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RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF ISOQUERCETIN - A FLAVONOID FROM BRYONIA LACINIOSA LINN.

Shashikala Metri¹, Dr. Ravindra Pal Singh² and Dr. Mamta Shah³.

1. Research scholar, department of pharmacognosy, gokaraju rangaraju college of pharmacy, hyderabad.
2. Professor, department of pharmaceutics, nims institute of pharmacy, jaipur.
3. Professor, department of pharmacognosy, I. M. College of pharmacy, gujarat.

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Abstract

Bryonia laciniosa Linn. (Family: Cucurbitaceae), is an annual climbing herb. Traditionally, it is mentioned in the Ayurvedic text in Vrishya Rasayana category and highly valued as a female fertility booster. The present study involves the isolation and identification of isoquercetin from methanolic extract of *Bryonia laciniosa* by using TLC, UV, FTIR, ¹HNMR and HR-LCMS techniques. The structure of the isolated compound was established by High-Resolution Liquid Chromatogram Mass Spectrometry and proton-NMR. The mass spectra of compound produced molecular ion peak (m/z) at 469.07. Proton-NMR showed two proton signals of H-6 and H-8 of ring 'A' and three aromatic proton signals of H-6', H-2' and H-5' of ring 'B' confirming 3',4' dihydroxy structure in the isolated compound corresponding to the aromatic ring 'B' of quercetin nucleus. The signal of glucose moiety at δ 5.299 indicated the isolated compound has β - linked glucose. Thus, confirming the isolated compound as isoquercetin, a flavonoid glucoside. This is the first paper to report a flavonoid from *Bryonia laciniosa*.

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Introduction:-

The traditional system of medicine consists of numerous medicinal plants, which have their potential therapeutic utilities. *Bryonia laciniosa* Linn. (Syn. *Diplocyclos palmatus* (Linn.) Jeffrey, Family: Cucurbitaceae), is an annual climbing herb, belongs to the genus *Bryonia*. It is commonly known as Shivlingi and found growing throughout India.

Traditionally, in Ayurveda, *B. laciniosa* is considered in Vrishya Rasayana category and is highly valued as a female fertility booster (Kirtikar *et al.*, 1988; Dwivedi *et al.*, 2007). It is also used in the treatment of skin diseases, paralysis of the tongue, snakebite. Extensive pharmacological activity has been reported which include anti-inflammatory (Gupta *et al.*, 2003), antidiabetic (Patel *et al.*, 2012) analgesic, antipyretic (Sivakumar *et al.*, 2004), antimicrobial (Bonyadi *et al.*), anti-asthmatic, anticonvulsant and antioxidant activities (Jayaram *et al.*, 2010). Preliminary phytochemical analysis of *B. laciniosa* reveals the presence of alkaloids, triterpenoids, steroids, saponins, flavonoids, proteins, and fixed oils and fats. Punicic acid (Gowrikumar *et al.*, 1981), goniotalamin (Mosaddik *et al.*, 2000) and glucomannan (Vandana *et al.*, 2006) were reported from seeds of the plant. But there are fewer reports on extraction and isolation of secondary metabolites from this plant. Flavonoids constitute an

Corresponding Author:- Shashikala Metri.

Address:- Research scholar, Ddepartment of Pharmacognosy, Gokaraju Rangaraju College of Pharmacy, Hyderabad.

important group of secondary metabolites having a various biological role in the plant and the humans. Thus, the present investigation aims to extract, isolate and identify the flavonoid from *B. laciniosa*.

Materials and methods:-

Plant material:-

The plant was collected from the agricultural hedges of Vijayapur District, Karnataka India. It was identified and authenticated by Botanist, Dr. P. S. Nagar, Botany Department, M.S University, Vadodara. The voucher specimen (No. LMCP/MSU/BOT/177/BI/Dp) was deposited in the herbarium of Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad.

Preparation of the extract:-

Shade dried whole plant material was crushed into small pieces and ground to a coarse powder. The coarsely powdered sample was successively extracted with the pet. ether, chloroform in Soxhlet apparatus to remove chlorophyll and fatty material and then extracted with 70% methanol. Methanolic extract was concentrated under reduced pressure in a rotary evaporator at $60^{\circ} \pm 2^{\circ}$ C and evaporated to dryness. The residue was suspended in water and shaken with ethyl acetate to get the flavonoid-rich extract. The process was repeated to ensure complete extraction. The ethyl acetate layer was pooled, combined and concentrated under reduced pressure. The dark brown colored mass was obtained and dissolved in methanol and used for preparative TLC.

Preparative Thin Layer Chromatography (PTLC):-

PTLC was performed according to the standard method (Markham, 1975; Wagner *et al.*, 1996). Methanolic extract was applied in the form of a band on silica gel G plates (0.3-0.4 mm thickness) and developed in mobile phase containing ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26), previously saturated for 20 minutes. After $\frac{3}{4}$ development, the plates were air-dried and visualized under UV TLC viewer at 366 nm. The prominent fluorescent band at Rf 0.45 was marked and scrapped into the beaker and suspended in 70 % methanol (Gaurav *et al.*, 2012). The liquid was centrifuged for 10 min and supernatants were collected, concentrated and vacuum evaporated. The residue was crystallized with chloroform. The purified compound was tested for the presence of flavonoids and subjected to TLC for the confirmation of the band at the same Rf with the extract.

Characterization of isolated compound:-

The isolated compound was characterized by UV, FTIR, HR-LCMS, and ^1H NMR studies.

Ultra Violet (UV)/ Visible Spectroscopy:-

The isolated compound was dissolved in methanol and scanned in a UV/visible double beam spectrometer (UV/Visible spectrometer LAB INDIA UV 3000+) in the range of 200-500 nm to record the spectra. Spectral shift was studied by adding 5% AlCl_3 powdered sodium acetate (NaOAc) and boric acid (H_3BO_3).

Fourier Transform Infrared (FTIR) spectroscopy:- KBR pressed pellet technique was employed to record FTIR spectra of isolated compounds to identify the functional groups present. Few crystals were mixed with KBR to ensure its uniform distribution and pressed in the pellet machine. The pellet was scanned in the FTIR spectrometer (FTIR 8400S, Shimadzu) in the range of $4000 - 500 \text{ cm}^{-1}$.

HR-LCMS (High-Resolution Liquid Chromatogram Mass Spectrometry) study:-

HR-LCMS of isolated Compound was carried out using Agilent Technologies, USA, mass spectrometry model-G6550AQ, instrument, version TOF B.05.01, attached to ESI-MFE ionization scanner. High-resolution liquid chromatography, coupled with a mass spectrometer (LC/MS) determines the molecular formula, molecular weight, retention time and structure of unknown compound through the fragmentation process. It was carried out at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Pawai, Mumbai. The spectra were recorded in the range of 100-950 m/z in +ve mode using 175.0 voltage. The data acquisition was done using the acquisition software 6200 series, TOF/6500 series.

^1H (Proton) NMR studies:-

^1H NMR of isolated Compound was recorded on Varian-NMR- Mercury 300. The instrument was operated at 300 MHz in the region from 0-12. The compound was dissolved in DMSO to record the spectra and spectra obtained was compared with data available in the literature.

Results and discussion:-

PTLC:-

PTLC separates the compounds on a microscale using a thick layer of adsorbent, sufficient for characterization and identification (Harborne *et al.*, 1975). Isoquercetin was isolated by Preparative TLC using flavonoid-rich ethyl acetate extract in the solvent system containing ethyl acetate: glacial acetic acid: formic acid: water. The extract showed a fluorescent band under UV at 366 nm and yellowish-brown under visible light which was collected and purified. The compound appeared as yellowish-white crystals, soluble in water and insoluble in alcohol and non-polar solvents. It gave positive tests for flavonoids, the Shinoda test and 10% NaOH test. It gave positive test for reducing sugars (Fehling's test) and confirms the presence of sugar moiety (Fig.1 A) (Harborne, 1998).

TLC studies:-

The isolated compound showed sharp single fluorescent spot on precoated silica gel GF₂₅₄ corresponding to the spot in the methanolic extract using ethyl acetate: glacial acetic acid: formic acid: water as solvent system. The R_f value of the isolated compound was matched with the R_f value of the spot in extract (Fig.1 B).

UV/ Visible and FTIR Spectroscopy:-

The methanol spectra of flavonoids exhibit two major absorption peaks in the region of 240-400 nm. These two peaks are commonly known as Band I (300-380 nm) and Band II (240-280). The isolated compound gave two main absorption peaks (Fig. 2), band I at 327.5 nm due to B-ring of cinnamoyl system and band II was observed at 270.2 nm due to A-ring benzoyl system of flavonoid moiety. UV Spectral shifts were also studied, the details of which are represented in Table 1 (Mabry *et al.*, 1970).

FTIR spectra mainly used to identify the types of functional group present in the compound. The isolated compound exhibited a broad peak at 3327.32 cm⁻¹ indicates the presence of hydroxylic (-OH) group, 1396.51 cm⁻¹ due to aromatic ring, 1654.98 cm⁻¹ was due to the carbonyl (C=O) group and -OH bending vibrations at 1288.49 cm⁻¹, 1159.26 cm⁻¹, 1080.17 cm⁻¹ (Fig. 3).

HR-LCMS study:-

HR-LCMS helps in the identification of unknown compounds. The spectrum of an unknown compound is compared with the spectrum of the known compounds stored in the software library. It gives information about the name, retention time, molecular weight, molecular formula and structure of compounds of the sample under the study. It can also be used when the standard is not available (Asha *et al.*, 2016). HR-LCMS of isolated compound was conducted using the database of Sophisticated Analytical Instrument Facility (SAIF) IIT Bombay, having more than 60,000 patterns. According to HR-LCMS, the mass of isolated compounds was found to be 464.09 and manufactured formula was found to be C₂₁H₂₀O₁₂. The spectrum produced molecular ion peak (m/z) at 469.07 corresponds to [(M+Na) +(H₂O)] and signal at (ESI, EIC) 464.092 corresponds to molecular ion peak (M⁺) (Fig. 4). Thus, confirming the mass of isolated compound.

¹H NMR study:-

It was recorded on Varian NMR–Mercury 300, showed two proton signals at δ 6.193, (1H, d, J = 2.1 Hz, H-6) and δ 6.385 (1H, d, J = 2.1 Hz, H-8) were due to protons of ring 'A'. Three aromatic proton signals at 7.548 (1 H, d, J = 2.0 Hz H-6'), 7.524 (1H, d, 2.0 Hz, H-2'), 6.849 (1H, d, J = 8.7, H-5') were assigned to the protons of ring 'B'. This indicates 3',4' dihydroxy structure present in the isolated compound, corresponding to the aromatic ring B of quercetin nucleus (Fig. 5). The signal of glucose moiety was observed as triplet at δ 5.299 (1H') indicating the compound to contain β- linked glucose (Maria *et al.*, 2000). Multiplet signals at δ 3.0 - 3.70 (6 H, m) showed six sugar protons and signals at δ 12.601 (H-13), 10.93 (H-12), 9.70 (H-15) and signals at 9.1 (H-14) for four -OH groups attached to the flavonoids core structure (Fig. 6). This confirmed the isolated compound is a flavonoid glycoside.

Based on TLC and spectral study, viz., UV, FTIR, HR-LCMS, ¹H NMR and comparison with previously reported data (Agus *et al.*, 2014; Maria *et al.*, 2000), the isolated compound was identified as a flavonoid glycoside and structure was established as Quercetin-3-O-β-D-glucopyranoside. It is commonly known as isoquercetin and the molecular formula was found to be C₂₁H₂₀O₁₂. This is being the first report on the isolation of phytoconstituent from this plant.

Table 1:-UV spectral shifts of isolated compound

Methanol solution	λ_{\max} (nm)			Spectral shift	Structural diagnosis
	Band I	Band II	Band III		
Alone	327.5	270.0	222.5	-	3-OH group
5% AlCl ₃	334.5	280.0	227.0	10 nm of band II and 7 nm of band I bathochromic shift	5-OH free
NaOAc	325.5	270.0	220.5	2nm hypsochromic shift of band I & III	7-OH free
H ₃ BO ₃	330.0	275.0	222.5	5 nm of band II and 3 nm of band I bathochromic shift	3',4'dihydroxy free

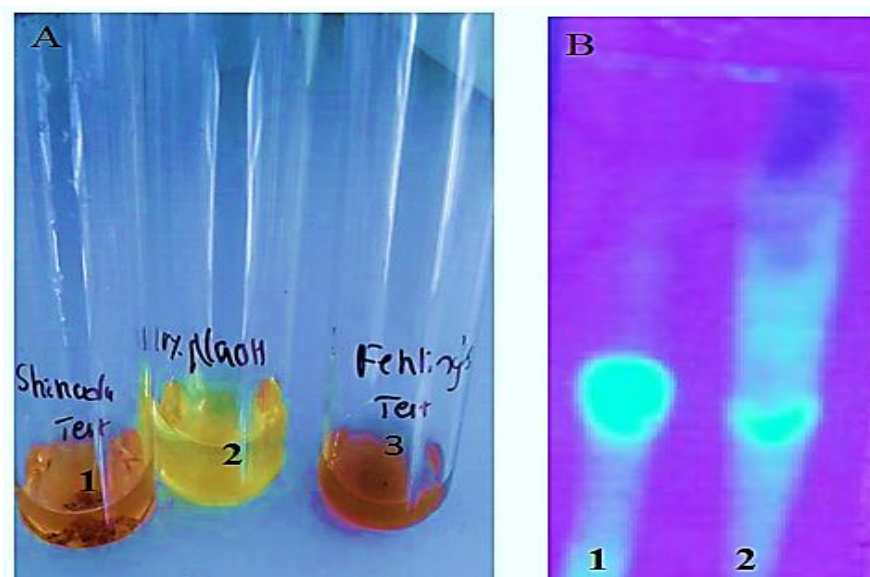


Fig 1:-A. Identification tests for the isolated compound Shinoda test. 2. 10% NaOH test 3. Fehling's test B. TLC of *Bryonia laciniosa* Linn. under UV 366nm Isolated compound 2. Crude methanolic extract

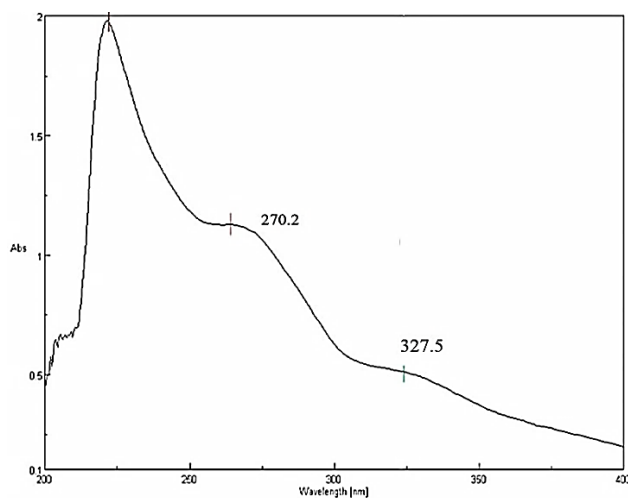


Fig 2:-UV spectra of isolated compound

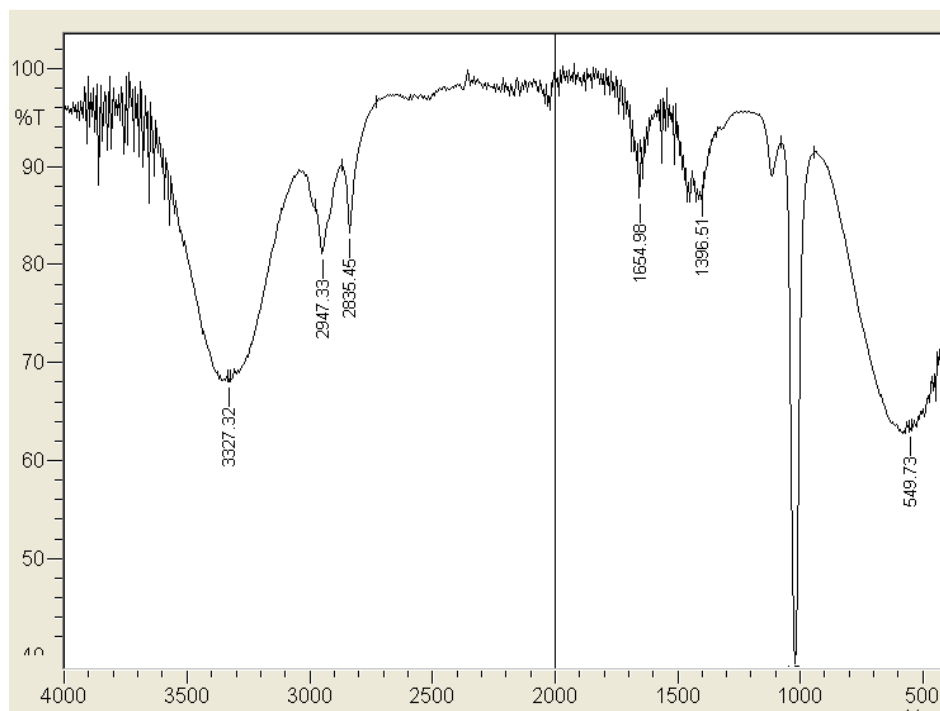


Fig 3:-FTIR spectra of the isolated compound

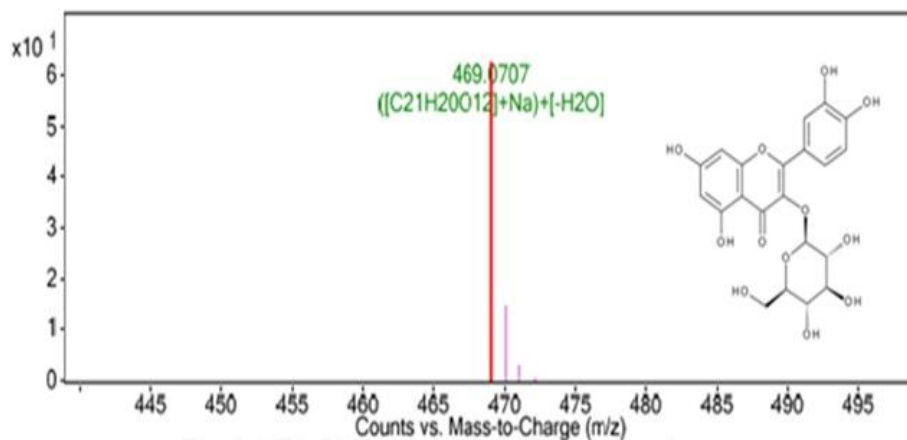


Fig 4:-HR-LCMS spectra of the isolated compound

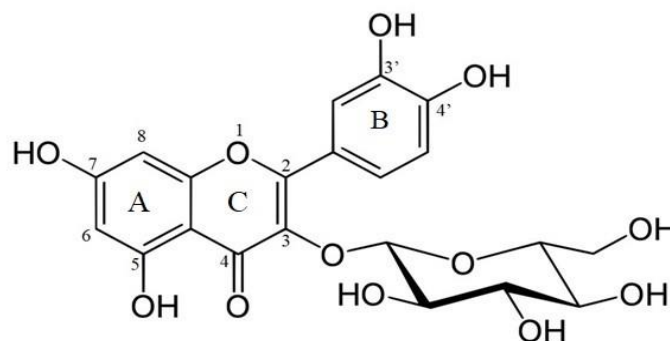


Fig 5:-Structure of isolated compound

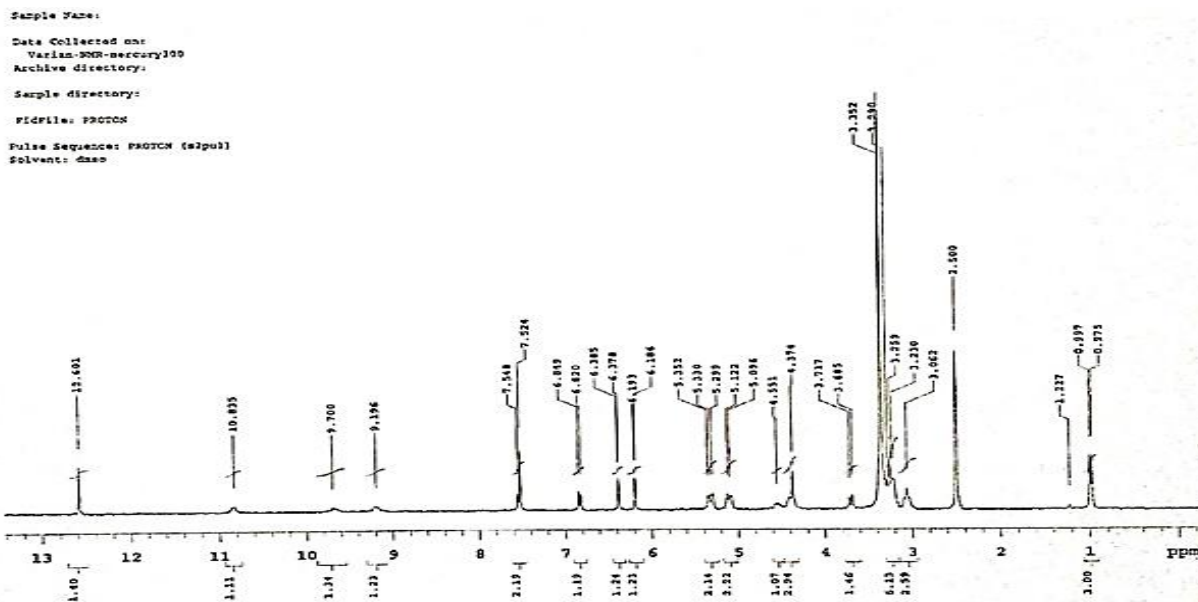


Fig 6:- ^1H NMR spectra of isolated compound

Conclusion:-

Isoquercetin, a flavonoid glycoside was successfully isolated from the methanolic extract of *Bryonia laciniosa*. Based on HR-LCMS and other spectral data the structure was established as Quercetin-O-Glucoside. Recently chemical markers have been used for the standardization of the medicinal plants, this flavonoid can be used as a chemical marker for the standardization of *Bryonia laciniosa*. Nowadays isoquercetin has been under evaluation for the treatment of kidney cancer, renal cell carcinoma, advanced renal cell carcinoma, thromboembolism of vein in colorectal cancer, among others. Thus, the plant, *Bryonia laciniosa* can be used as a source for the isolation of isoquercetin.

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