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Assessment of the Isolation of Bioactive Molecules from the Root Extract of Nardostachys Jatamansi

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KEYWORDS

Isolation, *N. Jatamansi*, Chromatograph, Sesquiterpenes, Characterization

ABSTRACT:

Introduction: *Nardostachys jatamansi,* a perennial herb renowned for its therapeutic properties in traditional medicine, has been the focus of extensive research due to its potential pharmacological significance.

Objective: This study aimed to isolate and characterize sesquiterpene compounds from the root extract of *N. jatamansi*.

Methods: Chromatographic techniques were used in a stepwise manner for solvent extraction, and isolation process. The isolated sesquiterpene was subjected to various spectroscopic analyses, including UV-visible spectroscopy, nuclear magnetic resonance (NMR) and mass spectrometry (MS), to elucidate its structural composition and confirm its purity.

Result: Results indicated that the *Nardostachys jatamansi* and Std. Sesquiterpenoid Rf values were determined to be 0.53 and 0.53, respectively. For column chromatography, toluene: ethyl acetate: acetic acid (9:1:0.2) was chosen as the mobile phase. Using mass spectroscopy, a mass spectrum of the isolated component (Fraction H) of the alcoholic extract from *Nardostachys jatamansi* was captured. The successful isolation of a sesquiterpene compound from *N. jatamansi* root extract, with spectral data supporting its structural identification. The elucidation of the sesquiterpene's chemical structure contributes to the understanding of the plant's chemical composition and potential therapeutic applications.

Conclusion: This research provides a foundation for further investigations into the pharmacological properties of *N. jatamansi* and its constituents, potentially paving the way for further novel pharmaceutical evaluation.

1. Introduction

Natural products, also known as "natural sources deriving compounds," are found in plants, animals, and microorganisms. In particular, a wide range of species from the kingdom of plants are still utilized as medicine to treat a number of illnesses in various regions of the world, including South America **(Bolzani et al., 2012),** Asia **(Durajpandiyan et al., 2006; Grover et al., 2002),** Africa **(Jurg et al., 1991; Nugueym et al., 2008; Brusotti et al., 2011; Khalid et al., 2012).**"Natural product and/or natural product structures continued to play a highly significant role in the drug discovery and development process," according to Newman and Cragg's recent review **(New man and Cragg, 2012)**.Biodiversity provides numerous novel chemical entities (NCEs) for therapeutic purposes. These secondary metabolites, produced by plants as defense against pathogens and herbivores, belong to three primary chemical families: phenolic compounds, terpenoids, and alkaloids. Understanding plant parts used for treatments is crucial for ethno pharmacological methods. However, due to lack of information about the secondary metabolite's nature, extraction, purification,

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and separation procedures are conducted to isolate and identify the bioactive compounds **(Brusotti et al., 2014).***Nardostachys jatamansi* (D.Don) DC. (Caprifoliaceae Juss.: Dipsacales Batsch) has a long history of use in ayurveda and traditional medicine systems **(Sharma et al., 2000; Chen and Mukherji, 2016).** Phytochemical research demonstrated the presence of essential oils (coumarins and sesquiterpenes) in both the roots and rhizomes of *N. jatamansi,* which are directly related with several medicinal applications **(Mishra et al., 1995; Nautiyal, 2013; Singh et al., 2015; Rekha et al., 2013; Liu and Liu, 2014).** It is also one of the top 20 most traded plants in India and is heavily exploited **(Rai et al., 2000; Olsen, 2005; Chauhan et al., 2011; Dhiman and Bhattacharyya, 2020).** It is presently on the verge of extinction due to its vast range of uses in traditional medicine, severe over-exploitation, and small reproductive phase and poor germination rate (10-20%) **(Nautiyal et al., 2003)**.The tiny, perennial rhizomatous herb *Nardostachys jatamansi DC* grows from 2200 m to 5000 m above sea level on steep, damp, rocky, undisturbed grassy slopes in India, Nepal, China, Tibet, and Bhutan **(Ghimire et al., 2005).**The Ayurvedic classics Charaka Samhita, Nighantus Chikitsagranthas, and Sushruta Samhita all contain descriptions of jatamansi. It is explained in the Charaka Samhita asSangyasthapa Mahakashaya is utilized in Kushtha as a dhumvarti for hikkashwasa. Used in Kasa, Hriveradighrita is used in Arsha. Used in unmade is Mahapaishachikaghrita. It is known as Kumararasayana in the Sushruta Samhita **(Airi et al., 2000)**.It's also referred to as spikenard, Indian nard, jatamansi, and balchar. Several medical systems use its rhizomes in traditional remedies **(Yang, 1996).**It has been tested for depressing effects in herbal mixtures containing other plants. According to Ayurveda, Nardostachys jatamansi roots and rhizome have different effects on the doshas. In the end, trido shashamak, but kapha-pitta nashak in particular, has been used medicinally due to its anti-ischemic, antioxidant, neuroprotective, and anticonvulsant properties. Additionally, Nardostachys jatamansi improves memory. It is also intended to treat amnesia brought on by aging because mice age naturally. The rhizome of Nardostachys jatamansi DC has been shown to be an effective antistress and memory-restoring medication for dementia patients **(Jadhav etal., 2009).**For millennia, India has utilized jatamansi extensively in perfumes and medicine. Numerous therapeutic qualities are associated with it, including anti-lipid peroxidative, hypolipidemic, antioxidant, hepatoprotective, sedative, tranquilizing, antihypertensive, anti-inflammatory, antidepressant-like, anticonvulsant, hypotensive, anti-asthmatic, and anti-estrogenic activity **(Rahman et al., 2011)**. In addition, it is used to treat a number of nervous problems, including excitement, epilepsy, neurosis, insomnia, Alzheimer's disease, and learning and memory impairments **(Joshi and Parle, 2006; Rahman et al., 2011).** It is also used to cure hair loss, growth, and shine **(Bagchi et al., 1991).** Additionally, the antispasmodic and stimulating qualities of their extracts can be utilized to control constipation, urine, menstruation, and digestion in addition to treating fits and heart palpitations **(Anon, 1993).**

2. Materials and Method

2.1 Plant Material

The roots and rhizomes of *Nardostachys jatamansi* were collected from Garhwal region in Uttarakhand in the month of September, 2021. The plant was authenticated by Dr. V.P Bhatt, Scientist, Herbal research and development Institute, Chamoli, Uttarakhand. A voucher specimen (Ref No 320/HRDI/21-1/2020-21) has been deposited in the herbarium of our institute, Herbal research and development Institute, for future reference.

2.2 Plant Extraction

On the basis of last publication extraction of dried leaves powder was done with petroleum ether and by placing in a thimble of Soxhlet apparatus using Hydroalcoholic solvent system at 40-60 ºC temperature of the heating mantle for 8-10 hours **(Pokhriyal et al., 2023).** Further evaluation of that extract like isolation of phenols and characterization is carried out in this paper.

2.3 Preliminary Thin Layer Chromatography

Thin Layer Chromatography was performed utilizing a different solvent system that included standard Sesquiterpenoid on TLC plates of silica gel 60 F254 precoated with a layer thickness of 0.2 mm. The capillary tube was used to manually apply the spots, the plates were allowed to air dry, and a solvent system was used to produce the TLC chamber at room temperature.

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Spots on TLC plates were seen using an iodine chamber in visible light and UV light 254, 365. Rf values were computed **(Kagan *et al.,* 2014).**

$$R_f \text{ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

A solvent system was created for the initial TLC analysis of the *Nardostachys jatamansi,* extract, wherein the standard Sesquiterpenoid was observed in the Toluene: Ethyl acetate: Acetic acid (9:1:0.2) mobile phase. For column chromatography, toluene: ethyl acetate: acetic acid (9:1:0.2) solvent was used as the mobile phase.

2.4 Column Chromatography

Sesquiterpenoid was separated from *Nardostachys jatamansi* extract using silica gel column chromatography using hydro alcoholic extract. Chromatography was performed using a vertical glass column composed of borosilicate material. Before packing, the column was thoroughly dried and cleaned with acetone. Wet packing was used to pack the column, with silica gel (60–120) serving as the adsorbent. Toluene was used to produce the slurry, which was then added to the column. One gram of extract was applied to the column's top. Column chromatography was performed using the gradient elusion technique. Toluene was used to elude the column: Nineteen elutes of ethyl acetate: acetic acid (9:1:02) were obtained. To find a single compound, TLC was used to concentrate the fractions or elutes that had been collected **(Srivastava *et al.,* 2021).**

2.5 Spectroscopic Characterization

2.5.1 UV-visible Spectroscopy

The isolated fraction H of *Nardostachys jatamansi*extract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded **(Patel *et al.,* 2022)**.

2.5.2 FT-IR

FT-IR spectroscopy was carried out using a Perkin Spectrum BX spectrophotometer to determine whether the functional groups were present in the separated fraction H of *Nardostachys jatamansi* extract. After being dried and crushed using KBr pellets, the samples were examined using a Thermo Nicolet model 6700 spectrum analyzer. A mixture of 2% finely dried sample was used to make a disk containing 100 mg of KBr, which was subsequently analyzed using an IR spectrometer. Spectra of infrared light were obtained between 400 and 4,000 cm-1. **(Luciene *et al.,* 2008)**

2.5.3 NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction H of *Nardostachys jatamansi* extract to identify the structure of the compound present in the isolated fraction. NMR spectroscopy for this purpose was Fourier Transform Nuclear Magnetic Resonance spectroscopy, Model AVNACENEO500 Ascend Bruker BioSpin International AG, Switzerland **(Zia *et al.,* 2019).**

2.5.4 Mass Spectroscopy

Mass spectrometry converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of the compound was mass spectrometer instrument micrOTOF-Q 228888.10348**(Wiley *et al.,* 1995).**

3. Results

3.1 Preliminary TLC preparation for the estimation of active constitutes- TLC of *Nardostachys jatamansi* Hydro alcoholic extract

For Sesquiterpenoid:-

Mobile Phase- Toluene: Ethyl acetate: Acetic acid (9: 1: 0.2)

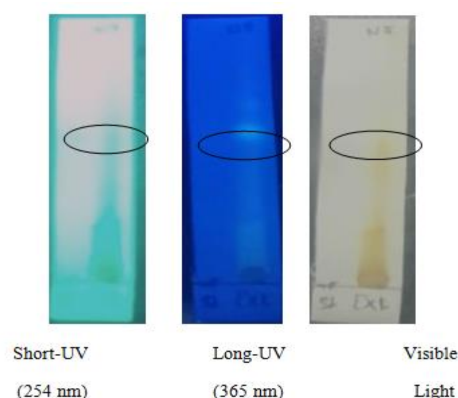

Figure 1: TLC estimation by UV lamp for TLC of *NJ* with Std. Sesquiterpenoid

(Std. = Standard, *NJ* = *Nardostachys Jatamansi*)

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Table 1TLC of *Nardostachys Jatamansi* Hydro alcoholic extract

S. No.	Solvent system	No. of spots	Colour of spots at Wavelength (254 & 365nm)	Rf value (Extract)	Rf value (Std. Sesquiterpenoid)
`1.	Toluene: Ethyl Acetate: Acetic acid (9:1:02)	06	Light Green (Std) Florescence Light Blue Florescence Florescence (Green) Dark Florescence Florescence	- 0.17 0.20 0.26 0.53 0.62 0.67	0.53

TLC of *Nardostachys jatamansi* extract was performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in Toluene: Ethyl Acetate: Acetic acid (9:1:02) that were clearly visible bands of *Nardostachys jatamansi* extract with Std. Sesquiterpenoid. The Rf values of N*ardostachys jatamansi* extract with std. Sesquiterpenoid were found to be 0.53 and 0.53.

3.2 Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of *Nardostachys Jatamansi* Hydro alcoholic extract were tested for the detection of various phyto compounds using TLC. The collected fractions/elutes were taken properly and do the UV spectrum.

3.2.1Column Chromatography of *Nardostachys jatamansi* Hydro alcoholic extract –

Table 1: Fraction collected from Column Chromatography of *Nardostachys jatamansi* Hydro alcoholic extract

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

3.2.2 TLC of all collected fractions-

A) TLC of all collected fractions of *Nardostachys jatamansi* Hydro alcoholic extract –

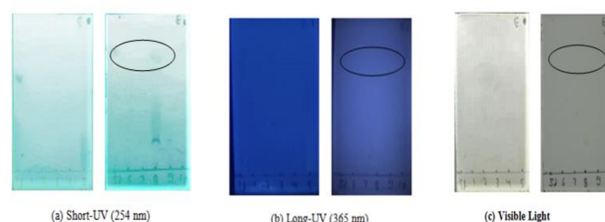

Figure 2: TLC estimation by UV lamp for *NJ* fractions after column chromatography with std.

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Std. Sesquiterpenoid. a) Short-UV (254 nm), b) Long-UV (365 nm), c) visible light

(Std. = Standard, *NJ = Nardostachys Jatamansi*)

TLC of fractions (A, B, C, D, E, F, G, H, I & J) of *Nardostachys jatamansi* Hydro alcoholic extract -

Table 2: Rf values of all collected fractions of *Nardostachys jatamansi* after column chromatography

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelenth (254 & 365nm)	Rf value (Extract)	Rf value (std.Sesquiter-peniod)
1.	A	Toluene: Ethyl Acetate: Acetic acid (9:1:02)	-	-	-	0.54
2.	B		01	Light Fluorescence	0.46	
3.	C		01	Light Blue	0.18	
4.	D		01	Light Blue	0.18	
5	E		01	Fluorescence	0.26	
6	F		01	Light Green	0.29	
7	G		01	Light Green	0.29	
8	H		02	Green Dark Green	0.54 0.23	
9	I		01	Dark Green	0.23	
10	J		-	-	-	

Rf value resulted after performing the TLC estimation was also done for the confirmation of active constituent in fraction H of *Nardostachys jatamansi* Hydro alcoholic extract with mobile phase Toluene: Ethyl Acetate: Acetic acid (9:1:02)by comparing with std.Sesquiterpeniod.

3.3 Spectroscopic Characterization

3.3.1 Active constituents estimation By UV-Spectroscopy and FTIR – Spectroscopy

UV spectra of the isolated fraction H of *Nardostachys jatamansi* was recorded over a scanning range of 200-800 nm and λmax of isolated compound were determined. The Blank was Toluene: Ethyl acetate: Acetic acid (9:1:0.2). The wavelength of isolated fraction H of *Nardostachys jatamansi* Hydro alcoholic extract was found to be 251 nm.

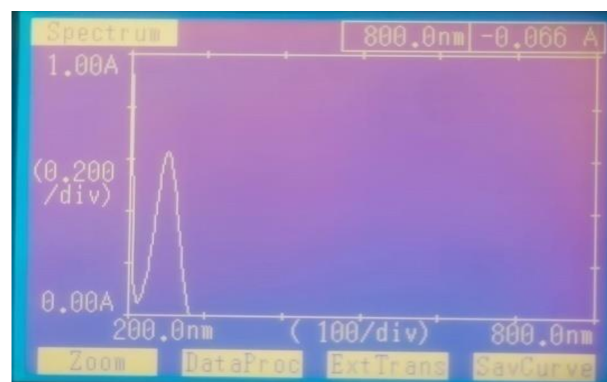

Figure 3: Active constitutes estimation By UV-Spectra of H fraction of *Nardostachys jatamansi* Hydro alcoholic extract after column chromatography

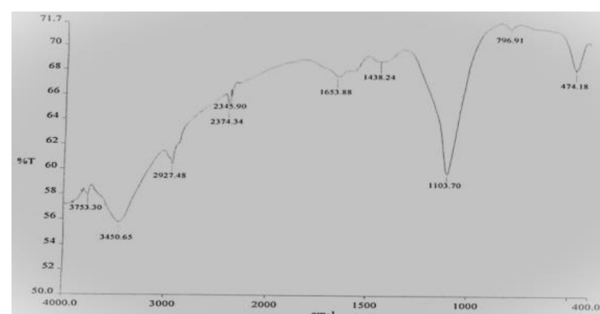

Figure 4: IR spectra of the isolated Fraction H of *Nardostachys jatamansi* Hydro alcoholic extract

Table 3: FTIR- Spectrum Frequency Range of the isolated Fraction H of *Nardostachys jatamansi* Hydro alcoholic extract

Sr.No.	Fraction	Frequency Range	Group Absorption (cm^{-1})	Appearance	Group	Compound Class
1	H	4000-3000 (cm^{-1})	3450.65	Strong, Broad	O-H stretching	Hydroxyl Group
		3000-2500 (cm^{-1})	2927.48	Medium	C-H stretching	Alkane
		2400-	2345.9	Stron	O=C=O	Carbon

		2000 (cm^{-1})	0	g	stretching	dioxide
		2000-1600 (cm^{-1})	1653.88	Medium	C-O stretching	Carbonyl group
		1400-1100 (cm^{-1})	1103.70	Weak	C-C stretching	Alkane
		1600-1400 (cm^{-1})	1438.24	Strong	C=C stretching	Benzene Ring
		840-790 (cm^{-1})	796.91	Medium	C=C bending	Alkene

The IR Spectra of isolated fraction H of *Nardostachys jatamansi* Hydro alcoholic extract showed that -OH group broad absorption peak appeared at 3450.65 cm-1. The C-H stretching peak appeared at 2927.48 cm-1 for Alkane. O=C=O stretching peak appeared at 2345.90cm-1, Carbonyl group C-O stretching absorption peak appeared at 1653.88 cm-1 and C-C stretching peak at 1103.70 cm-1 for Alkane. The C=C stretching absorption peak at 1438.24 cm-1 for Benzene ring and C=C bending peak appeared at 796.91 cm-1.

3.3.3 ^{1}H NMR-Spectroscopy

^{1}H NMR spectra of isolated fraction H of *Nardostachys jatamansi* Hydro alcoholic extract was recorded on NMR Spectrometer. Tetramethylsilane used as an internal standard. The signals are denoted with the symbols s, d, t, and m for singlet, doublet, triplet, and multiplet, respectively.

(A) 1H NMR spectra of the isolated compound (Fraction H) of Nardostachys jatamansi –

In ^{1}H NMR spectra of isolated fraction H of *Nardostachys jatamansi* Hydro alcoholic extract showed that ^{1}H-3 protons appeared at 0.93 (d) ppm, ^{1}H-9 protons appeared at 1.15-1.34 ppm (1.20 (s) ppm, 1.25 (s) ppm, 1.30 (s) ppm), ^{1}H-2 protons appeared at 1.55-1.75 ppm (1.60 (dddd) ppm, 1.62 (dddd) ppm), ^{1}H-2 protons appeared at 1.92-2.15 ppm (1.99 (dqd) ppm, 2.05 (d) ppm), ^{1}H-2 protons appeared at 2.20-2.47 ppm (2.29 (dtd) ppm, 2.38 (dddd) ppm), ^{1}H-2 protons appeared at 2.70-3.00 ppm (2.85 (dd) ppm, 3.01 (dd) ppm), ^{1}H-1 proton appeared at 4.60 (ddd) ppm and ^{1}H-1 proton appeared at 5.68 (dd) ppm.

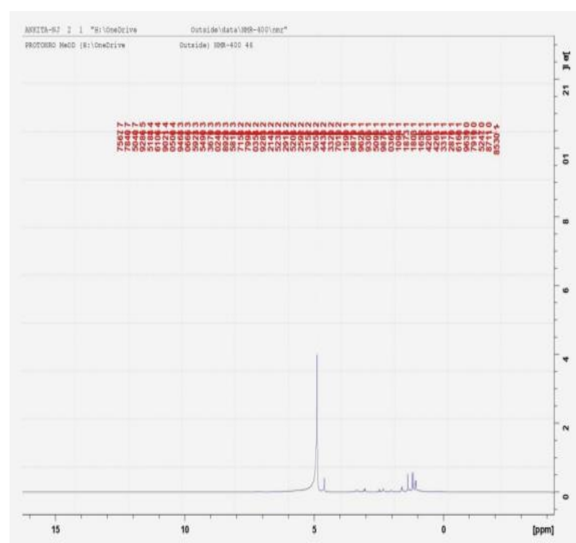

Figure 5: ^{1}H-NMR spectra of the isolated compound (Fraction H) of *Nardostachys jatamansi* Hydro alcoholic extract

3.3.4 Mass – Spectroscopy-

A mass spectrum of isolated compound (Fraction H) of *Nardostachys jatamansi* Hydro alcoholic extract was recorded on Mass Spectroscopy. Mass spectra of isolated fraction (Fraction H) of *Nardostachys jatamansi* Hydro alcoholic extract showed molecular ion [M^+] peaks at mlz 251.1615 which corresponds to the molecular formula $C_{15}H_{22}O_3$ according to their fragments.

(A)Mass spectra of the isolated compound (Fraction H) of Nardostachys jatamansi Hydro alcoholic extract-

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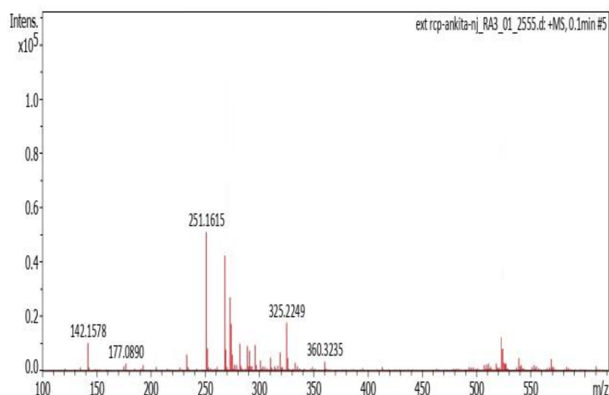
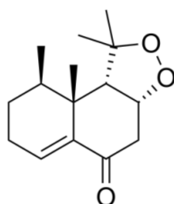


Figure 6: **Mass spectra of the isolated compound (Fraction H) of *Nardostachys jatamansi* Hydro alcoholic extract**



Nardosinone

IUPAC NAME: (3aR,9R,9aR,9bS)-1,1,9,9a-Tetramethyl-1,3a,4,7,8,9,9a,9b-octahydro-5H-naphtho[2,1-c][1,2]dioxol-5-one

4. Discussion

Different solvent systems were used for the preliminary TLC of *Nardostachys Jatamansi* Hydro alcoholic extract (solvent system was selected on the basis of literature review). TLC was done in Toluene: Ethyl acetate: Acetic acid (9:1:0.2) using standard Sesquiterpenoid, which was observed in the bands of *Nardostachys jatamansi* Hydroalcoholic extract. The *Nardostachys jatamansi* and Std. Sesquiterpenoid Rf values were determined to be 0.53 and 0.53, respectively. For column chromatography, toluene: ethyl acetate: acetic acid (9:1:0.2) was chosen as the mobile phase. The mobile phase of Toluene: Ethyl acetate: Acetic acid (9:1:0.2) for *Nardostachys jatamansi* is used in column chromatography to isolate the active ingredients, yielding Fractions 01 (A), 02 (B), 03 (C), 04 (D), 05 (E), 06 (F), 07 (G), 08 (H), 09 (I), and 10 (J) **(Table 2).**TLC estimation was performed for the validation of active ingredients in fractions H of *Nardostachys jatamansi* using mobile phase Toluene: Ethyl acetate: Acetic acid (9:1:0.2) by comparing with standard Sesquiterpenoid **(Fig 2, Table 3).** The fractions were appropriately collected, and a UV spectrum studies was carried out. The UV spectra of the isolated fractions H of *Nardostachys jatamansi* were recorded throughout a scanning range of 200-800 nm, and the maximum of fractions H was measured, and the wavelength of *Nardostachys jatamansi,* H fraction was found to be 251 nm **(Fig 3).** The separated fraction H of the *Nardostachys jatamansi* Hydro alcoholic extract's IR spectrum revealed that a wide absorption peak for the -OH group had emerged at 3450.65 cm-1. For alkane, the C-H stretching peak was seen at 2927.48 cm-1. For alkane, the O=C=O stretching peak was seen at 2345.90 cm-1, the C-O stretching absorption peak for the carbonyl group was at 1653.88 cm-1, and the C-C stretching peak was at 1103.70 cm-1. The benzene ring's C=C stretching absorption peak was observed at 1438.24 cm-1, whereas the C=C bending peak was observed at 796.91 cm-1**(Table 4, Figure 4).**In 1H NMR spectra of isolated fraction H of *Nardostachys jatamansi* Hydro alcoholic extract showed that lH-3 protons appeared at 0.93 (d) ppm, lH-9 protons appeared at 1.15-1.34 ppm (1.20 (s) ppm, 1.25 (s) ppm, 1.30 (s) ppm), lH-2 protons appeared at 1.55-1.75 ppm (1.60 (dddd) ppm, 1.62 (dddd) ppm), lH-2 protons appeared at 1.92-2.15 ppm (1.99 (dqd) ppm, 2.05 (d) ppm), lH-2 protons appeared at 2.20-2.47 ppm (2.29 (dtd) ppm, 2.38 (dddd) ppm), lH-2 protons appeared at 2.70-3.00 ppm (2.85 (dd) ppm, 3.01 (dd) ppm), lH-1 proton appeared at 4.60 (ddd) ppm and lH-1 proton appeared at 5.68 (dd) ppm**(Fig 5).**Using mass spectroscopy, a mass spectrum of the isolated component (Fraction H) of the alcoholic extract from *Nardostachys jatamansi* was captured. The isolated fraction (Fraction H) of the alcoholic extract from *Nardostachys jatamansi* displayed mass spectra with molecular ion [M+] peaks at mlz 251.1615, which, based on their fragmentation, corresponds to the chemical formula C15H22O3 **(Fig 6).** It was determined from this physical, chemical, and spectroscopic analysis that fraction H of the *Nardostachys jatamansi* Hydro alcoholic extract contained Nardosinone.

5. Conclusion

Nardostachys jatamansi contains a number of bioactive chemicals, including crystalline acid, Jatamansic acid,

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hydrocarbons, a polyoxygenated crystalline solid together with A-endesmol, B-eudesmol, ethanol, angelicin, 4-hydroxythymol dimithyl ether. The range of examination of the Hydro alcoholic extracts of plant *Nardostachys jatamansi* belonging to the family Caprifoliaceae was effectively carried out. From these physically, chemically and spectral investigation were confirmed the presence of **Nardosinone** in fraction H *Nardostachys jatamansi* Hydro alcoholic extract. The identification and characterization of the bioactive molecule, achieved through advanced analytical techniques, have paved the way for a deeper understanding of the plant's pharmacological properties. The identified compound has antioxidant, anti-inflammatory, and neuroprotective effects. Nardosinone can suppress the generation and differentiation of OCs from mouse bone marrow macrophages (BMMs) through JNK, ERK, PLCγ2, c-Fos, and NFATc1 signaling pathways in association with scavenging the RANKL-induced ROS. These findings highlight the plant's medicinal significance and the need for further research to explore its therapeutic potential. The outcomes of this study lay the groundwork for further research and development, fostering a bridge between traditional knowledge and contemporary scientific advancements in the pursuit of effective and natural healthcare solutions.

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