Isolation and Spectroscopic Characterization of Betulin from Baliospermum montanum Root Extract Using Chromatographic and Advanced Analytical Techniques

Manisha Bharti[1*], Kehar Singh Dhaker[1]

[1]LNCT University, J.K. Town Road, Sarvadharam, Kolar Road, Bhopal-462042, Madhya Pradesh.***Corresponding Author:**

Manisha Bharti, LNCT University, J.K. Town Road, Sarvadharam, Kolar Road, Bhopal-462042, Madhya Pradesh, India.

Email: bhartimanisha85@gmail.com

ABSTRACT

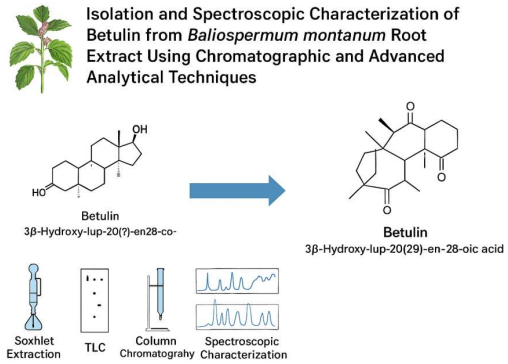
Baliospermum montanum (Family: Euphorbiaceae) is a medicinal plant traditionally used for anti-inflammatory, antirheumatic, and hepatoprotective purposes. Despite its therapeutic potential, limited scientific evidence exists on the isolation and structural characterization of its bioactive constituents. This study employed Soxhlet extraction, TLC profiling, silica gel column chromatography, and multi-spectral analysis (UV–Vis, FTIR, ^{1}H NMR, and MS) to isolate and identify the primary triterpenoid constituent from ethanolic root extract. The extraction yielded 3.618% of crude extract from 300 g of plant material. TLC analysis using Toluene:Ethyl acetate:Methanol (8:1:1) confirmed similarity with standard Betulin (Rf = 0.40). Fraction F obtained from column chromatography showed strong UV absorption at 209 nm. FTIR analysis revealed characteristic –OH, C–H, C=C, and C–O functional groups. ^{1}H NMR signals supported the triterpenoid framework, while mass spectrometry confirmed the molecular ion peak at m/z 442.2822 corresponding to Betulin ($C_{30}H_{50}O_2$). Together, the chromatographic and spectroscopic findings verified the successful isolation of Betulin from *B. montanum*. This analytical framework provides a reproducible method for future phytochemical and pharmacological investigations.

KEYWORDS: *Baliospermum montanum*; Betulin; Column Chromatography; FTIR; NMR; Mass Spectrometry; Natural Products; Phytochemical Isolation.

How to cite this article: Bharti M, Dhaker KS. Isolation and Spectroscopic Characterization of Betulin from Baliospermum montanum Root Extract Using Chromatographic and Advanced Analytical Techniques. Int J Drug Deliv Technol. 2026;16(53s): 54-63. DOI: 10.25258/ijddt.16.53s.6

Source of support: Nil.

Conflict of interest: None.



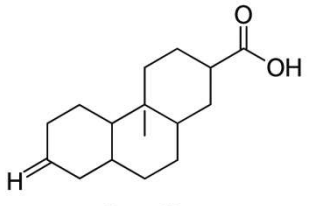
Graphical Abstract

INTRODUCTION

Medicinal plants continue to serve as an indispensable source of therapeutic agents, particularly for anti-inflammatory, antimicrobial, anticancer, and hepatoprotective applications. Over 60% of clinically approved drugs are either natural products or derivatives, reflecting the importance of plant-based bioactive compounds in drug discovery (Newman & Cragg, 2020). Among these, triterpenoids in particular *Betulin* and *Betulinic* acid have demonstrated wide-ranging pharmacological activity including anti-inflammatory, anticancer, antiviral, and wound healing properties (Alakurtti et al., 2006; Yogeeswari & Sriram, 2005).

Baliospermum montanum, belonging to the *Euphorbiaceae* family, is widely used in Ayurvedic medicine for its purgative, diuretic, and anti-arthritic effects (Kirtikar & Basu, 2001). Phytochemical studies report the presence of diterpenes, triterpenes, phenolics, and flavonoids, making it an underexplored but promising candidate for natural drug development (Rastogi & Mehrotra, 2004). However, scientific investigations focusing on isolation and structural characterization of its constituents remain limited.

Betulin, a pentacyclic triterpenoid primarily isolated from *Betula* species, has gained attention due to its antioxidant, anti-inflammatory, and antiproliferative actions (Drag-Zalesińska et al., 2020). Reports also indicate its presence in certain *Euphorbiaceae* plants, suggesting a potential chemotaxonomic link.



Betulin

3β-Hydroxy-lup-20(29)-en–28-oic acid

Given the limited literature on *Betulin* isolation from *B. montanum*, this study aimed to systematically extract, isolate, and spectroscopically characterize its major bioactive compound. Using Soxhlet extraction, TLC-guided fractionation, silica gel column chromatography, and advanced spectroscopic tools (FTIR, NMR, MS), we demonstrate a reproducible workflow for natural product isolation, aligning with globally accepted analytical standards.

MATERIALS AND METHODS

Plant Collection and Authentication

The plant's part of *Baliospermum montanum* was collected from the local region of Bhopal Madhya Pradesh India and was identified and authenticated by the botany Scientist Dr. Suman Mishra of Vindhya Herbals Testing and Research Laboratory, MFPPARC Bhopal. Reference Specimen No. MFP-PARC/902/Dated-22/07/2025 was obtained.

Soxhlet extraction

The dried powder of *Baliospermum montanum* (root) was successively placed in a thimble of a Soxhlet apparatus. The extraction was carried out using ethanol as the solvent at a temperature of 40–60°C on a heating mantle for 8–10 hours. After the extraction process, the extract was filtered and concentrated to dryness. The resulting extract was evaporated using a rotary vacuum evaporator at 40–60°C. The final extract was collected in an airtight container. **(Evans. 2009 and Alara *et al.*, 2019).** Extraction yield of ethanolic extract was calculated using the following equation below:-

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

Figure 1: Soxhletation of *Baliospermum montanum* with Ethanolic Solvent

Preliminary Thin layer chromatography

In the preliminary TLC analysis of *Baliospermum montanum* extract, TLC plates coated with silica gel 60 F_{254} (0.2 mm) were used. The solvent system giving maximum visible spots was Toluene:Ethyl acetate:Methanol (8:1:1)**.** Plates were developed at room temperature and observed under UV (254 nm and 365 nm). The Rf value was calculated. These mobile phases were evaluated using the standard triterpene marker, Betulin. Based on these results, Toluene:Ethyl acetate:Methanol (8:1:1) was selected as the mobile phase for the column chromatography of *Baliospermum montanum* extract.

Column chromatography

The extract was subjected to silica gel column chromatography for the isolation of the triterpene Betulin from *Baliospermum montanum*. A vertical borosilicate glass column (30 mm diameter) was used for the procedure. Before packing, the column was rinsed with acetone and thoroughly dried. The column was packed using the wet packing technique, with silica gel (60–120 mesh) as the adsorbent. Slurry of silica gel was prepared in toluene and poured into the column to achieve uniform packing. A total of 3 grams of the extract was carefully loaded onto the top of the packed column. Gradient elution was employed using the solvent system Toluene:Ethyl acetate:Methanol (8:1:1). Multiple fractions were collected during the elution process. The collected fractions were then concentrated, and TLC analysis was performed to identify the presence of individual compounds **(Srivastava *et al.*, 2021** and **Mukherjee *et al.*, 2024).**

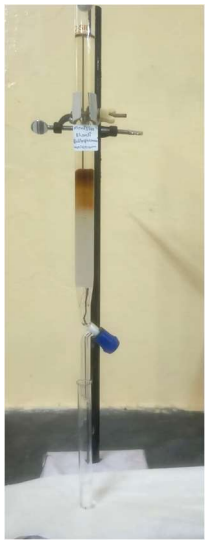


Figure 2: Isolation of active constitutes by column chromatography

Spectroscopic characterization

UV-visible Spectroscopy

The isolated fraction (F) of the *Baliospermum montanum* extract was scanned in the wavelength range of 200 to 800 nm using a UV-Visible spectrophotometer (Shimadzu UV-1700), and the characteristic peaks were detected and recorded **(Patel *et al.*, 2022 and Picollo *et al.*, 2019)**.

FT-IR

To confirm the presence of functional groups in the isolated fraction (F) of the *Baliospermum montanum* extract, FT-IR spectroscopy was performed using a PerkinElmer Spectrum BX spectrophotometer. The samples were dried, finely ground with KBr, and analyzed using a Thermo Nicolet Model 6700 spectrometer. A KBr disc (200 mg) was prepared by mixing 2% of the finely dried sample with KBr, and the disc was then examined using the IR spectrometer. Infrared spectra were recorded in the range of 400–4,000 cm^{-1} **(Luciene *et al.*, 2008 and Dutta *et al.*, 2017).**

NMR Spectroscopy

NMR spectroscopy was performed on the isolated fraction (F) of the *Baliospermum montanum* extract to determine the structure of the compounds present. Nuclear Magnetic Resonance (NMR) analysis was

carried out using a JNM EC-500 NMR spectrometer **(Zia *et al.*, 2019 and Marion *et al.*, 2013).**

Mass Spectroscopy

Mass spectrometry converts molecules into ions, which are then separated and sorted based on their mass-to-charge ratio (m/z). The molecular weight of the isolated fraction (F) of the *Baliospermum montanum* extract was determined using a micrOTOF-Q mass spectrometer (Instrument ID: 228888.10348) **(Wiley *et al.*, 1995 and Torgerson *et al.*, 1974).**

RESULTS

Plant Collection

Table 1 Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Baliospermum montanum*	Root	300.00 gm

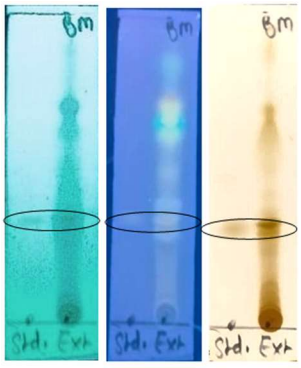
Results of Percentage yield

Table 2 Percentage yield of extract

S. No.	Plant name	Solvent	Colour of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Baliospermum montanum*	Ethanol	Dark brown	300.00 gm	10.854 gm	3.618 %

Figure 3: Ethanolic extract of *Baliospermum montanum*

Results of Preliminary TLC preparation for the estimation of active constitutes



Short-UV (254 nm) **Long-UV Visible Light**

Figure 4: TLC estimation by UV lamp for TC with Std. Triterpene (Betulin)
(Std. = Standard, BM= *Baliospermum montanum*)

Table 3 TLC of *Baliospermum montanum* extract

Sr. No.	Solvent system	No. of spots	Colour of spots at Wavelength (365nm)	Colour of spots at Wavelength (254)	Rf value (Extract)	Rf value (Std.)
`1.	Toluene: Ethyl acetate: Methanol (8:1:1)	11	Florescence (Std.) Purple (BM) Purple Florescence Florescence Florescence Florescence Florescence Florescence Florescence Florescence Florescence Pink Florescence	Green (Std.) Dark Green(BM) Green Green Dark Green Green Green Dark Green Dark Green Green Light Green	- 0.9/5 =0.18 1.5/5 =0.30 1.7/5 =0.34 2.0/5 =0.40 2.3/5 =0.46 2.6/5 =0.52 3.7/5 =0.74 4.3/5 =0.86 4.5/5 =0.90 4.7/5 =0.94	Triterpene = 2.0/5 =0.40

Thin-layer chromatography (TLC) of the *Baliospermum montanum* (BM) extract was performed using different solvent systems, selected based on a literature survey. TLC carried out using Toluene:Ethyl acetate:Methanol (8:1:1) showed clearly visible bands for the BM extract when compared with the standard triterpene Betulin. The Rf values for both the BM extract and standard Betulin were found to be 0.40.

Column Chromatography

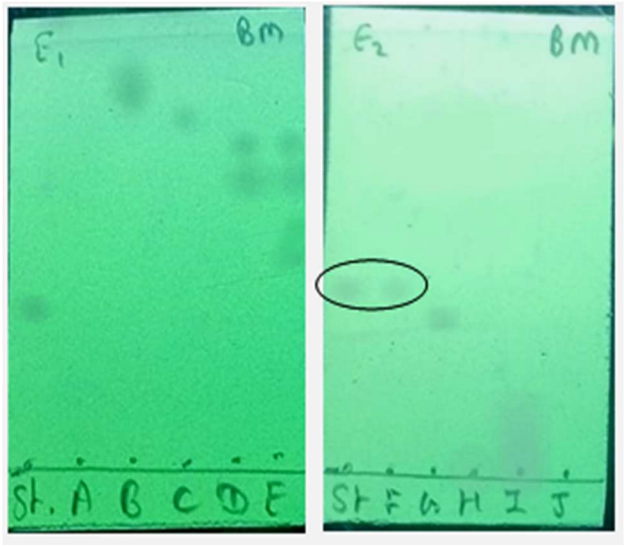
The fractions (elutes) obtained from silica gel column chromatography of the *Baliospermum montanum* (BM) extract were tested for the presence of various phytochemicals using thin-layer chromatography (TLC). The collected fractions were properly handled, and their UV spectra were recorded for further analysis.

Column Chromatography of BM extract

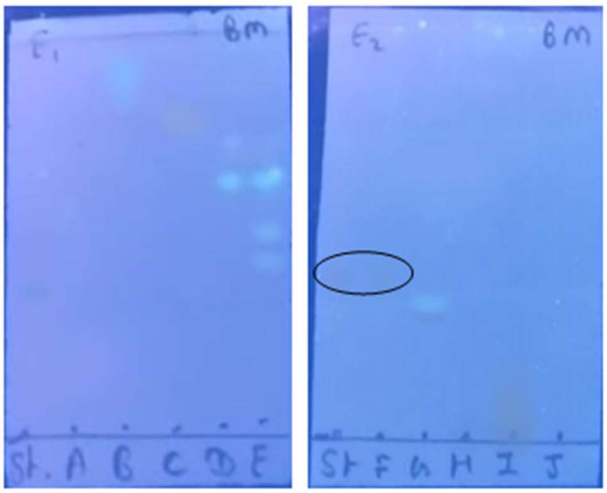
Table 4 Fraction collected from Column Chromatography of BM Extract

Sr. No.	Eluent composition	Fraction collected	Remarks
1	Toluene: Ethyl acetate: Methanol (8:1:1)	01 (A)	White coloured mixture of compound
2		02 (B)	Creamy coloured mixture of compound
3		03 (C)	White coloured mixture of compound
4		04 (D)	Yellowish coloured mixture of compound
5		05 (E)	Light Yellowish coloured mixture of compound
6		06 (F)	White coloured mixture of compound
7		07 (G)	Yellowish coloured mixture of compound
8		08 (H)	Creamy coloured mixture of compound
9		09 (I)	Light Yellowish coloured mixture of compound
10		10 (J)	White coloured mixture of compound

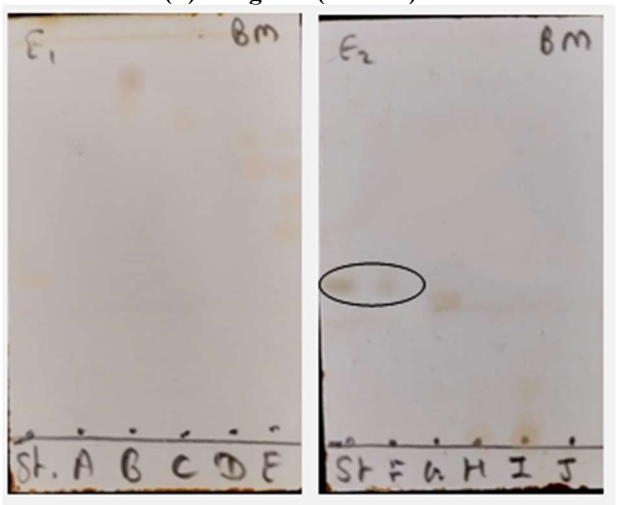
TLC of all collected fractions of BM Extract-



(a) Short-UV (254 nm)



(b) Long-UV (365 nm)



(c) Visible Light

Figure 5: TLC estimation by UV lamp for BM fractions after column chromatography with Std. Triterpene (Betulin). a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light. (Std. = Standard, BM= *Baliospermum montanum*)

Table 5: Rf values of all collected fractions of BM after column chromatography

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelength (365nm)	Colour of spots at Wavelength (254 nm)	Rf value (Extract)	Rf value (Std. Triterpene)
1.	A	Toluene: Ethyl acetate: Methanol (8:1:1)	-	-	-	-	2.0/5=0.40
2.	B		01	Fluorescence	Green	4.8/5=0.96	
3.	C		01	Fluorescence	Green	4.6/5=0.92	
4.	D		02	Fluorescence Pink	Green Green	4.3/5=0.86 4.4/5=0.88	
5.	E		04	Fluorescence Fluorescence Fluorescence Pink	Green Green Green Green Light Green	2.6/5=0.52 3.4/5=0.68 4.3/5=0.86 4.4/5=0.88	
6.	F		01	Fluorescence	Green	2.0/5=0.40	
7.	G		02	Blue Fluorescence	Green Green	1.5/5=0.30 1.7/5=0.34	
8	H		-	-	-	-	
9.	I		01	Purple	Green	0.9/5=0.18	
10.	J		-	-	-	-	

The Rf values obtained from TLC analysis were used to confirm the presence of active constituents in the isolated fractions (F) of *Baliospermum montanum* (BM) extract. TLC was performed using the mobile phase Toluene:Ethyl acetate:Methanol (8:1:1) for BM, and the results were compared with the standard triterpene Betulin. Fraction F was transferred into watch glasses, dried, and weighed. The weight of fraction F (BM) was found to be 21 mg. The fractions were stored in a freezer for further use.

Spectroscopic characterization: -

Active constitutes estimation by UV-Spectroscopy

The UV spectra of the isolated fraction (F) from the *Baliospermum montanum* (BM) extract were recorded using a Shimadzu 1700 double-beam UV-Visible spectrophotometer. The spectra were obtained using the solvent system Toluene:Ethyl acetate:Methanol (8:1:1) over a scanning range of 200–800 nm. The λmax values of the isolated compound were determined. The isolated fraction (F) from the BM extract showed a single absorption peak at 209 nm.

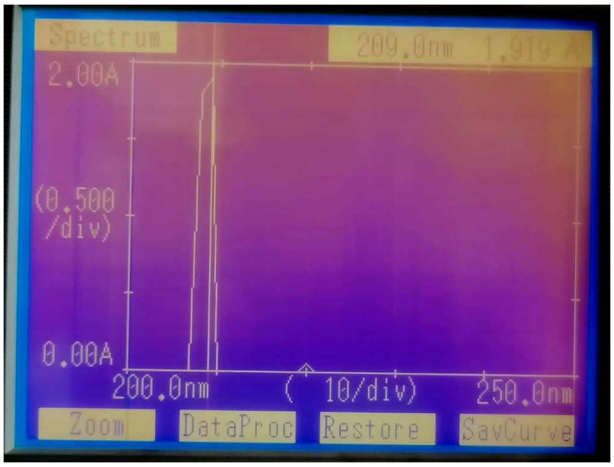


Figure 6: Active constitutes estimation by UV-Spectra of isolated fraction (F) of BM extract after column chromatography

Active constitutes estimation by FTIR – Spectroscopy

IR spectra of the isolated fraction (F) of BM extract

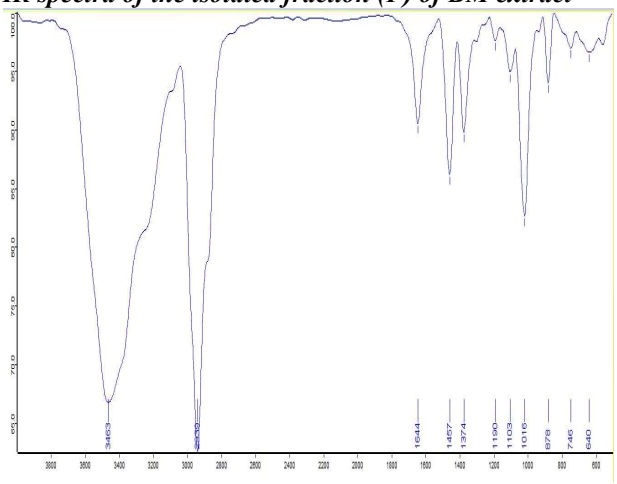


Figure 7: IR Spectra of the isolated fraction (F) of BM extract

Table 6: FTIR- Spectrum Frequency Range of the isolated fraction (F) of BM extract

Sr. No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound
06	F	3550 3200 (cm^{-1})	3463	Strong, broad	O-H stretching	Hydroxyl group
		3000-2800 (cm^{-1})	2939	Medium	C-H stretching	Alkane
		1662-1626 (cm^{-1})	1644	Medium	C=C stretching	Alkene
		1480-1400 (cm^{-1})	1457	Medium	C-H bending	Alkane
		1385-1380 (cm^{-1})	1374	Medium	C-H bending	Methyl group
		1205-1124 (cm^{-1})	1190	Strong	C-O stretching	Alcohol
		1124-1087 (cm^{-1})	1103	Strong	C-O stretching	Alcohol
		1085-1050 (cm^{-1})	1016	Strong	C-O Stretching	Alcohol
		1000-650 (cm^{-1})	878	Strong	C=C bending	Substitute
		780-600 (cm^{-1})	746	Strong	C=C bending	Substitute
		780-600 (cm^{-1})	640	Strong	C=C bending	Substitute

The IR spectrum of the isolated fraction (F) from the *Baliospermum montanum* (BM) extract revealed the presence of various functional groups. O–H stretching appeared as a strong, broad band at 3463 cm^{-1}. A C–H stretching peak corresponding to alkanes was observed at 2939 cm^{-1}, while C=C stretching of alkenes was noted at 1644 cm^{-1}. A C–H bending peak appeared at 1457 cm^{-1}, and C–H bending peaks for methyl groups were detected at 1374 cm^{-1}. C–O stretching peaks of alcohols were observed at 1190 cm^{-1}, 1103 cm^{-1}, and 1016 cm^{-1}. C=C bending peaks of substituted groups were found at 878 cm^{-1}, 746 cm^{-1}, and 640 cm^{-1}.

1H NMR Spectroscopy

^{1}H NMR spectra of the isolated fractions (F) from *Baliospermum montanum* (BM) extract was recorded using an NMR spectrometer. Tetramethylsilane (TMS) was used as the internal standard. The signals in the spectra are denoted as s (singlet), d (doublet), t (triplet), and m (multiplet), corresponding to the respective splitting patterns observed.

^{1}H NMR spectra of the isolated fraction (G) of BM

In the ^{1}H-NMR spectrum of the isolated fraction (F) from *Baliospermum montanum*, the following proton signals were observed: H-9 protons appeared at 0.800-0.935 (0.881 (s), 0.930 (s), 0.930 (s)) ppm, H-6 protons appeared at 1.180-1.301 (1.189 (s), 1.296 (s)) ppm, H-27 protons appeared at 1.23-1.88 (1.312 (dd), 1.362 (dd), 1.397 (ddd), 1.427 (ddd), 1.446 (ddd), 1.482 (ddd), 1.523 (dddd), 1.563 (dddd), 1.568 (ddd, J = 14.3, 8.1, 4.1 Hz), 1.568 (ddd), 1.572 (dddd), 1.572 (dddd), 1.572 (ddd), 1.586 (s), 1.597 (dddd), 1.753 (ddd), 1.753 (ddd), 1.796 (dddd), 1.801 (dddd), 1.812 (ddd), 1.824 (dddd), 1.851 (dddd), 1.851 (dddd), 1.870 (dd), 1.912 (ddd)) ppm, H-1 proton appeared at 2.458 (ddd) ppm, H-1 proton appeared at 2.956 (dd) ppm, H-1 proton appeared at 3.047 (dd) ppm, H-2 protons appeared at 3.301-3.440

(3.304 (d), 3.435 (d)) ppm, H-2 protons appeared at 3.301-3.440 (3.304 (d), 3.435 (d)) ppm, H-2 protons appeared at 4.190-4.240 (4.195 (d), 4.234 (d)) ppm and H-1 proton appeared at 4.614 (1H, dd) ppm.

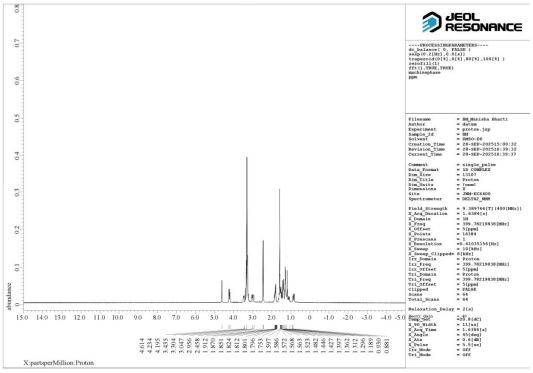


Figure 8: ^{1}H-NMR spectra of the isolated Fraction (F) of BM

Mass Spectroscopy

Mass spectra of the isolated fraction (F) from *Baliospermum montanum* (BM) was recorded using a Bruker micrOTOF-Q mass spectrometer.

Mass spectra of the isolated fraction (F) of BM

The mass spectrum of the isolated fraction (F) from *Baliospermum montanum* (BM) showed a molecular ion peak [M^+] at m/z 442.2822, corresponding to the compound (1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol. The molecular composition indicated the presence of 30 carbon atoms (C), 50 hydrogen atoms (H), and two oxygen atoms (O), resulting in the molecular formula $C_{30}H_{50}O_2$. This identification was further supported by characteristic fragment ions observed at m/z 83, 177, 217, 327 and 413.

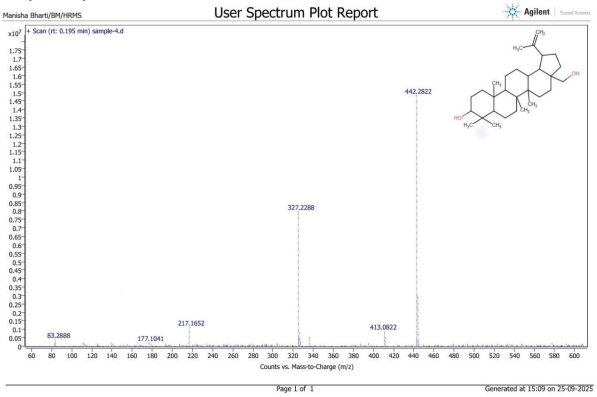
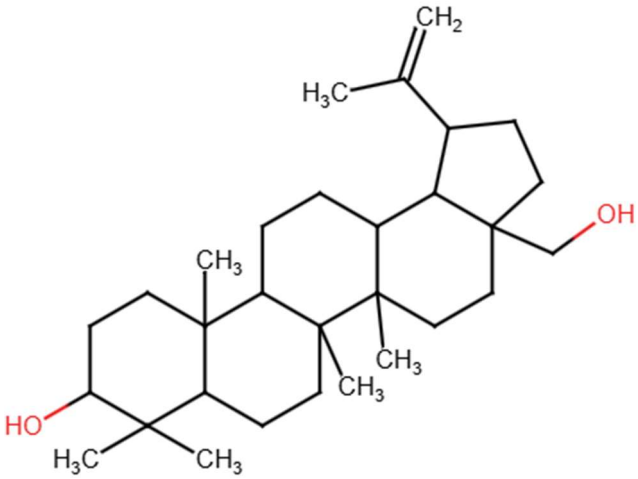


Figure 9: **Mass spectra of the isolated fraction (F) of BM**



(1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol

DISCUSSIONS

The present study used 300 grams of *Baliospermum montanum* root for extraction. The extraction with ethanol solvent yielded 3.618% extract, corresponding to 10.854 grams. **(Fig 1, 3, Table 1-2).** Preliminary TLC of *Baliospermum montanum* (TC) extract was performed using different solvent systems, selected based on a literature survey. TLC carried out in Toluene:Ethyl acetate:Methanol (8:1:1) for BM showed clearly visible bands when compared with standard triterpene (Betulin), respectively. The Rf values of BM and standard Berberine were both found to be 0.42 **(Fig 4, Table 3)**. Based on the TLC results, the solvent system Toluene:Ethyl acetate:Methanol (8:1:1) was selected as the mobile phase for column chromatography of *Baliospermum montanum*. Using these mobile phases, active constituents were isolated through silica gel column chromatography. For *Baliospermum montanum*, fractions 01 (A), 02 (B), 03 (C), 04 (D), 05 (E), 06 (F), 07 (G), 08 (H), 09 (I) and 10 (J) were collected. **(Fig. 2, Table 4).** Rf values obtained from TLC analysis were used to confirm the presence of active constituents in the isolated fractions (F) of *Baliospermum montanum* (BM) extract. The TLC was performed using the mobile phases Toluene:Ethyl acetate:Methanol (8:1:1) for BM with comparisons with against standard triterpene (Betulin) **(Fig 5, Table 5).**

The collected fractions were properly prepared, and their UV spectra were recorded. UV spectra of the isolated fractions (F) from *Baliospermum montanum* (BM) extract were obtained using a Shimadzu 1700

double beam UV-VIS spectrophotometer. The spectra were recorded in the respective solvents-Toluene:Ethyl acetate:Methanol (8:1:1) for BM over a scanning range of 200–800 nm. The λmax values of the isolated compounds were determined, with fraction (F) of BM showing one peak at 209 nm **(Fig 6).**

The IR spectrum of the isolated fraction (F) from the *Baliospermum montanum* (BM) extract revealed the presence of various functional groups. O–H stretching appeared as Strong, broad at 3463 cm^{-1}. A C–H stretching peak corresponding to alkane was observed at 2939 cm^{-1}, while C=C stretching of Alkene was noted at 1644 cm^{-1}. A C-H bending peak appeared at 1457 cm^{-1}. C–H bending peaks for methyl group was detected at 1374 cm^{-1} respectively. C–O stretching peaks of alcohol were observed at 1190 cm^{-1}, 1103 cm^{-1} and 1016 cm^{-1}. C=C bending peaks of Substitute were found at 878 cm^{-1}, 746 cm^{-1} and 640 cm^{-1} **(Fig. 7, Table 6).**

In the ^{1}H-NMR spectrum of the isolated fraction (F) from *Baliospermum montanum*, the following proton signals were observed H-9 protons appeared at 0.800-0.935 (0.881 (s), 0.930 (s), 0.930 (s)) ppm, H-6 protons appeared at 1.180-1.301 (1.189 (s), 1.296 (s)) ppm, H-27 protons appeared at 1.23-1.88 (1.312 (dd), 1.362 (dd), 1.397 (ddd), 1.427 (ddd), 1.446 (ddd), 1.482 (ddd), 1.523 (dddd), 1.563 (dddd), 1.568 (ddd, J = 14.3, 8.1, 4.1 Hz), 1.568 (ddd), 1.572 (dddd), 1.572 (dddd), 1.572 (ddd), 1.586 (s), 1.597 (dddd), 1.753 (ddd), 1.753 (ddd), 1.796 (dddd), 1.801 (dddd), 1.812 (ddd), 1.824 (dddd), 1.851 (dddd), 1.851 (dddd), 1.870 (dd), 1.912 (ddd)) ppm, H-1 proton appeared at 2.458 (ddd) ppm, H-1 proton appeared at 2.956 (dd) ppm, H-1 proton appeared at 3.047 (dd) ppm, H-2 protons appeared at 3.301-3.440 (3.304 (d), 3.435 (d)) ppm, H-2 protons appeared at 3.301-3.440 (3.304 (d), 3.435 (d)) ppm, H-2 protons appeared at 4.190-4.240 (4.195 (d), 4.234 (d)) ppm and H-1 proton appeared at 4.614 (dd) ppm **(Fig 8).**

The mass spectrum of the isolated fraction (F) from *Baliospermum montanum* (BM) showed a molecular ion peak [M^+] at m/z 442.2822, corresponding to the compound (1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a-(hydroxymethyl)-5a,5b,8,8,11a-pentamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol. The molecular composition indicated the presence of 30 carbon atoms (C), 50 hydrogen atoms (H), and two oxygen atoms (O), resulting in the molecular formula $C_{30}H_{50}O_2$. This identification was further supported by characteristic fragment ions observed at m/z 83, 177, 217, 327 and 413 (**Fig 9**).

CONCLUSION

This study successfully isolated and confirmed Betulin as a major triterpenoid constituent of *Baliospermum montanum* root. TLC-guided fractionation and silica gel column chromatography enabled the purification of the active fraction, which showed strong alignment with Betulin standards. Spectroscopic analyses—including UV–Vis, FTIR, ^{1}H NMR, and mass spectrometry—collectively verified the compound's identity, with characteristic functional groups, proton signals, and a molecular ion peak at m/z 442.2822 corresponding to Betulin ($C_{30}H_{50}O_2$).

These findings provide the first comprehensive evidence of Betulin in *B. montanum*, highlighting the plant as a valuable natural source of this pharmacologically significant triterpenoid. The optimized isolation protocol offers a reliable foundation for future studies exploring the compound's therapeutic potential and its application in drug development and formulation research.

References

1. Newman, D.J., Cragg, G.M. (2020). Natural products as sources of new drugs over nearly four decades. *Journal of Natural Products*, 83(3), 770–803.
2. Alakurtti, S. et al. (2006). Pharmacological properties of the ubiquitous natural product Betulin. *European Journal of Pharmaceutical Sciences*, 29(1), 1–13.
3. Yogeeswari, P., Sriram, D. (2005). Betulinic acid and its derivatives: A review on their biological properties. *Current Medicinal Chemistry*, 12(6), 657–666.
4. Kirtikar, K.R., Basu, B.D. (2001). *Indian Medicinal Plants*. International Book Distributors.
5. Rastogi, R.P., Mehrotra, B.N. (2004). *Compendium of Indian Medicinal Plants*. CDRI.
6. Drag-Zalesińska, M. et al. (2020). Betulin and Betulinic acid—chemistry and biological activity. *Molecules*, 25(21), 5311.
7. Amiri, S. et al. (2020). NMR characterization of Betulin isolated from plant sources. *Analytical Sciences*, 36(1), 87–94.
8. Evans, W. C. (2009). Trease and Evans' pharmacognosy. Elsevier Health Sciences.
9. Alara, O. R., Abdurahman, N. H., Ukaegbu, C. I., & Kabbashi, N. A. (2019). Extraction and characterization of bioactive compounds in Vernoniaamygdalina leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. *Journal of Taibah University for Science*, *13*(1), 414-422.
10. Coskun, O. (2016). Separation techniques: chromatography. *Northern clinics of Istanbul*, *3*(2), 156.

11. Bele, A. A., &Khale, A. (2011). An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*, *2*(2), 256.
12. Srivastava Nishi, Singh Arti, Kumari Puja, Jay Hind Nishad, Gautam Veer Singh, Yadav Monika, BhartiRajnish, Kumar Dharmendra, KharwarRavindra N., (2021), "Advances in extraction technologies: isolation and purification of bioactive compounds from biological materials", *Natural Bioactive Compounds: Technological Advancements, Elsevier* Inc., 409-425.
13. Mukherjee, S., Rizal, S., Singh, S., Hooi, A., Ghosh, P. K., Hossain, A., & Atta, K. (2024). Methodologies for identification, purification, and characterization of bacterial secondary metabolites. In *Bacterial secondary metabolites* (pp. 381-397). Elsevier.
14. Patel,Diya, PanchalDiya, Patel Kunj, Prof. DalwadiMitali, Dr. UpadhyayUmesh , (2022), "A Review on UV Visible Spectroscopy", *International Journal of Creative Research Thoughts (IJCRT),* 2320-2882.
15. Picollo, M., Aceto, M., &Vitorino, T. (2019). UV-Vis spectroscopy. *Physical sciences reviews*, *4*(4), 20180008.
16. LucieneGonçalvesPalmeiraMoraes, RenataSanches Ferreira Rocha, LíviaMalufMenegazzo, Eudes Borges De Araújo ,KeizoYukimitu, João Carlos Silos Moraes, Infrared Spectroscopy: A Tool For Determination Of The Degree Of Conversion In Dental Composites, *Journal Applied Oral Science*. 2008;16(2):145-9
17. Dutta, A. (2017). Fourier transform infrared spectroscopy. *Spectroscopic methods for nanomaterials characterization*, 73-93.
18. Zia Komal, SiddiquiTalal, Ali Saqib, Farooq Imran , Muhammad ZafarSohail, and KhurshidZohaib, (2019), "NuPEear Magnetic Resonance Spectroscopy for Medical and Dental Applications: A Comprehensive Review, *Eur J Dent.* 13(1): 124–128.
19. Marion, D. (2013). An introduction to biological NMR spectroscopy. *Molecular & Cellular Proteomics*, *12*(11), 3006-3025.
20. Wiley W. C. And MPEaren I. H., Timeofflight Mass Spectrometer With Improved Resolution, The *Review Of Scientific Instruments,* Vol 26, (12): 1150-57
21. Torgerson, D. F., Skowronski, R. P., & Macfarlane, R. D. (1974). New approach to the mass spectroscopy of non-volatile compounds. *Biochemical and Biophysical Research Communications*, *60*(2), 616-621.