IJPSR (2021), Volume 12, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 March 2020; received in revised form, 25 June 2020; accepted, 21 July 2020; published 01 March 2021

PHYTOCHEMICAL INVESTIGATION, ISOLATION AND CHARACTERIZATION OF BETULIN FROM LEAF OF *GYMNOSPORIA MONTANA*

Dhru Bhavita [*1], B. Lakshmi [2] and Maitreyi Zaveri [1]

K. B. Institute of Pharmaceutical Education and Research [1], Sector-23, GH-6, Gandhinagar - 382023, Gujarat, India.
Department of Biotechnology [2], K. S. V. University, Gandhinagar - 382023, Gujarat, India.

Keywords:

Gymnosporia montana, Phytoconstituents, Characterization, Betulin

Correspondence to Author:
Dr. Bhavita Dhru

Lecturer,
K. B. Institute of Pharmaceutical Education and Research, Sector-23, GH-6, Gandhinagar - 382023, Gujarat, India.

E-mail: dhru1909@gmail.com

ABSTRACT: Ethnomedicinal plant-like *Gymnosporia montana* belonging to the family Celastraceae is commonly known as Vikalo in Gujarat. Ethanomedicinally, fresh leaves of Vikalo are chewed in trible regions of Gujarat to cure jaundice. Phytochemical screening of the plant showed the presence of phytoconstituents like phenol, flavonoids, alkaloids, triterpenoids, and saponin. The present study was undertaken for Phytochemical Investigation, Isolation and Characterization of Betulin from the leaf of *Gymnosporia montana*. On the basis of characterization studies, the novel compound betulin was isolated by column chromatography; the isolated compound has been evaluated for its physical properties (colour, state, solubility, melting range, and R_f value), which identically resembles the standard betulin.

INTRODUCTION: The use of medicinal plants for the treatment of various diseases is as old as human civilization and has obtained worldwide significance in the primary healthcare system. In spite of their structural complexcity and many unknown chemical constituents, they have been frequently prescribed because of their use and efficacy, contributing to the disclosure of their therapeutic properties. Globally, at least 121 chemical substances of known structure are still extracted from plants for use as drugs. Lately, the chemical basis of these plant-derived medicines and their therapeutic efficacies have been the center of attention.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(3).1752-56

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(3).1752-56

Out of the known 17,000 higher plants in India, 7,600 are known to become medicinal plants and are used in Ayurvedic / other medicinal systems for treatment from ancient times [1]. Therefore, medicinal plants have played a key role in world health. They are distributed worldwide, but they are most abundant in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants [2-4]. Herbal medicine is the oldest form of healthcare known to mankind.

Herbs had been used by all cultures throughout history. According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [5]. Ethano-medicinally, one of the important plants such as

*Gymnosporia montana*has been used in the treatment of Jaundice in tribal area [6]. *Gymnosporia montana,* a plant native in Gujarat, belonging to the family *Celeastracea,* commonly known as Vikalo is a shrub or tree growing wild in dry areas [7]. In South Africa, leaves are used as a vermifuge for children. They have also found utility in the treatment of Jaundice. Wood and leaf ash are reported to be used in Nigeria as a substitute for salt. Leaf ash mixed with ghee is applied for the treatment of sores. A decoction of the leafy twigs has been used as a mouth wash and to relieve toothache. In the traditional system of medicine the root, stem, and leaves are valued for their medicinal properties but major medicinal properties are attributed to the leaf of *Gymnosporia montana* [8].

Several compounds *viz.* tingenone, 3-O-acetyloleanolic acid, hexacosane, hexacosanol, n-triacontanol, betulin, β -amyrone, β-amyrin, δ-amyrin, β - sitosterol, celacinnine and kaempferol have been isolated [9-12] from the leaves of *G. montana*. The presence of Galactose as free sugar and seven free amino acids, including arginine, glutamic acid, alanine, proline, γ-aminobutyric acid, have also been reported by De *et al.* [13]. The same group also has reported[14]the presence of seven fatty acids, of which palmitic acid is the major one (72.03%), in the leaf. The present study was undertaken for Phytochemical Investigation, Isolation and Characterization of Betulin from the leaf of *Gymnosporia montana.*

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The plant *Gymnosporia montana* was collected from the Vijapur, Gandhinagar, Gujarat, India during November 2008 and was authenticated by Dr. S. K. Patel, Head of the Botany Department, Government Science College, Gandhinagar. The voucher specimen KB/O8/0011 was deposited in K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

Preparation of Extract: The selected plant parts of *G. montana*was separated and dried under sunlight. Dried powdered passed through a sieve of 60 mesh (#) size and stored in airtight containers and then used for present work. The shade-dried leaf powder was extracted with methanol. The extraction was carried out by Soxhlet assembly for 6-8 h. Then the solvent was filtered and repeat the process for three times in the same manner. The extracts were concentrated and dried under a controlled temp of 60 ºC on a water bath and reported the % yield. Dried extract of the leaf was used for further investigation.

Isolation of Compound: 10 gm of methanolic extract was suspended in water and then extracted with n-hexane and dichloromethane. Then both the fractions are concentrated in a vacuum rotary evaporator, and the dried extract was labeled and stored in an airtight container and was used for further study. Then both the fractions were characterized by TLC and found that the R_f of the Betulin matches with the TLC of dichloromethane fraction. So, the dichloromethane fraction was used for further isolation by column chromatography.

The column was prepared with silica gel in n-hexane by the wet method, and the column is put overnight. Then the dichloromethane fraction was poured to silica gel open column chromatography, and step gradient technique was used to run the column. Various ratios of ethyl acetate and n-hexane (1:5, 1:3, 1:2 and 1:1) were used for the isolation of active components. The fractions of 50 ml were collected; and it was characterized by TLC and same type of fractions are mixed; then again characterized by TLC and found that the betulin is present as a single spot in the fraction collected from the 1:3 (ethyl acetate and n-hexane) solvent system. After isolation of the desired compound, it was subjected to characterization. For characterization studies, melting point, TLC and modern analytical techniques such as HPLC, Mass, NMR were performed.

Preparation of Sample and Standard Solutions: Isolated compound solution and standard solutions of betulin was prepared from the pure product by dissolving appropriate weights in methanol, and stored in a refrigerator. Working solutions were prepared freshly every day by an appropriate dissolution of a standard solution in methanol.

Apparatus and Chromatographic Conditions for Determination of Betulin by RP-HPLC: Shimadzu 2010 C integrated High performance liquid chromatographic system was used for this experiment.

Shimadzu 2010 C system equipped with 2010 quaternary gradient pump, 2010 UV-VIS detector, 2010 Column Oven, 2010 programmable auto sampler controlled by CLASS-VP software. The confirmation of isolated compound botulin was performed by the HPLC method on a base deactivated RP-phase. Complete separation of the betulin was achieved on 250 × 4.6 mm i.d. Hypersil BDSRP-C18 5 µm column. The mobile phase consisted of Acetonitrile: Water: (85:15, v/v). The injection volume was 20 µL used. The isocratic method was run for 30 min. The flow rate was 1ml/min at room temperature. The botulin was detected at 210 nm (UV-VIS detector). The oven temperature was ambient. HPLC analysis of isolated compounds was carried out for developing fingerprinting and also to verify the presence of botulin in the leaf of *Gymnosporia montana.*

Determination of Betulin by Mass Spectroscopy: The mass spectrometric structural examinations were carried out using a mass spectrometer. Recording traces completed the mass spectrometric examinations with a Perkin-Elmer Sigma System gas chromatography using a FID detector and nitrogen as a carrier gas for 25 ml/min.

Determination of Betulin by UV Spectroscopy: UV-visible spectrophotometric analysis was conducted on the isolated compound betulin using a UV-visible spectrophotometer Perkin Elmer, the USA at room temperature.

Determination of Betulin by NMR Spectroscopy: The NMR experiment was performed on a Bruker NMR spectrometer at room temperature.

RESULTS AND DISCUSSION: Melting range of the isolated compound, determined by the open capillary method, was found 246-248 ºC that was compared with standard betulin is 248-251 °C, they are yellowish-white in color and solid in the state. Isolated compounds are soluble in chloroform and ethyl acetate. TLC co-chromatography was performed for an isolated compound solution was spotted on the TLC plate along with a standard solution of betulin. The stationary phase consisted of TLC Aluminum sheets pre-coated with silica gel 60 F254, thickness 0.2 mm, (20 × 20 cm) (Emerck, Germany), mobile phase consisted of toluene: methanol (9:1) detected at daylight, and derivatization was done with anisaldehyde in sulphuric acid scanned at 254 nm, 366 nm and after derivatization. TLC study was aimed at checking the presence of a similar kind of compound, if any, in methanolic fraction. Betulin was reported to be present in leaf of *G. montana.* Our observations on TLC support the presence of betulin in methanolic fraction of leaf of *G. montana*as shown in **Fig. 1**.

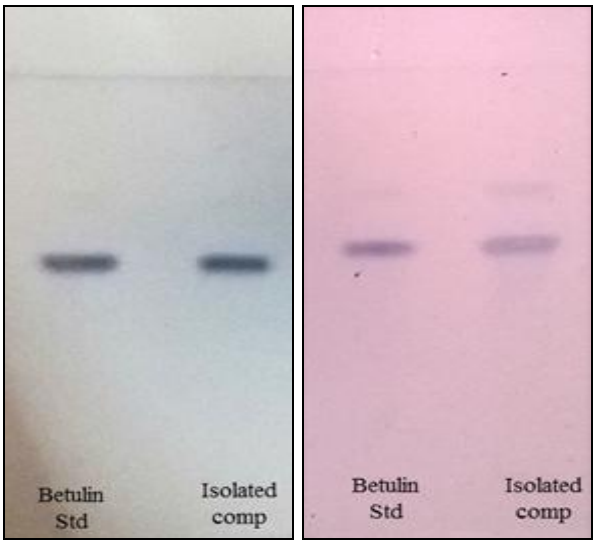

FIG. 1: CO-CHROMATOGRAPHY OF ISOLATED COMPOUND BETULIN FROM LEAF OF *G. MONTANA* AND BETILIN

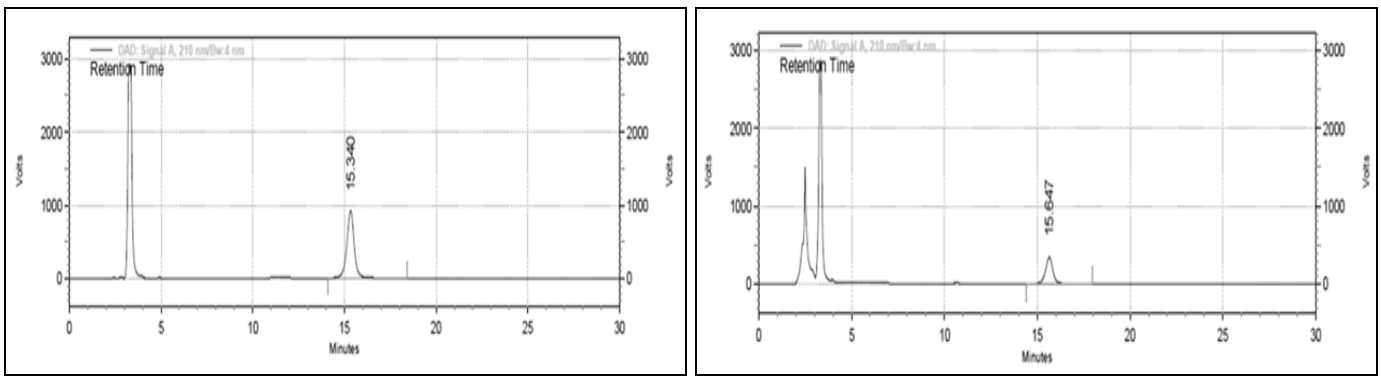

FIG. 2: REPRESENTATIVE CHROMATOGRAM OF BETILIN DETECTED AT210NM (A) BETULIN IN STANDARD SOLUTION AND (B) ISOLATED COMPOUND BETALIN LEAF OF *G. MONTANA*

In our study, different ratios of acetonitrile-water were tried for the separation of betulin, but acetonitrile-water 85:15 (v/v) was found to be the best for the separation of betulin in leaf of *G. montana* using reverse-phase Hypersil BDSRP - C18 column at a flow rate of 1.0 ml/min. It was observed that betulin showed the best peak resolutions at 210 nm using a UV-VIS detector. It can be seen from **Fig. 2** that good separation can be achieved within 20 min using the described conditions as the retention time of betulin in standard and sample solution was 15.34 and 15.64 min, respectively. The HPLC method mentioned here represented an excellent technique for the determination of betulin in standard and isolated compound betulin from leaf of *G. montana*. The method gives a good resolution of betulin with a short analysis time. To the best of our knowledge and literature surveyed, it was revealed that no systematic work had been carried out for the determination of betulin from leaf of *G. montana* using the RP-HPLC technique. The HPLC method described is simple, accurate, sensitive, and reproducible and falls best for the determination of betulin with good peak resolution and short analysis time. Mass spectroscopy of isolated compound was performed, showing a molecular ion peak at M++1 *i.e.*, at 445.00, while the calculated molecular weight of betulin as per molecular formula-$C_{30}H_{50}O_2$ is 442. The betulin was scanned in the wavelength ranging from 300-800 nm by using a UV-VIS spectrophotometer, and the characteristic peaks were detected. The wavelength of maximum absorbance (λ_{max}) of the isolated compound was found to be 316 nm in concentrated sulphuric acid, which is the same as standard betulin in concentrated sulphuric acid. ^{1}H-NMR spectroscopy was used for the confirmation of the structure of the isolated compound, in which various peaks in DMSO were found at different δ values, and it shows the presence of 50hydrogen in the compound. On the basis of characterization studies of isolated compounds, it was concluded that the isolated compound is betulin.

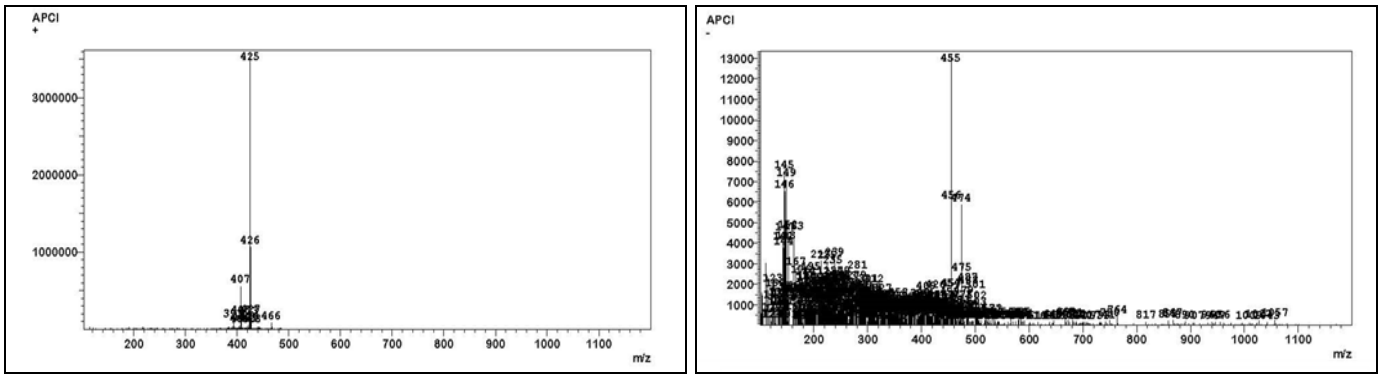

FIG. 3: MASS SPECTRA OF ISOLATED COMPOUND BETULIN FROM LEAF *G. MONTANA*

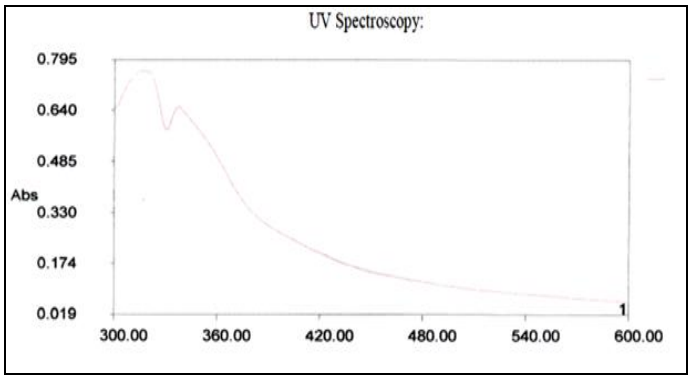

FIG. 4: UV SPECTRA OF ISOLATED COMPOUND BETULIN FROM LEAF OF *G. MONTANA*

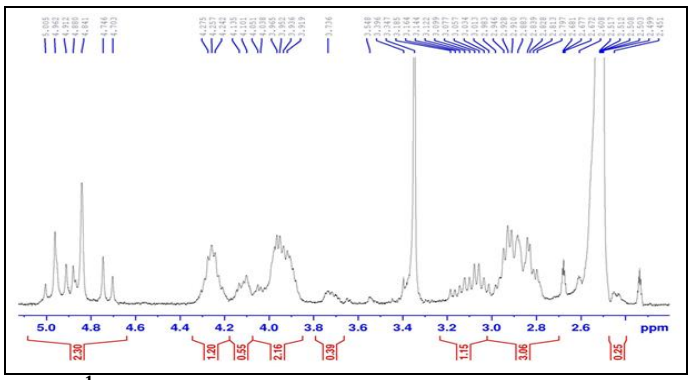

FIG. 5: ^{1}H-NMR SPECTRA OF ISOLATED COMPOUND BETULIN FROM LEAF OF *G. MONTANA*

CONCLUSION: On the basis of characterization studies, the novel compound betulin was isolated by column chromatography; the isolated compound has physical properties (colour, state, solubility, melting range, and R_f value) that identically resemble the standard betulin. Spectral data shows that the isolated compound has $\lambda_{max,}$ which is mostly similar to standard betulin; $M^{++}1$ peak was

also obtained in the mass spectra of the isolated compound, and above all, 50 protons are found in the 1H-NMR spectra of isolated compound. So, it can be concluded that the isolated compound is similar to the molecular formula $C_{30}H_{50}O_2$, which corresponds to the molecular formula of betulin. So, the isolated compound was found to be betulin. Easy availability of leaf of *G. montana* due to its acceptable geographical conditions in India, it can be used as a natural source of betulin and can find its way in pharmaceutical industries.

ACKNOWLEDGEMENT: The authors are thankful to Gujcost for providing financial assistance under Minor Research Project.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Anonymous. WHO. WHO Monographs on Selected Medicinal Plants. World Health Organization Geneva 2002; 2: 2435.
2. Anonymous. WHO. Ancient Remedies, New Disease. World Health Organization Geneva 2002: 111.
3. Abraham Z: Glimpses of Indian ethnobotany, oxford and publishing co. New Delhi 1981; 308-20.
4. Holiman A: Plants in medicine chelsea physic garden. The Chelse Phys Gar Co Ltd Pub Co New Delhi 1989; 308-20.
5. Prusti A, Mishra SR., Sahoo S and Mishra SK: Antibacterial activity of some indian medicinal plants. Ethnobot Leaflets 2008; 12: 227-30.
6. Anonymous. Ayurvedic Pharmacopoeia of India. Government of India, Ministry of health and family welfare department of Indian system of medicine and Homeopathy New Delhi 1986, 3: 209-210.
7. Kirtikar and Basu BD: Indian medicinal plants international book distributors. Book seller and Publisher Rajpur Road Dehradun India 1993; 1: 577.
8. Trease GE and Evans WC: Pharmacognosy. Ed 11th BraillarTiridel Cannada Macmillian Publishers 1989; 222.
9. Joshi CK, Bansal RK and Patni R: Chemical constituents of *Gymnosporia montana Euonymus pendulus*. Planta Med 1978; 34: 211.
10. Akshaya Kumar K and Srimannarayana G: Chemical constituents of leaves and roots of *Gymnosporia montana*. J Nat Prod 1981; 44(5): 625-28.
11. Khan MA and Mabry TJ: Revista *Latinoa mericana* de quimica. Med Aromat Plants Abstr 1990; 21(2): 55.
12. De S and Bhavsar GC: Phytochemical studies of *Gymnosporia montana* leaf. Indian J Pharm Sci 1993; 55(6): 234-35.
13. De S: Ph. D. Thesis, Gujarat University. Ahmedabad, India 1992.
14. De S, Dave KK and Bhavsar GC: Fatty acid compositions of *Gymnosporia montana* and *Paederia foetida* leaves. Ind J Pharm Sci 1993; 55(3): 110-12.

How to cite this article:
Bhavita D, Lakshmi B and Zaveri M: Phytochemical investigation, isolation and characterization of betulin from leaf of *Gymnosporia montana*. Int J Pharm Sci & Res 2021; 12(3): 1752-56. doi: 10.13040/IJPSR.0975-8232.12(3).1752-56.