AND METHODS.

2.1 Extraction

Leaves of *Prosopis cineraria, Family*fabacea**.** The aerial part (leaves) of *Prosopis cineraria* (Shami) were collected in month of November (15/09/2017) from Rajasthan .They were authenticated from Botanical survey of India. Barks of *prosopis cineraria* were collect and kept for Shade drying at a room temperature. Dried leaves convert into course powdered with the help of mixer. Then powders were extract withvarious solvents is used according to polarity Petroleum ether, Chloroform, Ethyl-aceatate, Methanol and water. All extracts are dried with the help of rotary evaporator. Then dry methanolic extract treated with Petroleum ether in a separating funnel. This process is repeat in two times. Discard the layer of ether and alcoholic layers were used for continus study[12].

Selection of part of plants (bark)

↓

Barks, convert into small coarse powder (100gm)

↓

Extraction with various solvents by Soxhletion

↓

All extracts are dried with the help of rotary evaporator

↓

Methanolic extract (17.6 gm)

↓

Extract treated with Petroleum ether (II)

↓

Discard the alcoholic layer and blackish brown precipites were obtained.

↓

Phytochemical screening

↓

TLC & Characterization by UV, IR, HPLC.

2.2 Phytochemical chemical screening.

Photochemical screening: -Photochemical investigations were conducted employing different phytochemical test and the test phytochemical constituents were co detected as elaborated by Khandelwal (2001) and Kokate, (2001)[13]. Phytochemical screening is done by following procedure and result show in table no.1.

1. Sinoda test: 2 ml of filtrate, and few drops of conc. HCl then magnesium turnings were added .Magenda or pink red colour is obtained if flavonoides are present [14].

2. Alkaline reagents: 2 ml extract and 2ml NaOH solution were mixed then added few drops .Intense yellow color were obtained if flavonoids are present[15]

3. Lead acetate test: Firstly 2 ml of methanolicextraxt mixed with 10% solution of Lead acetate .White precipitates were obtained, if flavonoids are present.

4. Zinc Hydrochloric test: Zinc dust mixed with extract then added some few drop of HCL. Red colour is obtained, if flavonoids are present.

2.3 Thin layer Chromatographic analysis

Thin layer chromatography is a technique used for the separation, identification and estimation of mixture of component from different type of such as crude drug, synthesize component [16]. This technique work on the adsorption

principle. In which technique two phase one is mobile phase another phase is stationary phase. This technique is very effective for separation and identification.

Development of TLC Chamber: -

Developing the chamber for TLC can be designed, a beaker with watch glass on the top. Pour solvents into the chamber to deapth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapour and can be line part of the inside of the beaker with filter paper and cover the beaker with a watch glass.

Preparation of TLC plate: -

Thin glass plate (20x20) were coated with silica gel G .The fresh Prepared plates were air dried at a room temperature for 5 to 10 min, then prepared plates were activated at 100°C for 20 to 30 min and cooled at normal temperature.

Preparative TLC:-

TLC was performed on the precoated silica gel TLC plates (0.2mm thick) grade 60 F254 to determine the no. of spots are present in methanolic extract of *Prosopis cineraria*spotting the crud extract as well as standard marker (rutin), at 1cm from the bottom of TLC plates with the help of thin capillary tubes ,than the plat saw in UV chamber for justification of spot. Then development of chromatogram was done in chromatographic chamber saturated with mixture of solvent Toluene : ethyl acetate :formic acid (4: 6:0.5), then after some time developed plates were air dried at room temperature for 1 to 2 min . Then saw in UV-chamber in long wavelength and short wavelength.The isolates were also subjected to ultraviolet and infrared spectral studies.

Rfvalue : Distance travelled by solute / Distance travelled by solvents.

Calculated Rf value value Shaw in result & discussion,fig no.1.

2.4 Qualitative characterization of isolated compound

Qualitative characterization of isolated compound by various analytical techniques HPLC, IR Spectroscopy, LC-MS and NMR

2.4.1 Characterization by UV- Visible Spectrometry

Preparation of standard: - We preparedstock (1000 mcg/ml) solution of standardrutin ,by 10 mg standard rutin was dissolved in 2 ml methanol in a volumetric flask (10 ml) then volume makup to mark ,then prepared sub-stock solution (100 mcg/ml and show absorbance in Shimadzu UV- Spectrometer in between range 200 to 500 nm.

Preparation of sample: -

Dry isolated methanolic extract (isolate from plant) was dissolved methanol (volumetric flask). Methanol was taken as reference solvent .then volume makeup to the mark and show absorbance in Shimadzu UV Spectrometer

2.4.2.Characterization by High Performance Liquid Chromatography

High-performance liquid chromatography is a separation technique which is used for separation, isolation and identificatication of mixture of compound. HPLC analyses were performed by using Shimaddzu HPLC system, LC solution software.

Preparation of mobile phase: -

The mobile phase prepared by mixing of acetonitrile and water with 0.5% formic acid (70 : 30) and maintain pH 3.2 .The mobile phase were degassed by sonication with the help of sonicatore and they were filtered through vacuums filtration before HPLC analysis [17]..

Preparation of standards: -

We prepared stock solution (1000 mcg/ml), by 10 mg standard rutin was accurately weighed and dissolved into 2 to 3 ml methanol in a volumetric flask (10 ml) and volume make by methanol up to the mark .The methanol is a reference solvent. Then sub stock solution (100 mcg/ml) was prepared from previously

prepared fresh stock solution .Then prepared further dilution 10 mcg /ml solution was prepared from sub-stock solution ,then it was filtered through 0.4 µm membrane filter. The samples were analyzed by RP- HPLC for qualitative estimation of standard rutin by Shimadzu model HPLC equipped with , O.D.S. Hypersil C18 Column (250 mm × 4.6 mm, 5 µm particle size), flow rate 1 ml/min. and wavelength (270nm and 340nm) programmable UV/VIS detector and built-in L.C. solution software was used for drug analysis[18].

Preparation of sample:-

Take 1mg of isolated compound dissolved in 2 to 3 ml of methanol in a volumetric flask (10 ml) and make up the volume by methanol up to mark . Than it was filtered through 0.4 µm membrane filter. The samples were analyzed by RP- HPLC for qualitative estimation of rutin by Shimadzu model HPLC equipped with , O.D.S. Hypersil C18 Column (250 mm × 4.6 mm, 5 µm particle size), flow rate 1 ml/min. and wavelength (270nm and 340nm)programmable UV/VIS detector and built-in L.C. solution software was used for drug analysis.

Then compared chromatogram of standard rutin and isolated rutin. And compared RT value of both standards rutin and isolated compound.

2.4.3. Characterization by IR:-

IR spectroscopy is the most convenient technique which is used for identification functional group of the compound .The bond between the vibrate atom and vibration motion-Stretching and bending. In stretching, change the bond length and the in bending change the bond angle .Bonding devided into different type Wagging, Scissoring, Rocking and Twisting. The infra red portion of the electromagnetic spectrum is generally classified into three regions, near, mid and far-infrared. Visible reason of IR spectra is approximately 1400 to 4000.

The IR spectrum of isolated constituents was determined by Jasco Japan spectrometer. We prepared sample for IR characterization few amount of isolated compound (1 to 2 mg) mixed with KBR (2mg to 4mg) prepared sample. And Sample take in a sample holder, then placed into spectrometer and Shaw spectra. IR spectra of isolated compound were compared with IR spectra of standard rutin.

3. RESULT AND DISCUSSION

3.1Phytochemical screening.

The result of different type of qualitative test performed in the laboratory which is Shaw table. The presence various phytochemical constituents like Alkaloids, Flavonoides, Phenolic compound, Steroids' and Amino acid. Posivative (+) sign is indicates presence and Negative (-) sign is indicates absence of phytochemical constituent.

Observation table No.1 Test for Flavonoides

S.No.	Reagent & Test	Methanol Extract
1.	Shinoda Test	+
2.	Alkaline Reagent Test	+
3.	Lead Acetate Test	+
4.	Zinc Hydrochloride Test	+

Thin Layer Chromatography analysis :

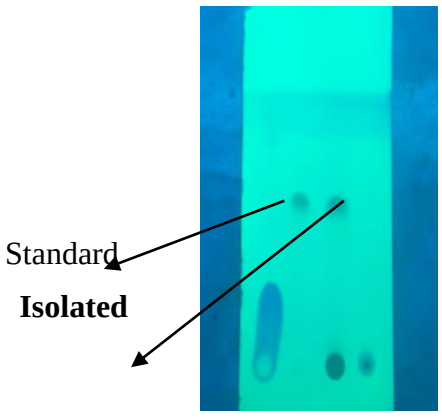

Fig. 1. TLC plate

Distance travelled by solute = 3.1 cm

Distance travelled by solvent = 5 cm

Rf value = Distance travelled by solute / Distance travelled by solvent

Rf value = 3.1/5

= 0.6

Rf value of test = 0.62

3.2 Characterization by UV-Visible spectroscopy:

UV Spectra: - The UV spectra of rutin in a methanolic solution show two absorbance bands at 257 and 360 nm

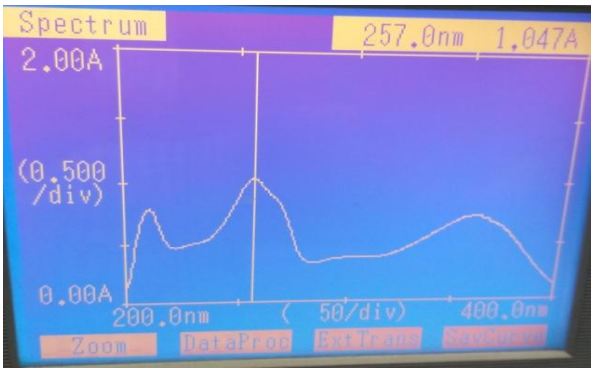

Fig. .2 UV Spectra of standard rutin

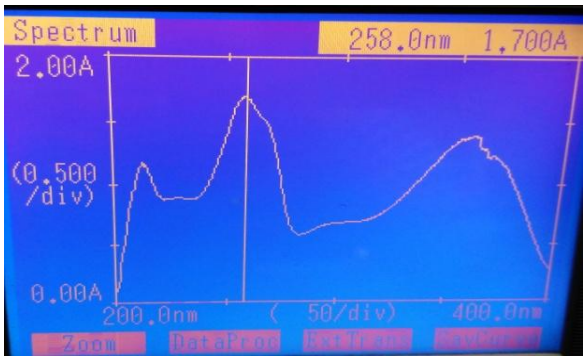

Fig..3.UV Spectra of isolated compound

Observation: λmax of standard rutin is 257 in a methanol and λmax of isolated compound were to be observed.

3.3 Characterization by HPLC:

Isolatedcompoundwas characterized by most convenient technique HPLC. Compared chromatogram of standard rutin and isolated rutin. And compared RT value of both standards rutin and isolated compound. Which showin Fig no.

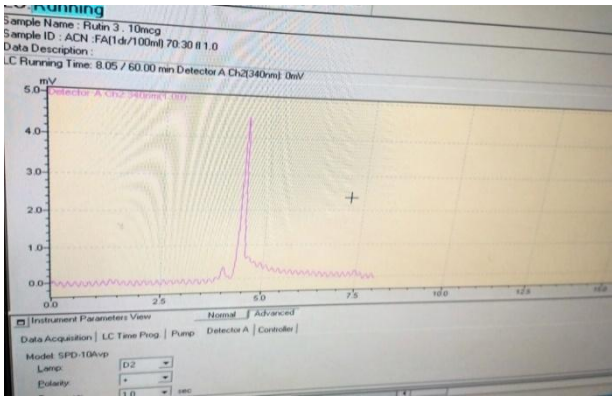

Fig.4 St.rutinchrometogram

Fig. 5 .Chrometogram of isolated compoun

Observation Table .2.

3.4Characterization by IR:

It's a most convenient technique for identification of functional groupIdentification of isolated compound by using IR spectroscopy Compare IR spectra of standard rutin ,and IR spectra of isolated compound which is show in given spectra.

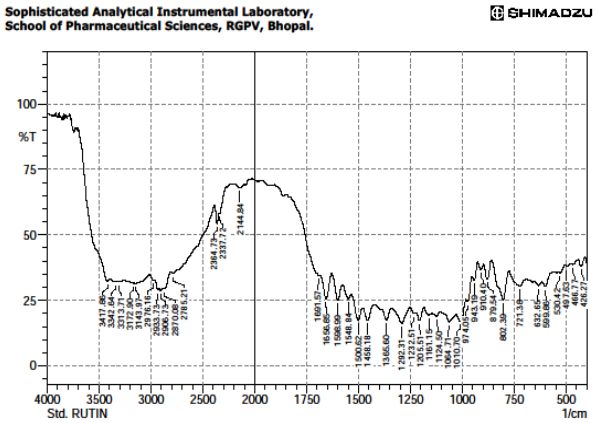

Fig. 6 IR Spectra of standard rutin

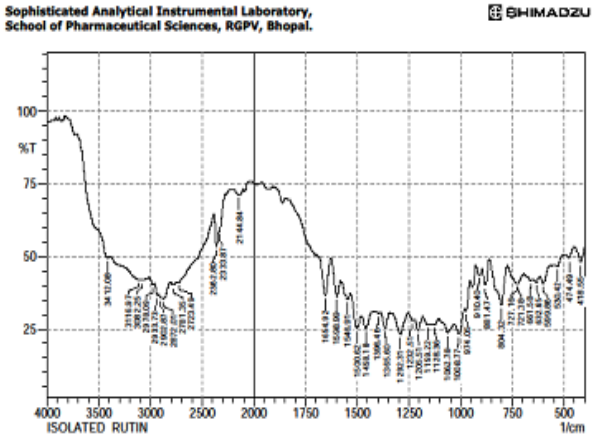

Fig. 7. IR spectra of isolated compound

S.No.	Sample	Mobile phase& ratio	λ max value	RT value
1.	Standard	ACN :water with formic acid(70:30)	272	4.43
2.	Isolated compound	ACN :water with formic acid(70:30)	272	4.41

S. No.	Functional group	Interpitate value
1	-OH	3412.08
2	-OH str.	3082.25
3	Phenolic -OH	3412.08
4	C-H starching	2978.09
5	C=O straching	1654.98
6	C=C straching	1558.99
7	CH	2872.01

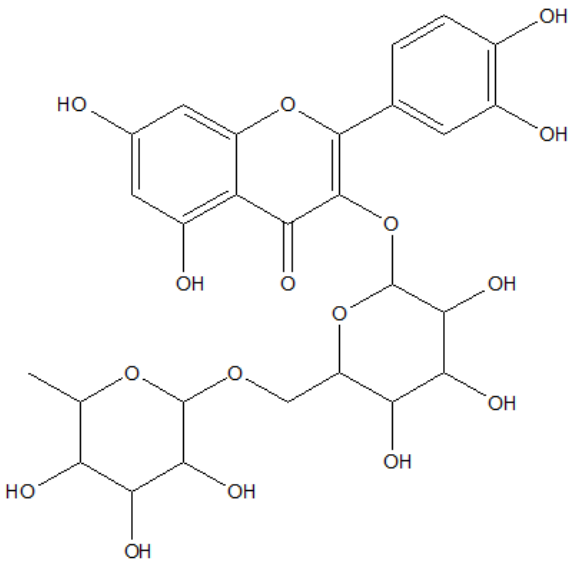

Fig .8 . Structure of rutin.

4.CONCLUSION

Prosopis cineraria are small tree generally found in dry arid regions of Arabia and India mainly in Rajasthan. Its state tree of Rajasthan. The flavonoids contained in *Prosopis cineraria* barks were, identified and characterized. The extraction of bark of *prosopis cineraria* by using sequential soxhelation, various solvents is used according to polarity Petroleum ether, Chloroform, Ethyl-aceatate, Methanol and water. The metabolic extract was subjected to phytochemical screening. TLC Thin layer chromatography and HPLC for isolation of compound and characterization of isolated compound was done by UV, HPLC and IR. onthe basis of comparisons of spectral data of isolated compound with respects to spectral data of standard rutin. The result show maximum yield of the flavonoids (17.6 gm) was obtained from methanolic extract; phytochemical screenings were done by various specific tests of flavonoids. The Rf value (0.62) of isolated compound has been compared with Rf value of standard rutin. Rf value of isolated compound 4.41 in mobile phase ACN: Water with 0.5 formic acid at lamda max 272. Characterization of isolated compound was done by UV, HPLC,IR ,on the basis spectral analysis. Rutin was firt isolate in the bark of *prosopis cineraria...*

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