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GC-MS MEDIATED PHYTOCHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY SCREENING OF *VALERIANA JATAMANSI* JONES. FROM THE KASHMIR HIMALAYA

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ABSTRACT
Background: *Valeriana jatamansi*, an important medicinal plant native to the Himalayan region, is valued for its diverse therapeutic applications. However, comprehensive characterization of its phytochemical composition and systematic evaluation of its biological activities remain essential for establishing its potential as a source of bioactive compounds. **Material methods**: The present study investigated the phytochemical profile, antioxidant capacity, and antibacterial activity of the methanolic rhizome extract of *Valeriana jatamansi*. Rhizomes were extracted using methanol, and the extraction yield was determined, followed by preliminary phytochemical screening and GC-MS analysis. Antioxidant potential was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical-scavenging and ferric reducing antioxidant power assay. Antibacterial activity was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* by determining the zones of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).
Results: The methanolic extract exhibited an extraction yield of 41.20% and showed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, and tannins. GC–MS analysis identified 57 compounds, collectively accounting for 91.84% of the total chromatographic peak area, with a predominance of sesquiterpenoid-related constituents. Epicurzerenone was the major constituent (18.67%), followed by eucalyptol (10.76%), levulinic acid (6.12%), khusimone (5.64%), and camphor (5.24%). The extract exhibited concentration-dependent antioxidant and antibacterial activities. Among the tested bacterial strains, Staphylococcus aureus and Bacillus subtilis showed the highest susceptibility, with MIC and MBC values of 250 and 500 µg/mL, respectively.
Conclusion: The study provides an integrated chemical and biological assessment of *Valeriana jatamansi* rhizome extract, demonstrating its sesquiterpenoid-rich composition alongside promising *in vitro* antioxidant and antibacterial activities. These findings highlight the potential of *Valeriana jatamansi* as a source of bioactive phytochemicals and provide a basis for further investigation. Future studies should focus on the isolation and structural characterization of individual constituents, evaluation of their specific bioactivities, and elucidation of the underlying mechanisms to better understand their contributions to the observed effects and assess their potential applications.

KEYWORDS: Medicinal plants, *Valeriana jatamansi,* GC-MS Analysis, sesquiterpenes, Antioxidant and Antibacterial activities.

1. INTRODUCTION

Medicinal and aromatic plants can be regarded as one of the richest sources of biologically active natural products that have not been sufficiently explored till date. One of the most well-known hotspots for Himalayan biodiversity, the Kashmir Himalaya is home to a remarkable array of high-range medicinal plants that have evolved unique secondary metabolome adaptations to withstand harsh environmental conditions like low temperatures, high radiation, and short vegetation periods (Peerzada et al., 2026). The genus *Valeriana* (Caprifoliaceae family), one of the most traded groups of medicinal plants worldwide and a natural source of sedative, anxiolytic, and neuroprotective plant components, has drawn a lot of attention due to its significance in pharmacology and economy (Quan et al., 2019; Peerzada et al., 2022). It is a small herb native to the Himalayas and grow at an altitudes ranging between 1,000 and 3,000 meters above sea level in regions extending from Afghanistan to Southwest China, including India, Nepal, Bhutan, and Myanmar. The genus *Valeriana* of family Caprifoliaceae consists of several biologically active species (Rather et al., 2012; Liu et al., 2017; Jugran et al., 2019).) *Valeriana jatamansi* is a hairy, rhizomatous, perennial dwarf herb that forms a cluster of thick, fiber-covered roots . Due to overuse, habitat degradation and other biotic interferences, this plant is at the verge of extinction and becoming an endangered species (Chakraborty et al., 2015; Lin et al., 2017). In drug industry valerenic acid and valepotriates isolated fron *Valeriana jatamansi* are used as a source of drug valerian and act

as natural source of valepotriates and psychopharmacological agent (Bhatt et al., 2012; Singh et al., 2006).The drug valerian is among the top selling herbal supplements and ranked eighth place. The various activities like anticancer, anti-inflammatory, anticoagulant, antioxidative, antifungal, antibacterial, neuroprotective, antiprotozoal and hepatoprotective, are due to valepotriates/iridoids isolated form *Valeriana jatamansi* (Dinda, Chowdhury, &Mohanta, 2009). In traditional Oriental medicine, the roots and rhizomes of *Valeriana jatamansi* have been used to manage various ailments, including snake poisoning, nervous disorders, skin diseases, and obesity (Quan et al., 2019). Studies on *Valeriana jatamansi* revealed a considerable number of iridoids having cytotoxic activity (chlorovaltrate, chlorovaltrates K -N, rupesin B, valejatanins A-C ,valtrals A-C, valeridoid A, valerialloside A , valerianoside A desacylbaldrinal, jatamanvaltrate P and jatamanvaltrates (Liu et al., 2017; Tian et al., 2018 ; Quan et al., 2020; Maurya et al., 2022). The UPLC- ESI-QqQ LIT − MS/MS quantification study revealed the presence of forty seven chemical compounds out of which nine were isolated from leaves and roots of *Valeriana jatamansi* (Shukla et al., 2021). GC–MS analysis of the essential oil obtained from the roots/rhizomes has identified several major sesquiterpenes, including patchouli alcohol, δ-guaiene, α-selinene, α-patchoulene, seychellene, β-patchoulene, maaliol, seychellenecalarene, and β-gurjunene, which may contribute to its medicinal properties (Raina & Negi, 2015; Thakur et al., 2022). However, the chemical composition of the essential oil exhibits considerable variation depending on several factors, including the plant part, season of collection, geographical origin, extraction method, and distillation time (Suraya et al., 2026; Lalawmpuii et al., 2026). GC- MS analysis of essential oil from roots/rhizomes reveals patchouli alcohol, δ-guaiene, α-selinene, α-patchoulene, seychellene, and β-patchoulene, maaliol , seychellenecalarene/ β-gurjunene as major sesquiterpene responsible for its medicinal properties (Raina & Negi 2015; Thakur et al., 2022). Most previous phytochemical investigations of *Valeriana jatamansi* have focused on its essential oil and volatile constituents using GC–MS, particularly from populations growing in different Himalayan regions. However, limited information is available on the GC–MS-based chemical profile of *Valeriana jatamansi* growing in the remote and relatively inaccessible Tulail–Gurez region of the Kashmir Himalaya. Moreover, geographical and environmental variations may influence the abundance and composition of volatile and semi-volatile constituents, making region-specific chemical characterization important. Therefore, the present study was undertaken to characterize the GC–MS profile of the methanolic rhizome extract of *Valeriana jatamansi* from Tulail–Gurez and to evaluate its associated antioxidant and antibacterial potential. The specific objectives were to: (i) perform preliminary qualitative screening of the major phytochemical classes present in the methanolic rhizome extract; (ii) characterize the volatile and semi-volatile constituents of the extract using GC–MS and identify the major compounds based on mass spectral library matching; and (iii) evaluate the antioxidant and antibacterial activities of the extract.

2. MATERIAL AND METHODS

2.1 Plant material: Plant material was collected from Tulail, Gurez Valley of District Bandipora, Kashmir. The collected material was taxonomically identified and authenticated at KASH Herbarium, University of Kashmir, and National Bureau of Plant Genetic Resources (NBPGR), with voucher numbers 9898-KASHand IC655076, respectively.

2.2 Chemicals: All Reagents for qualitative phytochemical analysis, Hydrochloric acid, DPPH and solvents were purchased from Merck, Ltd, India. Yeast extract medium and agar medium were brought from HiMedia, Mumbai, India. All the chemicals used were analytical and don't require further purification.

2.3 Bacterial Cultures: The bacterial strains *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S.aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Bacillus subtilis*(*B subtilis*) were procured from Department of Nanotechnology University of Kashmir.

2.4 Preparation of *Valeriana jatamansi* extract

Plants were thoroughly cleaned, trimmed, air-dried at room temperature, and surface-sterilized with 0.1% (w/v) $HgCl_2$ for 4min. The sterilized material was then dried, coarsely powdered, and stored in airtight glass containers until further use. A measured quantity of the powdered material was extracted with methanol, and the resulting extract was filtered, concentrated under reduced pressure, and stored under cool, dry conditions until further analysis (Madhu et al., 2016). The percentage yield of the extract was calculated using the following formula:

$$\%\text{Yield} = \frac{W.extract}{W.sample} \times 100$$

where:

W.extract = weight of the dried extract (g)

W.sample = initial weight of the powder (g)

2.5 Qualitative phytochemical screening

Qualitative phytochemical screening of the methanolic rhizome extract was performed according to the methods of (Banu and Cathrine 2015; De Silva et al., 2017; Mishra, K., 2026 and Singh and Kumar 2017). The extract was qualitatively screened for major phytochemical groups using standard tests. Alkaloids were detected by Mayer's test based on the formation of a white/creamy precipitate. Flavonoids were identified by the alkaline reagent test, indicated by a yellow colour that disappeared upon acidification. Saponins were detected by the foam test based on the formation of persistent froth. Phenolic compounds were assessed using the ferric chloride test, indicated by the development of a dark green/blue-black colour. Steroids and triterpenoids were evaluated

using Salkowski's test, based on characteristic colour development in the chloroform and acid layers. Glycosides were detected using Keller–Killiani's test, indicated by the development of reddish-brown to bluish-green colour. Tannins were assessed by the ferric chloride test, with the development of a blue-green colour considered indicative of their presence. Proteins were detected using Millon's test, based on the formation of a characteristic precipitate. The results were recorded qualitatively as either present (+) or absent (−).

2.6 GC–MS-Based Chemical Profiling

The volatile constituents of the samples were analyzed using gas chromatography–mass spectrometry (GC–MS). Sample was injected in an GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Splitless. The operating conditions of the GC–MS set for the analysis were as follows: oven temperature 45 °C for 2 min then 140 °C at 5°C/ min and finally increased to 280 °C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out 70 eV. The running time of the GC was from 9.10 min – 52.0 min. NIST14.L library (2020) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST14.L).

2.7 Chemical Antioxidant Assays

The antioxidant capacity of the methanolic rhizome extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging and ferric-reducing antioxidant power (FRAP) assays (Polile. 2024). All measurements were performed in triplicate, and methanol was used as the solvent blank. Ascorbic acid was used as the reference standard.

2.7.1 DPPH radical-scavenging assay: The DPPH assay was performed according to the standard colorimetric method with minor modifications (Daphedar et al., 2024). Briefly, 100 µL of the methanolic rhizome extract at different concentrations (50, 100, 150, and 200 µg/mL) was mixed with 100 µL of 60 µM DPPH solution prepared in methanol in a 96-well microplate. The plate was shaken for 1 min and incubated at room temperature in the dark for 30 min. Absorbance was measured at 517 nm using a microplate reader. Ascorbic acid was used as standard while as DPPH solution without extract served as the control. All measurements were performed in triplicates and radical-scavenging activity was calculated as:

DPPH inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

2.7.2 FRAP assay: The ferric-reducing antioxidant capacity of the methanolic extract was evaluated using the FRAP assay (Fernandes et al., 2016). The FRAP working reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution prepared in 40 mM HCl, and 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution in a ratio of 10:1:1 (v/v/v). Briefly, 100µL of the methanolic extract at different concentrations was mixed with 100µL of freshly prepared FRAP reagent and incubated at 37 °C for 4–6 min. Absorbance was measured at 593 nm. A calibration curve was prepared using $FeSO_4 \cdot 7H_2O$ as the reference standard, and the reducing capacity was expressed as µmol Fe^{2+} equivalents. All measurements were performed in triplicate.

2.8 Antibacterial activity

The antibacterial activity of the methanolic rhizome extract was evaluated using the agar well diffusion method (Arfa et al., 2026). Briefly, the bacterial strains *E.coli*, *S.aureus*, *P. aeruginosa*, and *B. subtilis* were initially grown in YEPD (Yeast Extract Peptone Dextrose) broth and incubated at 37°C for 18–24 h. The bacterial inocula were standardized to a turbidity equivalent to 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL. YEPD agar was prepared according to the manufacturer's instructions, sterilized by autoclaving, and poured into sterile petri plates. After solidification, 100 µL of the standardized bacterial inoculums was uniformly spread over the agar surface using a sterile spreader. Wells of approximately 6 mm diameter were aseptically prepared in the solidified agar using a sterile cork borer. The methanolic rhizome extract was prepared at concentrations of 50, 100, 150, and 200µg/mL**,** and100 µL of each concentration was added to the respective wells. The plates were allowed to stand for approximately 30 min at room temperature to facilitate diffusion of the extract and were subsequently incubated at 37°C for 24 h**.** The corresponding solvent was used as the negative control, while streptomycin as standard antibiotic. Following incubation, antibacterial activity was evaluated by measuring the diameter of the zone of inhibition (mm) around each well using a calibrated ruler. All treatments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

2.9 MIC and MBC Determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of the methanolic rhizome extract was determined by the broth microdilution method using *E.coli*, *S.aureus*, *P. aeruginosa*, and *B. subtilis*. MIC was defined as the lowest concentration of the extract that completely inhibited visible bacterial growth under the experimental conditions. Different concentrations of the extract (25, 50, 100, 150, 200, 250, 500, and 1000 µg/mL) were prepared in dimethyl sulfoxide (DMSO). Briefly, 5 mL of YEPD broth was dispensed into sterile test tubes, followed by the addition of an appropriate volume of the respective extract concentration and 10 µL of standardized bacterial suspension adjusted to 0.5 McFarland standard. The tubes were incubated at 37°C for 24 h. A bacterial culture without extract was maintained as the growth control, while DMSO without extract served as the solvent control. Following incubation, bacterial growth was assessed

visually based on turbidity. The MIC was determined as the lowest concentration of the methanolic rhizome extract at which no visible turbidity was observed, indicating complete inhibition of bacterial growth (S. Jain et al., 2017). The MBC was determined by subculturing aliquots from the MIC tubes and the tubes containing higher extract concentrations onto YEPD agar plates. The inoculated plates were incubated at 37°C for 24 h. Following incubation, the plates were examined for the presence or absence of viable bacterial colonies. The MBC was defined as the lowest concentration of the extract that resulted in no visible bacterial growth on the agar plate, indicating a bactericidal effect.

2.10 Stastical analysis

Experimental data was expressed as mean ± standard deviation (SD) from replicate measurements. Antioxidant assay data (DPPH and FRAP) were analyzed using one-way ANOVA, followed by Tukey`s HSD multiple-comparison test. Concentration-response relationships were further assessed by regression analysis and IC_{50} were estimated from fitted regression equations. For the antimicrobial disc diffusion assay, two way ANOVA was performed to evaluate the effects of bacterial strain, extract concentration and their interaction on the zone of inhibition (ZOI), followed by Tukey`s HSD test. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using R Studio.

3 RESULTS

3.1 Extraction yield and qualitative phytochemical Screening

The methanolic rhizome extract of *Valeriana jatamansi* from Tulail, Gurez, showed an extraction yield of 41.20%. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, and tannins, while phenolic compounds and proteins were not detected. The presence of terpenoids and glycosides is consistent with the known phytochemical profile of *Valeriana jatamansi*, which includes iridoid-derived valepotriates and other terpenoid metabolites **(**Tang et al., 2002).

3.2 GC-MS Analysis

GC-MS analysis of the volatile constituents of *Valeriana jatamansi* revealed the identification of 57 compounds based on their retention time and mass spectral profiles comparison with NIST.14.L (2020) spectral library (Figure 1). The identified compounds accounted for approximately 91.84% of the total chromatographic peak area, based on a similarity index ≥80%. The identified constituents comprised several chemical classes, including monoterpene hydrocarbons (4.74%), oxygenated monoterpenes (26.33%), sesquiterpene hydrocarbons (8.27%), oxygenated sesquiterpenes (40.34%), and other compounds (12.16%).

The chromatographic profiles was predominantly characterized by terpeniod constituents, particularly oxygenated sesquiterpeniods. Among the identified compounds, epicurzerenone (18.67%) was the most predominant compound, followed by eucalyptol (10.76%), levulinic acid (6.12%), khusimone (5.64%), and camphor (5.24%). Other relatively abundant compounds were germacron (3.97%), β-elemene (2.64%), isoborneol (2.60%), diepicedrene-1-oxide (2.39%), curzerene (2.22%), curcumenol (2.16%), aristolochene (2.08%), and α-terpinyl propionate (1.93%).

The monoterpene fraction comprised both monoterpene hydrocarbons and oxygenated monoterpenes. The monoterpene hydrocarbons included α-pinene (0.62%), camphene (1.51%), α-sabinene (0.12%), β-pinene (1.74%), β-myrcene (0.30%), α-phellandrene (0.07%), and δ-limonene (0.38%), collectively accounting for 4.74% of the total chromatographic peak area. The oxygenated monoterpene fraction comprised eucalyptol (10.76%), linalool (0.58%), camphor (5.24%), isoborneol (2.60%), endo-borneol (1.64%), terpinen-4-ol (0.76%), α-terpinyl propionate (1.93%), cis-carveol (0.15%), carveol (0.29%), and isobornyl acetate (2.38%), contributing 26.33%. Thus, the total monoterpene fraction accounted for 31.07% of the chromatographic peak area.

The sesquiterpene fraction was comparatively diverse and comprised sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The sesquiterpene hydrocarbons included σ-elemene (0.18%), β-elemene (2.64%), β-cis-caryophyllene (0.55%), (+)-sativene (0.11%), (E)-β-farnesene (0.27%), germacrene D (0.67%), γ-selinene (0.51%), isoledene (0.11%), α-guaiene (0.01%), γ-elemene (0.62%), (E)-β-elemene (0.11%), aristolochene (2.08%), and β-selinene (0.41%), collectively contributing 8.27%. The oxygenated sesquiterpenes included curzerene (2.22%), elemol (0.11%), caryophyllene oxide (0.63%), globulol (0.53%), β-eudesmol (0.39%), epicurzerenone (18.67%), isospathulenol (0.79%), shyobunol (0.35%), τ-cadinol (0.14%), neointermedeol (1.41%), spatulenol (0.19%), germacron (3.97%), epiglobulol (0.21%), curcumenol (2.16%), diepicedrene-1-oxide (2.39%), linderane (0.24%), khusimone (5.64%), and hydroxyvalerenic acid (0.15%), collectively accounting for 40.19%. Therefore, the total sesquiterpene fraction contributed 48.46% of the chromatographic peak area. Among the non-terpenoid constituents, levulinic acid showed a relatively high peak area of 6.12%, while 2-heptanol (0.40%), 2-nonanone (0.62%), 2-nonanol (0.84%), 2-undecanone (0.35%), 1-heptatriacotanol (0.24%), butyl 9,12-octadecadienoate (1.20%), Z-3-hexadecen-7-yne (2.27%), and δ-nonyl-δ-valerolactone (0.27%) were detected at comparatively lower abundances. Collectively, these non-terpenoid constituents accounted for 12.31% of the total chromatographic peak area. The predominance of sesquiterpenoid constituents in the present sample is consistent with the general chemical characteristics reported for *Valeriana jatamansi* (Cornara et al., 2020; Raina and Negi 2015).

Table 1. Distribution of major chemical classes in the GC–MS profile of *Valeriana jatamansi*.

S. No.	Peak No.	R. Time (min)	Compound	Area %	Molecular formula
1	3	1.588	Levulinic acid	6.12	$C_5H_8O_3$
2	7	5.803	2-Heptanol	0.40	$C_7H_{16}O$
3	10	6.564	α-Pinene	0.62	$C_{10}H_{16}$
4	11	7.018	Camphene	1.51	$C_{10}H_{16}$
5	12	7.692	α-Sabinene	0.12	$C_{10}H_{16}$
6	13	7.823	β-Pinene	1.74	$C_{10}H_{16}$
7	14	8.207	β-Myrcene	0.30	$C_{10}H_{16}$
8	15	8.660	α-Phellandrene	0.07	$C_{10}H_{16}$
9	19	9.355	δ-Limonene	0.38	$C_{10}H_{16}$
10	20	9.569	Eucalyptol (1,8-cineole)	10.76	$C_{10}H_{18}O$
11	27	11.241	2-Nonanone	0.62	$C_9H_{18}O$
12	28	11.564	Linalool	0.58	$C_{10}H_{18}O$
13	29	11.669	2-Nonanol	0.84	$C_9H_{20}O$
14	35	12.933	Camphor	5.24	$C_{10}H_{16}O$
15	37	13.426	Isoborneol	2.60	$C_{10}H_{18}O$
16	39	13.679	endo-Borneol	1.64	$C_{10}H_{18}O$
17	40	13.898	Terpinen-4-ol	0.76	$C_{10}H_{18}O$
18	43	14.363	α-Terpinyl propionate	1.93	$C_{13}H_{22}O_2$
19	49	15.028	cis-Carveol	0.15	$C_{10}H_{16}O$
20	51	15.407	Carveol	0.29	$C_{10}H_{16}O$
21	61	16.897	Isobornyl acetate	2.38	$C_{12}H_{20}O_2$
22	62	17.054	2-Undecanone	0.35	$C_{11}H_{22}O$
23	65	18.179	σ-Elemene	0.18	$C_{15}H_{24}$
24	72	19.659	β-Elemene	2.64	$C_{15}H_{24}$
25	75	20.389	β-cis-Caryophyllene	0.55	$C_{15}H_{24}$
26	79	21.130	(+)-Sativene	0.11	$C_{15}H_{24}$
27	80	21.265	(E)-β-Farnesene	0.27	$C_{15}H_{24}$
28	85	22.020	Germacrene D	0.67	$C_{15}H_{24}$
29	86	22.248	γ-Selinene	0.51	$C_{15}H_{24}$
30	87	22.473	Curzerene	2.22	$C_{15}H_{20}$
31	93	23.189	Isoledene	0.11	$C_{15}H_{24}$
32	97	23.783	α-Guaiene	0.01	$C_{15}H_{24}$
33	101	24.291	Elemol	0.11	$C_{15}H_{26}O$
34	102	24.606	γ-Elemene	0.62	$C_{15}H_{24}$
35	103	24.793	(E)-β-Elemene	0.11	$C_{15}H_{24}$
36	106	25.541	Caryophyllene oxide	0.63	$C_{15}H_{24}O$
37	107	25.908	Globulol	0.53	$C_{15}H_{26}O$
38	108	26.205	β-Eudesmol	0.39	$C_{15}H_{26}O$
39	109	26.954	Epicurzerenone	18.67	$C_{15}H_{18}O_2$
40	113	27.373	Aristolochene	2.08	$C_{15}H_{24}$
41	115	27.556	Isospathulenol	0.79	$C_{15}H_{24}O$
42	116	27.670	Shyobunol	0.35	$C_{15}H_{26}O$
43	118	27.879	τ-Cadinol	0.14	$C_{15}H_{26}O$
44	122	28.202	β-Selinene	0.41	$C_{15}H_{26}O$
45	123	28.313	Neointermedeol	1.41	$C_{15}H_{26}O$
46	126	28.556	Spatulenol	0.19	$C_{15}H_{24}O$
47	128	28.765	1-Heptatriacotanol	0.24	$C_{37}H_{76}O$
48	130	29.190	Germacron (Germacrone)	3.97	$C_{15}H_{20}O$
49	133	29.566	Epiglobulol	0.21	$C_{15}H_{26}O$
50	134	29.798	Curcumenol	2.16	$C_{15}H_{24}O$
51	136	30.132	Butyl 9,12-octadecadienoate	1.20	$C_{22}H_{40}O_2$
52	137	30.244	Diepicedrene-1-oxide	2.39	$C_{15}H_{24}O$
53	141	30.590	Linderane	0.24	$C_{15}H_{16}O_4$

54	142	30.803	Z-3-Hexadecen-7-yne	2.27	$C_{16}H_{28}$
55	143	31.075	Khusimone	5.64	$C_{14}H_{20}O$
56	153	31.918	Hydroxyvalerenic acid	0.15	$C_{15}H_{22}O_3$
57	204	53.413	δ-Nonyl-δ-valerolactone	0.27	$C_{14}H_{26}O_2$

Notably, hydroxyvalerenic acid was detected in the GC–MS profile at peak no. 153, with a retention time of approximately 31.5–31.9 min and a relative abundance of ~0.16%. The detection of this compound is particularly noteworthy as hydroxyvalerenic acid is recognized as a characteristic quality marker of *Valeriana* rhizome preparations, supporting the chemical characterization of *Valeriana jatamansi*.

3.3 Antioxidant activity of *Valeriana jatamansi*

The antioxidant potential of the *Valeriana jatamansi* sample was evaluated using DPPH radical-scavenging and ferric reducing antioxidant power (FRAP) assays. Both assays demonstrated a concentration-dependent increase in antioxidant activity (Figure 2 A, B: Table 2). The DPPH radical-scavenging activity of the sample increased in a concentration-dependent manner, from 33.34 ± 1.61% at 50 µg/mL to 52.41 ± 0.79% at 200 µg/mL. In comparison, ascorbic acid exhibited higher radical-scavenging activity, increasing from 53.00 ± 1.00% to 75.00 ± 1.00% across the same concentration range. One-way ANOVA revealed a highly significant effect of treatment group on inhibition percentage ($p < 0.001$). Tukey`s HSD test further showed that all the treatments groups differed significantly from one another ($p < 0.05$), except for the sample at 200 µg/mL and ascorbic acid at 50 µg/mL, which shared the letter "d" and therefore were not significantly different. Regression analysis demostrated a strong positive relationship between concentration and inhibition for both the sample ($R^2 = 0.961$) and ascorbic acid ($R^2 = 0.974$). The regression-derived IC_{50} for the sample was estimated at 171.44 µg/mL, whereas that of ascorbic acid was estimated at 23.33 µg/mL. In the FRAP assay, the reducing capacity of the sample increased with concentration, from 251.51 ± 4.43 Fe^{2+} equivalents at 50 µg/mL to 399.75 ± 42.00 Fe^{2+} equivalents at 200 µg/mL, whereas ascorbic acid showed higher reducing capacity, increasing from 371.41 ± 10.68 to 600.33 ± 11.34 Fe^{2+} equivalents over the same concentration range. One-way ANOVA revealed significant differences among the treatment–concentration groups ($p< 0.001$). Tukey's HSD test showed that ascorbic acid at 150 and 200 µg/mL had the highest comparable FRAP values, while the sample at 50 and 100 µg/mL exhibited the lowest activity. Overall, the results from both assays demonstrate that *Valeriana jatamansi* possesses appreciable antioxidant activity, which increased with concentration, although its activity remained lower than that of the ascorbic acid reference standard.

Table 2.DPPH radical-scavenging activity and ferric reducing antioxidant power (FRAP) of the *Valeriana jatamansi* extract and ascorbic acid at different concentrations.

Treatment	Concentration (µg/mL)	DPPH inhibition (%)	FRAP (Fe^{2+} equivalents)
Extract	50	33.34 ± 1.61^{g}	251.51 ± 4.43^{e}
	100	39.64 ± 0.72^{f}	254.26 ± 27.10^{e}
	150	49.64 ± 0.57^{e}	323.86 ± 19.69^{d}
	200	52.41 ± 0.86^{de}	399.75 ± 42.00^{bc}
Ascorbic acid	50	53.00 ± 1.00^{d}	371.41 ± 10.68^{cd}
	100	62.00 ± 1.00^{c}	450.92 ± 19.81^{b}
	150	71.00 ± 1.00^{b}	572.88 ± 11.88^{a}
	200	75.00 ± 1.00^{a}	600.33 ± 11.34^{a}

3.4.1 Disc Diffusion Assay

The antibacterial activity of the *Valeriana jatamansi* rhizome extract increased in a concentration-dependent manner against all four bacterial strains tested. Against *E. coli*, the zone of inhibition (ZOI) increased from 7.17 ± 0.15 mm at 50 µg/mL to 13.47 ± 0.25 mm at 200 µg/mL. Similarly, *S. aureus* exhibited an increase in ZOI from 8.07 ± 0.15 mm to 15.07 ± 0.25 mm, while *P. aeruginosa* showed an increase from 6.50 ± 0.20 mm to 11.67 ± 0.25 mm across the same concentration range. *B. subtilis* demonstrated the greatest susceptibility, with the ZOI increasing from 8.50 ± 0.20 mm at 0 µg/mL to 15.80 ± 0.20 mm at 200 µg/mL. Overall, *B. subtilis* and *S. aureus* were more susceptible to the extract, whereas *P. aeruginosa* exhibited comparatively lower susceptibility (Figure 3A, Table 3). Two-way ANOVA revealed significant effects of bacterial strain and extract concentration on the ZOI (both $p < 0.001$), along with a significant strain × concentration interaction ($p < 0.001$), indicating that the antibacterial response to increasing extract concentrations varied among the bacterial strains. Tukey's HSD post hoc test further identified significant differences among strain–concentration combinations ($p < 0.05$). The maximum ZOI was recorded against *B. subtilis* at 200 µg/mL (15.80 ± 0.20 mm), whereas the minimum was observed against *P. aeruginosa* at 50 µg/mL (6.50 ± 0.20 mm).

3.4.2 Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC)

The MIC and MBC assays further confirmed the antibacterial potential of the extract, revealing differential susceptibility among the tested bacterial strains. *Staphylococcus aureus* and *Bacillus subtilis* exhibited the highest susceptibility, with MIC and MBC values of 250 and 500 µg/mL, respectively. In comparison, *Escherichia coli* and *Pseudomonas aeruginosa* showed comparatively lower susceptibility, with MIC and MBC values of 500 and 1000 µg/mL, respectively. Overall, the findings suggest greater antibacterial efficacy of the extract against Gram-positive bacteria than against Gram-negative bacteria. **(Fig 3B, Table 3).**

Table 3: Antimicrobial activity of *Valeriana jatamansi* extract against selected bacterial strains with MIC and MBC values.

Zone of Inhibition (mm)						
Bacterial strain	**50 µg/mL (mm)**	**100 µg/mL (mm)**	**150 µg/mL (mm)**	**200 µg/mL (mm)**	**MIC (µg/mL)**	**MBC (µg/mL)**
E. coli	7.17 ±0.15	9.10 ± 0.10	11.30 ±0.20	13.47 ± 0.25	500	1000
S. aureus	8.07 ± 0.15	10.20 ± 0.20	12.60 ± 0.20	15.07 ± 0.25	250	500
P.aeruginosa	6.50 ± 0.20	8.00 ± 0.20	9.80 ± 0.20	11.67 ± 0.25	500	1000
B. subtilis	8.50 ± 0.20	10.80 ± 0.20	13.20 ± 0.20	15.80 ± 0.20	250	500

4 DISCUSSION

Preliminary phytochemical screening revealed alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides and tannins, while phenolics and proteins were not detected. The occurrence of terpenoids and glycosides agrees with the known phytochemical characteristics of *Valeriana jatamansi*, which is recognized for its iridoid and sesquiterpenoid constituents (Tang et al., 2002). GC–MS analysis identified 57 compounds, representing 91.84% of the total chromatographic area. The profile was predominantly terpenoid, with oxygenated sesquiterpenes (40.34%) being the major chemical class, followed by oxygenated monoterpenes (26.33%) and sesquiterpene hydrocarbons (8.27%). Epicurzerenone (18.67%) was the major constituent, followed by eucalyptol (10.76%), levulinic acid (6.12%), khusimone (5.64%) and camphor (5.24%). The predominance of sesquiterpenoids agrees with previous reports on *Valeriana jatamansi*, although the major constituents differed considerably. Verma et al., 2011) reported patchouli alcohol as the dominant constituent in several Uttarakhand populations, whereas Raina and Negi (2015) observed oxygenated sesquiterpenes as the major fraction. These differences indicate considerable chemical and chemotypic variation within *Valeriana jatamansi*, potentially associated with geographical origin, altitude, environmental conditions and genetic variability. The high proportion of epicurzerenone and other oxygenated sesquiterpenes in the *Valeriana jatamansi* extract suggests that the extract may possess a distinct chemical profile compared with populations reported from other Himalayan regions. The *Valeriana jatamansi* extract demonstrated concentration-dependent antioxidant activity in both DPPH and FRAP assays. The progressive increase in DPPH radical-scavenging activity and FRAP reducing capacity with increasing concentration suggests the presence of redox-active phytoconstituents capable of donating electrons or hydrogen atoms (Nisa et al., 2026). Although the activity was slightly lower than that of ascorbic acid, the results indicate appreciable antioxidant potential. Similar antioxidant activity has previously been reported for *Valeriana jatamansi* and has been associated with its phenolic, flavonoid, and other bioactive constituents (Jugran et al., 2016). The present results also showed a comparatively substantial monoterpene contribution (31.07%), largely attributable to eucalyptol, camphor, isoborneol, endo-borneol and isobornyl acetate. Raina and Negi (2015) likewise reported a profile dominated by sesquiterpenoids and identified only relatively minor amounts of oxygenated monoterpenes in their germplasm. Such differences may arise from genetic variability, geographical origin, altitude, phenological stage, environmental conditions, plant age and differences in extraction and analytical procedures. Indeed, (Jugran et al., 2016) demonstrated that essential-oil composition in *Valeriana jatamansi* varies with both phenological stage and altitude, further supporting the environmental and developmental influence on metabolite accumulation. The MIC of the extract against *Escherichia coli* and *Pseudomonas aeruginosa* was 500 µg/mL, whereas *Staphylococcus aureus* and *Bacillus subtilis* exhibited comparatively greater sensitivity, with MIC values of 250 µg/mL. The corresponding MBC values were 1000 µg/mL for *E. coli* and *P. aeruginosa* and 500µg/mL for *S. aureus* and *B. subtilis*. These findings indicate that the methanolic extractof *Valeriana jatamansi* exhibited greater antibacterial activity against *S. aureus* and *B. subtilis* than against *E. coli* and *P. aeruginosa*. The observed differences in susceptibility may be associated with variations in bacterial cell-envelope architecture. Gram-positive bacteria possess a thick peptidoglycan layer containing teichoic acids, whereas Gram-negative bacteria have a thinner peptidoglycan layer surrounded by an outer membrane containing lipopolysaccharides. Such structural differences can influence the interaction, diffusion, and cellular uptake of bioactive constituents present in the extract.

CONCLUSION:

The present study provides an integrated phytochemical and biological assessment of *Valeriana jatamansi* rhizomes collected from Tulail, Gurez, highlighting their chemical complexity and in vitro bioactivity. Preliminary phytochemical screening confirmed the presence of alkaloids, terpenoids, tannins, and saponins, while GC–MS analysis identified 57 volatile constituents accounting for approximately 91.84% of the total chromatographic peak area (similarity index ≥80%). The chemical profile was predominantly characterized by terpenoid constituents, particularly oxygenated sesquiterpenoids, underscoring the importance of these metabolites in defining the phytochemical composition of the extract. Furthermore, the observed antioxidant and antibacterial activities indicate the potential biological relevance of the extract, although the contributions of individual constituents remain to be established. Collectively, these findings establish a baseline for the chemical characterization and biological evaluation of *Valeriana jatamansi* from the northwestern Himalayan region. Further investigations integrating bioassay-guided fractionation, compound-level characterization, and mechanistic validation are warranted to identify the metabolites responsible for the observed activities and assess their potential for future pharmaceutical or other bioactive-product applications.

REFERENCES

1. Arfa, J., Sofi, T. A., Haq, E., Peerzada, I. A., Anjum, S., Banoo, S., & Dar, M. U. D. (2026). Therapeutic potential of Dipsacus inermis-derived biogenic zinc oxide nanoparticles: Antibacterial activity and cancer cell cytotoxicity. Applied Biological Research, 28(2), 147–159.
2. Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science, 2(4), 25–32.
3. Bhatt, I. D., Dauthal, P., Rawat, S., Gaira, K. S., Jugran, A., Rawal, R. S., & Dhar, U. (2012). Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of Valeriana jatamansi Jones. Scientia Horticulturae, 136, 61–68.
4. Chakraborty, S., Mukherjee, D., & Baskey, S. (2015). Selection of lines of Valeriana jatamansi Jones, a high-value medicinal plant in North Eastern Himalayan region. Indian Journal of Genetics and Plant Breeding, 75, 404–407.
5. Cornara, L., Ambu, G., Trombetta, D., Denaro, M., Alloisio, S., Frigerio, J., Labra, M., Ghimire, G., Valussi, M., & Smeriglio, A. (2020). Comparative and functional screening of three species traditionally used as antidepressants: Valeriana officinalis L., Valeriana jatamansi Jones ex Roxb. and Nardostachys jatamansi (D. Don) DC. Plants, 9(8), 994.
6. Daphedar, A. B., Majani, S. S., Kaddipudi, P. J., Hujaratti, R. B., Kakkalmeli, S. B., Shati, A. A., & Kollur, S. P. (2024). Evaluation of antioxidant and antibacterial activities of silver nanoparticles derived from Limonia acidissima L. fruit extract. Current Research in Green and Sustainable Chemistry, 8, 100399.
7. De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. American Journal of Essential Oils and Natural Products, 5(2), 29–32.
8. Dinda, B., Chowdhury, D. R., & Mohanta, B. C. (2009). Naturally occurring iridoids, secoiridoids and their bioactivity: An updated review, part 3. Chemical and Pharmaceutical Bulletin, 57, 765–796.
9. Fernandes, R. D. P. P., Trindade, M. A., Tonin, F. G., Lima, C. G. D., Pugine, S. M. P., Munekata, P. E. S., & De Melo, M. P. (2016). Evaluation of antioxidant capacity of 13 plant extracts by three different methods: Cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. Journal of Food Science and Technology, 53(1), 451–460.
10. Jain, S., & Mehata, M. S. (2017). Medicinal plant leaf extract and pure flavonoid mediated green synthesis of silver nanoparticles and their enhanced antibacterial property. Scientific Reports, 7, 15867.
11. Jugran, A. K., Bahukhandi, A., Dhyani, P., Bhatt, I. D., Rawal, R. S., & Nandi, S. K. (2016). Impact of altitudes and habitats on valerenic acid, total phenolics, flavonoids, tannins, and antioxidant activity of Valeriana jatamansi. Applied Biochemistry and Biotechnology, 179(6), 911–926.
12. Jugran, A. K., Rawat, S., Bhatt, I. D., & Rawal, R. S. (2019). Valeriana jatamansi: An herbaceous plant with multiple medicinal uses. Phytotherapy Research, 33(3), 482–503.
13. Lalawmpuii, P. C., Peerzada, I. A., Sofi, P. A., Masoodi, T. H., Mushtaq, T., Atta, U., Mushtaq, M., Arfa, J., Mohammad, I., & Shabir, S. (2026). Phytochemical diversity of Digitalis purpurea L.: Correlating chemical profiles with environmental conditions. Genetics and Molecular Research, 25(19s).
14. Lin, S., Fu, P., Chen, T., Ye, J., Yang, X. W., & Zhang, W. D. (2017). Three minor valepotriate isomers from Valeriana jatamansi and their cytotoxicity. Journal of Asian Natural Products Research, 19, 15–21.
15. Liu, Y., Wu, P., Hu, Q., Pei, Y., Qi, F., Zhang, Z., & Fei, D. (2017). Cytotoxic and antibacterial activities of iridoids and sesquiterpenoids from Valeriana jatamansi. Fitoterapia, 123, 73–78.
16. Madhu, M., Sailaja, V., Satyadev, T. N. V. S. S., & Satyanarayana, M. V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry, 5(2), 25–29.
17. Maurya, A. K., Kumar, A., & Agnihotri, V. K. (2022). New iridoids from the roots of Valeriana jatamansi Jones. Natural Product Research, 36(13), 3360–3367.

18. Mishra, R. R. (2026). Phytochemical analysis and comparison of some selected plants. Vegetos, 39(1), 125–136.
19. Nisa, Mir Muskan Un, Ishtiyak Ahmad Peerzada, Rashid Mir, Talib Bashir Bhat, Naseh A. Algehainy, Mohammed M. Jalal, Malik A. Altayar, Faris J. Tayeb, Ruqaiah I. Bedaiwi, and Ibrahim Altedlawi Albalawi (2026). "Eco-Metabolomic Assessment of Aconitum heterophyllum in the Kashmir Himalaya; Integrating Morphological, Edaphic, Phytochemical, Antioxidant and Climatic Factors." Phyton-International Journal of Experimental Botany. 95(9), 16.
20. Peerzada, I. A., Islam, M. A., Chamberlain, J., Dhyani, S., Reddy, M., & Saha, S. (2022). Potential of NTFP-based bioeconomy in livelihood security and income inequality mitigation in Kashmir Himalayas. Sustainability, 14, 2281.
21. Peerzada, I. A., Sofi, P. A., Shabir, S., Mohammad, I., Gupta, L. M., Malik, A. R., & Rafeeq, J. (2026). ITS and rbcL DNA barcoding for molecular authentication of Thymus linearis (Lamiaceae) from the Kashmir Himalaya: A first report. Genetics and Molecular Research.25 (19s)
22 Quan, L. Q., Hegazy, A. M., Zhang, Z. J., Zhao, X. D., Li, H. M., & Li, R. T. (2020). Iridoids and bis-iridoids from Valeriana jatamansi and their cytotoxicity against human glioma stem cells. Phytochemistry, 175, 112372.
23. Quan, L., Su, L., Qi, S., Xue, Y., Yang, T., Liu, D., Zhao, X., Li, R., & Li, H. (2019). Bioactive 3,8-epoxy iridoids from Valeriana jatamansi. Chemistry & Biodiversity, 16(5), e1800474.
24. Raina, A. P., & Negi, K. S. (2015). Essential oil composition of Valeriana jatamansi Jones from Himalayan regions of India. Indian Journal of Pharmaceutical Sciences, 77(2), 218–222.
25. Rather, A. M., Nawchoo, I. A., Ganie, A. H., Singh, H., Dutt, B., & Wani, A. A. (2012). Bioactive compounds and medicinal properties of Valeriana jatamansi Jones: A review. Life Science Journal, 9, 847–850.
26. Shukla, V., Singh, P., Konwar, R., Singh, B., & Kumar, B. (2021). Phytochemical analysis of high-value medicinal plant Valeriana jatamansi using LC-MS and its in-vitro anti-proliferative screening. Phytomedicine Plus, 1(2), 100025.
27. Singh, N., Gupta, A. P., Singh, B., & Kaul, V. K. (2006). Quantification of valerenic acid in Valeriana jatamansi and Valeriana officinalis by HPTLC. Chromatographia, 63, 209–213.
28. Singh, V., & Kumar, R. (2017). Study of phytochemical analysis and antioxidant activity of Allium sativum of Bundelkhand region. International Journal of Life-Sciences Scientific Research, 3(6), 1451–1458.
29. Suraya, S., Sofi, P. A., Peerzada, I. A., Malik, A. R., Nazir, A. P., Arfa, J., Tahir, M., Mohammad, I., & Sabira, N. (2026). Influence of season, harvest time and plant part on the yield and chemical composition of rosemary (Salvia rosmarinus Spenn.) essential oil. Plant Science Today. 13(3). 01–07.
30. Tang, Y., Liu, Y., & Dai, H.-F. (2002). Iridoids from the rhizomes and roots of Valeriana jatamansi. Journal of Natural Products, 65, 1949–1952.
31. Thakur, B. K., Mahajan, M., & Pal, P. K. (2022). Chemical diversity of essential oil of Valeriana jatamansi from different altitudes of Himalaya and distillation methods. Molecules, 27(8), 2387.
32. Tian, S., Wang, Z., Wu, Z., Wei, Y., Yang, B., & Lou, S. (2018). Valtrate from Valeriana jatamansi Jones induces apoptosis and inhibits migration of human breast cancer cells in vitro. Natural Product Research, 34(18), 2660–2663.