Research Article

Advances in Applied Biological Research
Year 2026, Volume-3, Issue-2 (July - December)

Online ISSN:3048-9261

www.acspublisher.com

Morphological and Chemical Characterization of *Aconitum heterophyllum*, a Critically Endangered Himalayan Medicinal Plant

Mir Muskan Un Nisa[1], Ishtiyak Ahmad Peerzada[1], Sajad Ahmad Gangoo[2], Parvez Ahmad Sofi[1], Talib Bashir Bhat [3] and Sourav Singh[4]

[1]Division of Forest Products and Utilization, Faculty of Forestry, Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama Ganderbal J&K, Srinagar 191201, India
[2]Dean students Welfare Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir
[3]Division of Natural Resource Management, Faculty of Forestry, Sher-E-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama Ganderbal J&K, Srinagar 191201, India
[4]Division of Silviculture and Agroforestry, Sher-E-Kashmir University of Agricultural Sciences and Technology of Jammu 180009, India

ARTICLE INFO

Keywords: *Aconitum heterophyllum*; pharmacognosy; phytochemical screening; GC–MS; alkaloids; fatty acids.

DOI: 10.48165/aabr.2026.3.2.01

ABSTRACT

Aconitum heterophyllum Wall. ex Royle is a critically endangered Himalayan medicinal herb extensively used in traditional medicine for its analgesic, antipyretic, and anti-inflammatory properties. The present study evaluated the species through morphological characterization, qualitative phytochemical screening, and GC–MS profiling of methanolic root extracts. Morphological observations confirmed diagnostic features such as paired tuberous roots, palmately lobed leaves, and racemose inflorescences, supporting species authentication. Preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, lipids/fatty acids, and phenolic compounds, whereas flavonoids and saponins were absent. GC–MS analysis identified ten major compounds, predominantly α-linolenic acid, palmitic acid, fatty acid esters, carbohydrate derivatives, and nitrogen-containing metabolites. The findings indicate a chemically diverse metabolite profile with potential antioxidant and anti-inflammatory significance. However, the study also recognizes the limitations of GC–MS in detecting thermolabile diterpenoid alkaloids characteristic of *Aconitum* species. Overall, the study establishes baseline pharmacognostic and chemical markers useful for authentication, quality evaluation, conservation planning, and future LC–MS/MS- based metabolomic investigations of this threatened Himalayan medicinal plant.

Introduction

Medicinal plants continue to play a pivotal role in global healthcare systems, sustainable development, and biodiversity conservation due to their therapeutic, ecological, and economic value. For centuries, plant- derived remedies have served as primary healthcare resources, particularly in developing regions, where nearly 80% of the population relies on traditional herbal medicines for the treatment of common ailments

(Sen and Samanta, 2014). The pharmacological efficacy of these plants is largely attributed to their diverse repertoire of primary and secondary metabolites, including alkaloids, phenolics, terpenoids, fatty acids, and glycosides, many of which form the basis of modern pharmaceuticals and are often associated with fewer adverse effects than synthetic

*Corresponding author. Mir Muskan Un Nisa
E-mail address: mirmuskan@skuastkashmir.ac.in

drugs (Ahad et al., 2021). Beyond healthcare, medicinal plants contribute significantly to rural livelihoods and economic sustainability through cultivation and trade, while their conservation supports ecosystem stability and genetic diversity (Mofokeng et al., 2022). However, escalating commercial demand, habitat degradation, and unsustainable harvesting practices threaten numerous medicinal taxa worldwide, necessitating systematic documentation, quality control, and sustainable management supported by scientific and policy interventions (Chen et al., 2016 and Shukla, 2023) Among high-value Himalayan medicinal herbs, *Aconitum heterophyllum* Wall. ex Royle (Ranunculaceae), commonly known as Ativisha, occupies a prominent position in traditional systems of medicine due to its analgesic, antipyretic, anti-inflammatory, and antidiarrheal properties. The species is a perennial alpine geophyte native to the northwestern Himalaya, typically occurring at elevations of 2,400–3,600 m. Its erect habit, palmately lobed leaves, and paired tuberous roots represent morphological adaptations that enable survival under extreme alpine conditions. Despite its medicinal significance, the species faces severe conservation challenges. Its restricted endemic distribution, narrow ecological amplitude, and intensive harvesting of tuberous roots for pharmaceutical use have led to marked population declines and fragmentation. Consequently, *A. heterophyllum* is regarded as a conservation-priority and critically endangered medicinal plant in the Indian Himalaya (Ali et al., 2021; Ganie et al., 2019; Jeelani et al., 2015). Recent ecological and climate-based modeling studies predict further contractions in climatically suitable habitats, with potential reductions of 30–60% by the mid to late twenty-first century, driven by shifts in temperature, precipitation patterns, and elevational constraints (Tomar et al., 2025; Dad and Rashid, 2022; Chauhan et al., 2025). Field-based assessments corroborate these projections, documenting small, fragmented populations subjected to cumulative pressures from overharvesting, grazing, and habitat disturbance. These threats underscore the urgent need for integrated conservation strategies supported by robust pharmacognostic authentication, chemical characterization, and sustainable utilization practices. From a pharmacognostic perspective, accurate botanical identification and comprehensive phytochemical profiling are critical for ensuring the quality, safety, and efficacy of herbal drugs derived from *A. heterophyllum*. Species of the genus Aconitum are particularly known for their rich alkaloid content, including diterpenoid alkaloids that contribute significantly to their therapeutic properties but may also confer toxicity if improperly processed (Shen et al., 2025). Consequently, morphological standardization combined with chemical profiling serves not only as a tool for authentication but also as a foundation for understanding bioactivity and developing standardized formulations.

Although *Aconitum heterophyllum* Wall. ex Royle is recognized as a highly valuable and critically endangered Himalayan medicinal herb, previous investigations have largely focused on isolated phytochemical constituents, pharmacological properties, or targeted alkaloid characterization using advanced chromatographic techniques. Comprehensive studies integrating pharmacognostic authentication, quantitative morphological evaluation, preliminary phytochemical screening, and GC–MS- based metabolite profiling of Himalayan populations remain extremely limited. In particular, information regarding the volatile and semi-volatile phytochemical composition of *A. heterophyllum* root tubers from the Kashmir Himalaya is still poorly documented despite increasing concern over species adulteration, habitat degradation, and declining natural populations. Furthermore, most earlier analytical studies have primarily emphasized diterpenoid alkaloids using HPLC, UHPLC, and LC–MS/MS approaches, whereas comparatively little attention has been given to broad-spectrum metabolite profiling capable of identifying diverse bioactive compounds associated with antioxidant, anti-inflammatory, and therapeutic activities. The absence of integrated pharmacognostic and chemical characterization limits reliable species authentication, quality standardization, and conservation-oriented utilization of this medicinally important species. Therefore, the present study represents one of the few integrated investigations combining detailed morphological characterization, quantitative trait analysis, preliminary phytochemical screening, and GC–MS-based metabolite profiling of *Aconitum heterophyllum* roots collected from the Kashmir Himalaya. The study was undertaken to: (i) investigate the morphological and pharmacognostic characteristics of *A. heterophyllum* for accurate species authentication and characterization; and (ii) evaluate the phytochemical composition of the methanolic root extract through preliminary phytochemical screening and GC–MS analysis to identify bioactive metabolites of medicinal importance. The findings provide baseline pharmacognostic and phytochemical data that may contribute to quality evaluation, conservation planning, sustainable utilization, and future bioactivity-guided and metabolomic research on this critically endangered Himalayan medicinal plant.

Materials and Methods

Study Sites

The study was conducted in the Langate Forest Division (LFD) of the Kashmir Valley ,located at 34°15′22″ N latitude and 74°07′52″ E longitude, with an average elevation of 3,291 m a.s.l. The division covers an area of 36,061 ha and lies on the north-eastern slopes of the Kazinag and Shamsabari ranges. The terrain is characterized by predominantly east-facing slopes, with drainage largely flowing eastward. The climate is marked by mild summers and cold winters, with snowfall occurring mainly from December to February. Vegetation in the area comprises broadleaved species such as *Acer caesium*, *Prunus cornuta*, and *Robinia pseudoacacia*, along with conifers including *Abies pindrow*, *Picea smithiana*, *Cedrus deodara*, and *Pinus wallichiana*. The shrub layer is dominated by *Rosa webbiana*, *Berberis lyceum*, and *Indigofera heterantha* (Nisa et al., 2025).

Sample collection of plant specimens

Field surveys were carried out in the Langate Forest Division during July–August 2025 to collect plant specimens of *Aconitum heterophyllum* Wall. ex Royle from the Haril forest range. Morphological characteristics were recorded for 20 randomly selected plants following standard procedures (Jeelani et al., 2015). Herbarium specimens were prepared using established methods (Jain and Rao, 1977) and identified at the Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir. The voucher specimen was deposited under accession number 3742-KASH.

Morphological characterisation of plant specimens

Morphological data were collected from 20 randomly selected plants at each site following the method described by (Jain and Rao, 1977). The measured parameters included plant height (using a measuring scale), leaf length and width (with a ruler), and leaf area (using a leaf area meter and graph paper method). The length of the floral axis was measured with a ruler, whereas flower colour was determined using a colour chart and flower sex was identified using a pocket lens (Fig. 2).

Phytochemical screening

Dried root samples of *Aconitum heterophyllum* were shade dried, powdered, and sieved prior to extraction. Approximately 50 g of powdered material was successively extracted using petroleum ether, methanol, and distilled water in ascending order of polarity. Soxhlet extraction was carried out for 6–8 h at the respective boiling temperatures of each solvent using a solvent-to-sample ratio of 10:1 (v/w). The extracts were concentrated under reduced pressure using a rotary vacuum evaporator at 40°C and subsequently dried in a desiccator until constant weight was obtained. The percentage yield of the methanolic extract was calculated using the formula: Yield (%) = (Weight of dried extract / Weight of powdered sample) × 100 (Rasve et al., 2017). Preliminary phytochemical screening was conducted following standard qualitative procedures (Silva et al., 2017). The plant extracts were screened for alkaloids, flavonoids, carbohydrates, and saponins, using standard qualitative tests. Alkaloids were detected using Dragendorff's, Mayer's, and Wagner's tests. Flavonoids were identified using alkaline reagents and the lead acetate test. The presence of carbohydrates and saponins was confirmed using Molisch's, Fehling's, and foam tests (Table 1). GC–MS analysis of the methanolic root extract was performed using an Agilent 7890B gas chromatograph coupled with an Agilent 5977B mass selective detector. Separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The injection volume was 1 μL in splitless mode.The oven temperature program was set as follows: initial temperature 60°C (held for 2 min), increased to 280°C at a rate of 10°C min^{-1}, and maintained for 10 min. The injector temperature was maintained at 250°C. Mass spectra were recorded under electron ionization (EI) mode at 70 eV with a scan range of m/z 40–600.Compound identification was performed by comparing the obtained mass spectra with those available in the NIST 2020 Mass Spectral Library database. Only compounds showing similarity indices greater than 85% were considered tentatively identified. Procedural blanks and solvent controls were analyzed under identical conditions to minimize contamination and analytical artifacts (Uarrota et al., 2022).

Table 1. Preliminary qualitative phytochemical screening tests for detection of major metabolite classes in *Aconitum heterophyllum* root extract.

Phytochemical class	Test / Reagent used	Typical observation	Inference	References
Alkaloids	Dragendorff's test	Orange or reddish-brown precipitate	Alkaloids present	18
Alkaloids	Mayer's test	Cream or white precipitate	Alkaloids present	18
Alkaloids	Wagner's test	Reddish-brown precipitate	Alkaloids present	18
Flavonoids	Alkaline reagent test	Intense yellow color in NaOH, discharged on addition of acid	Flavonoids present	18; 19
Flavonoids	Lead acetate test	Yellow precipitate after addition of lead acetate	Flavonoids present	18;20
Carbohydrates	Molisch's test	Violet or purple ring at acid– aqueous interface	Carbohydrates present	18

Carbohydrates	Fehling's test	Brick-red cuprous oxide precipitate on heating	Reducing sugars present	18; 21
Saponins	Foam (froth) test	Stable persistent froth on shaking with water	Saponins present	18;19

Results and Discussion

The morphological characterization of *Aconitum heterophyllum* Wall. ex Royle confirmed several diagnostic features that are important for pharmacognostic authentication of the species (Figure 1; Table 2). The species was observed as a perennial geophytic herb with an erect growth habit and paired tuberous roots. Leaves were alternate and palmately lobed, with basal leaves larger than cauline leaves, while the inflorescence consisted of terminal racemes bearing zygomorphic bisexual flowers with a characteristic hood-shaped upper sepal. The flowers varied from pale yellowish-white to greenish or purplish in colour. Fruits were present as aggregates of follicles containing numerous seeds. These observations are consistent with earlier botanical descriptions of *A. heterophyllum* reported from the northwestern Himalaya (Ameera et al., 2016 and Mathew et al., 2021), thereby confirming the taxonomic identity and authenticity of the collected material.

Quantitative morphological evaluation of populations from Langate Forest Division (LFD) revealed substantial variation among different growth parameters (Table 3). The mean plant height recorded was 94.60 cm, with a comparatively high coefficient of variation (45.83%), indicating considerable phenotypic variability within the sampled population. Similarly, the number of leaves per plant showed moderate variation, with a mean value of 10.20 leaves plant^{-1} and a CV of 34.94%. Leaf dimensions also exhibited measurable variability, with mean leaf length and width recorded as 8.42 cm and 7.96 cm, respectively. The average leaf area was 78.02 cm^2, reflecting vigorous vegetative growth under favourable alpine environmental conditions of the study area. Reproductive traits demonstrated relatively lower variability compared with vegetative characters. The mean floral axis length was 20.96 cm, while the average number of flowers per plant was 11.00. Root-associated traits showed comparatively greater uniformity, particularly the number of root hairs plant^{-1}, which exhibited the lowest coefficient of variation (3.60%). Tuber length averaged 6.04 cm with moderate variability (CV = 12.64%). The observed development of tuberous roots likely represents an adaptive strategy for nutrient storage and survival under harsh alpine climatic conditions, where short growing seasons and low temperatures prevail. The comparatively high variability observed in plant height, leaf number, and leaf area may be attributed to microhabitat heterogeneity, variation in soil moisture, nutrient availability, altitude, and genetic differences among naturally occurring populations. Similar morphological plasticity has previously been reported in Himalayan populations of *A. heterophyllum* and other alpine medicinal herbs growing under diverse ecological conditions (13,22). Such variability may also reflect the influence of environmental stressors associated with alpine ecosystems, including temperature fluctuations and grazing pressure. Morphological authentication is particularly important in *Aconitum* species because adulteration and substitution of medicinal tubers are common in herbal trade due to high commercial demand and declining natural populations (Wani et al., 2021). Therefore, the combined qualitative and quantitative morphological characterization presented in the present study provides valuable baseline information for species identification, pharmacognostic standardization, conservation planning, and sustainable utilization of this critically endangered Himalayan medicinal herb.

Table 2: Morphological Characters of *Aconitum heterophyllum* Wall. ex Royle

Character	Description
Habit	Perennial, erect herb
Life form	Geophyte
Plant height	30–90 cm
Root system	Paired tuberous roots; light brownish-white
Stem	Erect, simple to sparsely branched
Leaf arrangement	Alternate
Leaves	Palmately lobed; basal leaves larger than cauline
Inflorescence	Terminal raceme
Flower symmetry	Zygomorphic
Flower sex	Bisexual
Flower colour	Pale yellowish-white to greenish or purple
Perianth	Sepals petaloid, 5
Upper sepal	Hood-shaped (helmet)
Petals	2, tubular, concealed
Stamens	Numerous
Gynoecium	Apocarpous
Fruit	Aggregate of follicles
Seeds	Numerous
Phenology	Flowering July–September
Altitudinal range	2,400–3,600 m

Table 3. Quantitative morphological characteristics of *Aconitum heterophyllum* in Langate Forest Division (LFD)

Trait	Mean	SE (Mean)	95% CI (LL–UL)	CV (%)
Plant height (cm)	94.60	19.39	40.77–148.43	45.83

Number of leaves plant^{-1}	10.20	1.59	5.78–14.62	34.94
Leaf length (cm)	8.42	0.76	6.32–10.53	20.13
Leaf width (cm)	7.96	0.36	6.95–8.97	10.19
Leaf area (cm^2)	78.02	7.21	58.01–98.03	20.65
Length of floral axis (cm)	20.96	2.50	11.40–25.20	26.70
Number of flowers plant^{-1}	11.00	0.54	10–13	10.90
Number of root hairs plant^{-1}	18.20	0.66	16–20	3.60
Tuber length (cm)	6.04	0.34	5.09–6.99	12.64

SE = Standard Error; CI = Confidence Interval; LL = Lower Limit; UL = Upper Limit; CV = Coefficient of Variation.

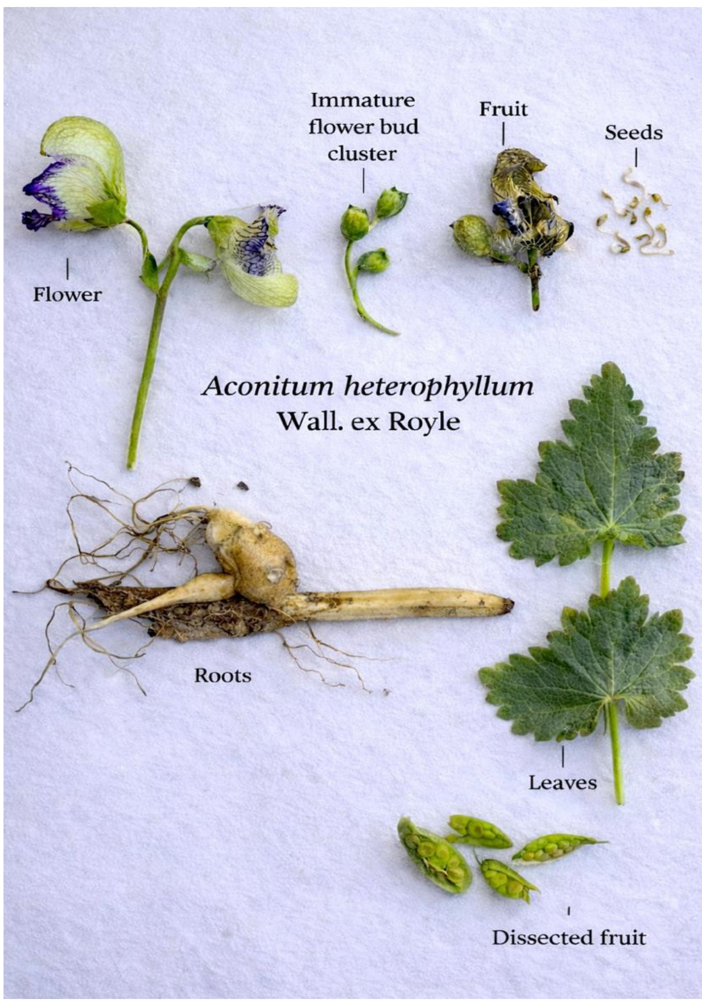


Fig 1. Morphological parts of *Aconitum heterophyllum* Wall. ex Royle showing flower, immature flower bud cluster, fruit, seeds, roots, leaves, and dissected fruit

The methanolic extract of *Aconitum heterophyllum* tubers collected from Langate Forest Division (LFD) yielded 3.3 g of crude extract from 20 g of dried powdered sample, corresponding to a percentage yield of 16.5%. The extract exhibited a pungent odour, honey-brown colour, and a viscous texture with crystalline appearance, indicating the presence of concentrated secondary metabolites and lipophilic bioactive constituents in the methanolic fraction. Preliminary phytochemical screening of the methanolic root extract (Fig. 2; Table 3) revealed the presence of alkaloids, carbohydrates, fatty acids/lipids, and alcohols, while flavonoids and saponins were absent. Positive reactions with Mayer's, Wagner's, and Dragendorff's reagents indicate a significant alkaloid content, consistent with the known chemistry of *Aconitum* species. Alkaloids from this genus are associated with analgesic, anti-inflammatory, antipyretic, and neuroactive activities, supporting the traditional medicinal applications of *A. heterophyllum* (Padma et al., 2019). The detection of carbohydrates and lipids suggests the occurrence of primary metabolites involved in energy storage and membrane stability, which may contribute indirectly to extract bioactivity and stability (Yang et al., 2013).

Table 3. Preliminary Phytochemical screening results of the methanolic extract (+ present, – absent).

Extract (s) Tests	Methanol
Alkaloids	
Mayer's test	+
Wagner's test	+
Dragendorff's test	+
Flavonoids	
Alkaline reagent test	–
Lead acetate test	–
Carbohydrates	
Fehling's test	+
Molisch's test	+
Saponins	
Foam test	–
Lipids/Fatty acids	
Spot test	+
Alcohols/Phenols	
Ferric chloride test	+

The absence of flavonoids and saponins may be attributed to organ specificity, solvent selectivity, or the limited sensitivity of qualitative colorimetric assays rather than their complete absence in the species. Previous LC–MS/MS studies on aerial parts of *A. heterophyllum* have reported diverse phenolic and flavonoid compounds not detectable through preliminary screening methods (Singh and Saloni, 2015; Tomar and Shalini, 2018; Omega et al., 2024; Ilyas et al., 2025). Therefore, the present phytochemical analysis should be considered a broad qualitative assessment rather than a comprehensive metabolomic profile.

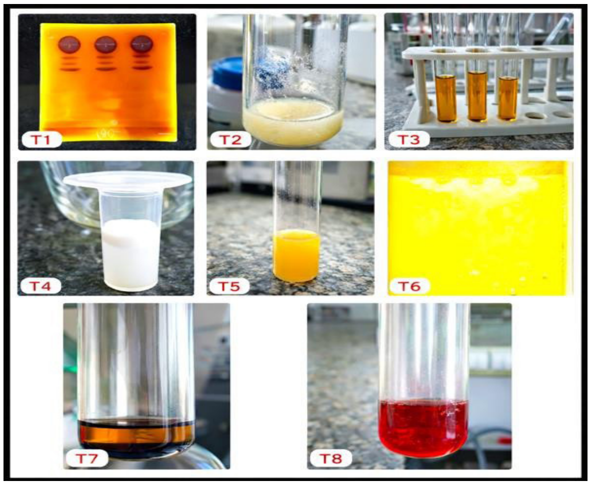


Figure 2: Preliminary Phytochemical Screening: T1: Dragendroffs test, T2: Mayers test, T3: Wagners test, T4: Foam test, T5: Alkaline reagent test, T6: Lead acetate test, T7: Molichs test, T8: Fehling's test

GC–MS characterization of the methanolic root extract of *Aconitum heterophyllum* identified ten major phytochemical constituents with varying retention times and relative abundances (Table 4; Fig. 3). The chromatographic profile was dominated by α-linolenic acid along with several fatty acids, esters, carbohydrate derivatives, heterocyclic amines, and organosilicon compounds. Structural representations of the detected metabolites are presented in Table 5. Similar lipid-rich phytochemical profiles have previously been reported in roots and aerial parts of *A. heterophyllum* and related medicinal species (Sekar et al., 2023; Nengroo et al., 2021; Rajakrishnan et al., 2016).

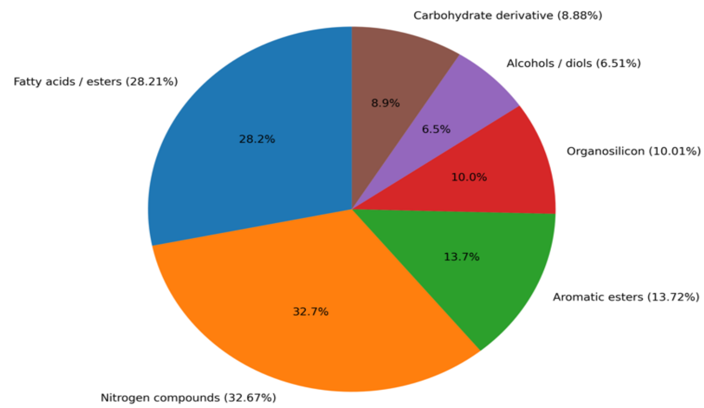
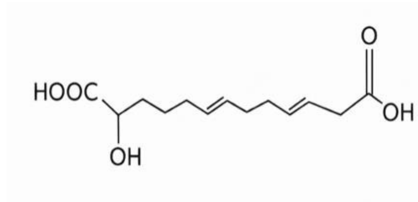
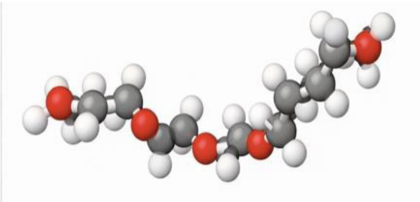
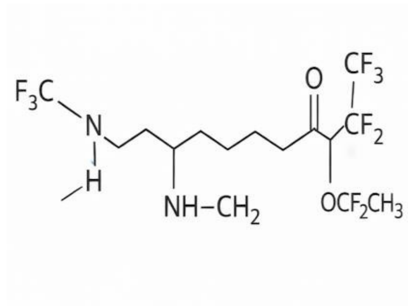
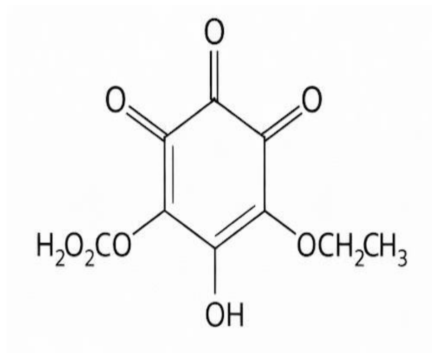
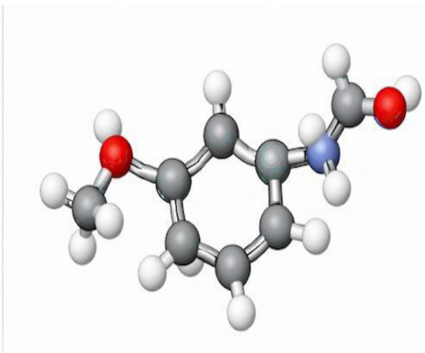
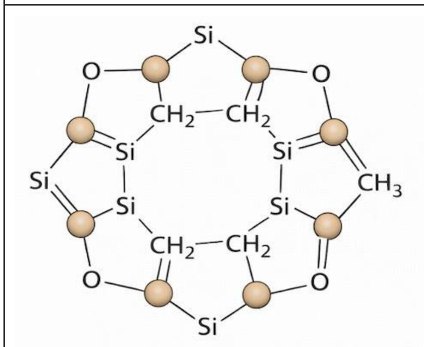
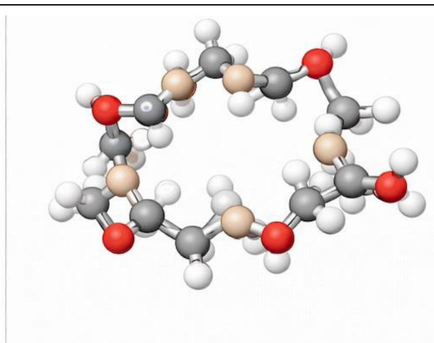
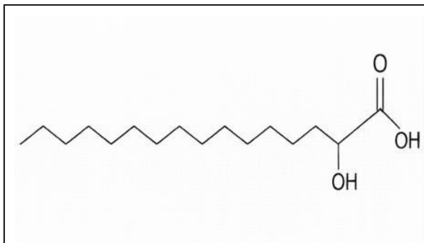
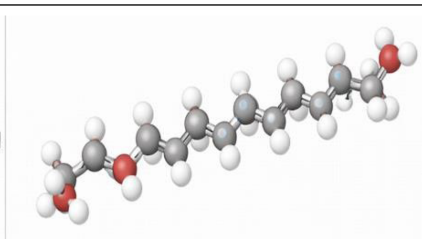
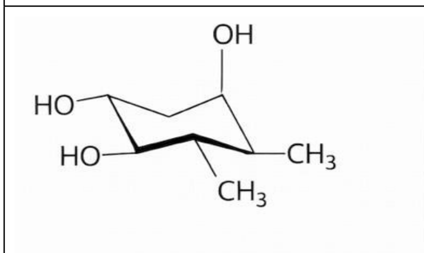
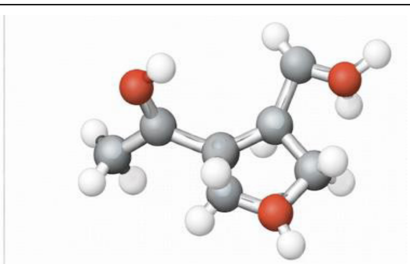


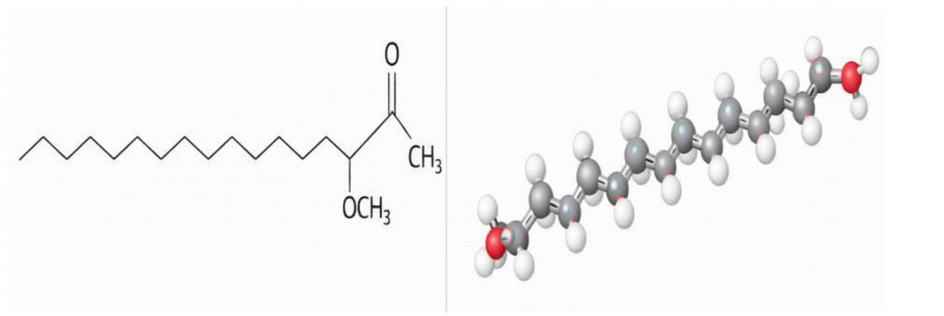
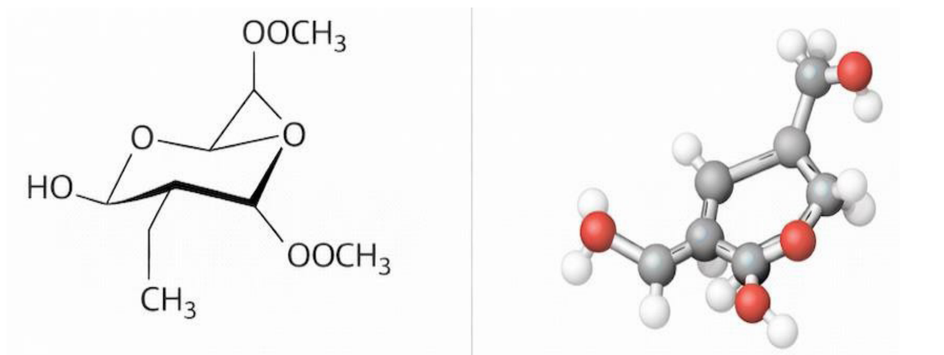
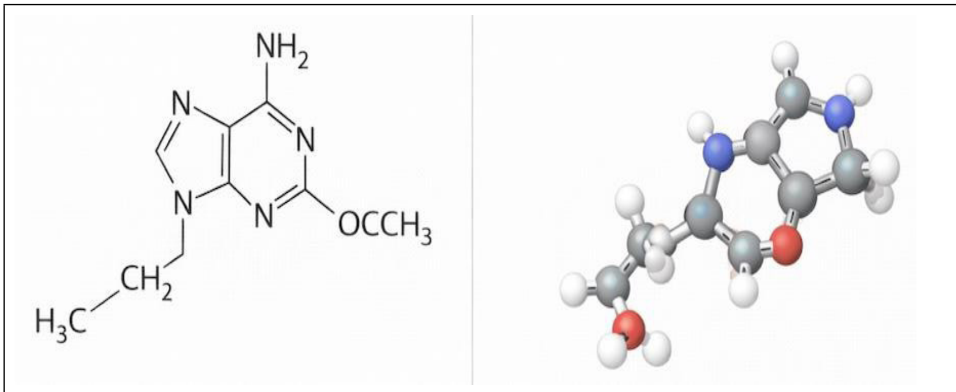
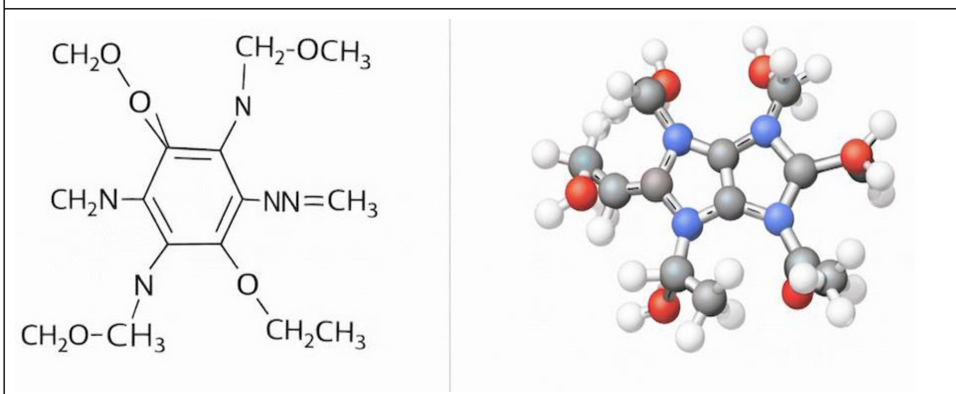
Fig 3. Relative percentage distribution of major phytochemical classes identified in the methanolic root extract of *Aconitum heterophyllum* by GC–MS analysis. Nitrogen-containing compounds constituted the largest fraction (52.7%), followed by fatty acids/ esters (26.2%), aromatic esters (13.2%), carbohydrate derivatives (8.9%), alcohols (5.5%), and minor contributions from dicarboxylic acids and related compounds, indicating predominance of lipophilic and nitrogenous metabolites in the extract.

Table 4. Chemical composition and relative percentage of compounds detected by GC–MS in the methanol extract of *Aconitum heterophyllum*

Peak No.	Retention Time (min)	Area %	Compound Name	Chemical Class / Classification	Key Functional Groups
1	4.405	19.24	cis,cis,cis-9,12,15-Octadecatrienoic acid (α-Linolenic acid)	Polyunsaturated fatty acid (PUFA)	Carboxylic acid, C=C (3 double bonds)
2	6.562	10.97	γ-Aminobutyric acid, N- heptafluorobutyryl-, trifluoro-2- propyl ester	Fluorinated amino acid derivative	Amide, ester, amine, fluorinated groups
3	12.625	13.72	Benzene-1,3-dicarboxylic acid, 5- hydroxymethyl-, diethyl ester	Aromatic diester (phthalate-type ester)	Aromatic ring, ester, alcohol
4	15.270	10.01	Hexa(trimethylene)-hexasila- hexaoxacyclododecane	Organosilicon cyclic siloxane	Si–O–Si (siloxane), cyclic ether
5	16.454	4.16	n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	Carboxylic acid
6	16.535	6.51	5-Methyl-1R,3-trans- cyclohexanediol	Cycloaliphatic diol (alcohol)	Two hydroxyl (–OH) groups
7	19.455	4.81	Hexadecanoic acid, methyl ester	Fatty acid methyl ester (FAME)	Ester
8	23.970	8.88	1,2,3-Tri-O-acetyl-5-deoxy-β-D- ribofuranose	Carbohydrate derivative (acetylated sugar)	Ester (acetate), ether
9	30.404	10.38	Carbamic acid, N-(pyrimidinyl)-, ethyl ester	Carbamate / heterocyclic amine derivative	Carbamate, amine, heterocycle
10	30.687	11.32	Hexa(methoxymethyl)melamine	Triazine resin precursor / melamine derivative	Triazine ring, ether, amine

Table 5. Molecular structures (2D and 3D representations) of phytochemicals detected in the methanol extract of *Aconitum heterophyllum* using GC–MS.

		cis,cis,cis-9,12,15- Octadecatrienoic acid
		γ-Aminobutyric acid, N- heptafluorobutyryl-, 1,1,1- trifluoro-2-propyl ester
		Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester
		1,3,5,7,9,11-Hexa(trimethylene)-1,3,5,7,9,11-hexasila-2,4,6,8,10,12-hexaoxacyclododecane
		n-Hexadecanoic acid
		5-Methyl-1R,3-trans- cyclohexanediol

	Hexadecanoic acid, methyl ester
	1,2,3-Tri-O-acetyl-5-deoxy-β-D- ribofuranose
	Carbamic acid, N-(4-amino-6- ethylaminopyrimidin-5-yl)-, ethyl ester
	Hexa(methoxymethyl)melamine

The predominance of α-linolenic acid is pharmacologically significant because this polyunsaturated fatty acid is widely recognized for its antioxidant, anti-inflammatory, cardioprotective, and neuroprotective activities through regulation of oxidative stress and inflammatory signaling pathways (Chelladurai and Chinnachamy, 2018). Likewise, palmitic acid and its ester derivatives have been reported to exhibit antimicrobial, antioxidant, and membrane-stabilizing properties that may contribute to cellular protection and physiological regulation (Suleiman et al., 2021). These metabolites may partly explain the antioxidant and therapeutic activities previously reported for *A. heterophyllum* extracts (Alam et al., 2023). In addition, the occurrence of heterocyclic amines, carbamate derivatives, and related secondary metabolites indicates considerable chemical diversity within the methanolic extract and suggests possible antimicrobial, cytoprotective, or enzyme inhibitory potential (Gad et al., 2021).

Previous phytochemical and metabolomic studies employing

LC–MS/MS and UHPLC-based analytical techniques have identified several diterpenoid alkaloids in *A. heterophyllum*, including atisinium- and hetisine-type alkaloids, which possess antiplasmodial, analgesic, anti-inflammatory, immunomodulatory, anticancer, and cardiomodulatory activities (24,29,38,39). Collectively, these bioactive constituents support the extensive ethnomedicinal use of *A. heterophyllum* in traditional Himalayan healthcare systems for the treatment of fever, inflammation, gastrointestinal disorders, respiratory ailments, weakness, and general debility. Therefore, the chemically diverse metabolites detected in the present investigation further reinforce the therapeutic relevance and pharmaceutical potential of this critically endangered medicinal herb.

GC–MS was employed in the present study as a rapid and reliable analytical tool for profiling volatile and semi-volatile metabolites, including fatty acids, esters, alcohols, and low-molecular-weight nitrogen- containing compounds. The technique provides valuable preliminary information regarding the lipophilic metabolite composition associated with antioxidant and anti-inflammatory activities. However, the pharmacologically important diterpenoid alkaloids of *Aconitum* species are generally polar, thermolabile, and structurally complex, making them less amenable to GC–MS detection without derivatization. Consequently, GC–MS alone cannot comprehensively represent the complete therapeutic metabolome of *A. heterophyllum*. Advanced LC–MS/MS and UHPLC-based metabolomic approaches are therefore necessary for accurate characterization and quantification of aconitine-, atisinium-, and hetisine-type alkaloids responsible for many of the medicinal properties of the species (Ali et al., 2021 and Anmol et al., 2021). Thus, the metabolites identified in the present study primarily represent the volatile and semi-volatile fraction of the root metabolome rather than the complete phytochemical composition.

Furthermore, certain compounds detected in the GC–MS chromatogram, particularly organosilicon derivatives and melamine-related compounds, may have originated from analytical contamination or instrumental artifacts such as column bleeding, derivatization residues, or contamination from laboratory plasticware. Similar analytical artifacts have been reported in previous GC–MS-based phytochemical investigations employing siloxane-containing stationary phases. Therefore, such compounds should be interpreted cautiously unless validated through repeated analyses and complementary analytical techniques. To minimize analytical interference, solvent blanks and procedural controls were included during analysis; however, definitive confirmation of these metabolites would require more sensitive and selective techniques such as LC–MS/MS or NMR spectroscopy.

Overall, the combined morphological authentication, phytochemical screening, and GC–MS profiling demonstrate that *A. heterophyllum* roots possess chemically diverse bioactive constituents, particularly alkaloids and fatty acid derivatives, supporting the plant's ethnomedicinal importance and therapeutic potential. The findings generated in the present study provide baseline pharmacognostic and phytochemical information that may contribute to species authentication, quality standardization, conservation planning, and future bioactivity-guided investigations of this critically endangered Himalayan medicinal herb.

Conclusion

The present study established baseline pharmacognostic and chemical characteristics of *Aconitum heterophyllum* through integrated morphological evaluation, qualitative phytochemical screening, and GC– MS profiling of methanolic root extracts. The results confirmed the presence of diverse bioactive metabolites, particularly alkaloids and fatty acid derivatives, supporting the species' traditional medicinal significance. However, the study also highlights the limitations of GC–MS in detecting thermolabile diterpenoid alkaloids characteristic of *Aconitum* species, emphasizing the need for advanced LC–MS/MS-based metabolomic investigations. Considering the critically endangered status of *A. heterophyllum*, conservation-oriented cultivation, sustainable harvesting protocols, habitat protection, and community-based resource management are essential for ensuring long-term utilization and preservation of this valuable Himalayan medicinal herb.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

References

Sen, T. and Samanta, S.K. 2014. Medicinal plants, human health and biodiversity: a broad review. *Advances in Biochemical Engineering/Biotechnology*, 147: 59–110. https://doi.org/10.1007/10_2014_273

Ahad, B., Shahri, W., Rasool, H., Reshi, Z.A., Rasool, S. and Hussain, T. 2021. Medicinal plants and herbal drugs: an overview. pp. 1–40. In: *Medicinal Plants and Herbal Drugs*. Springer, Switzerland. https://doi.org/10.1007/978-3-030-58975-2_1

Mofokeng, M.M., Du Plooy, C.P., Araya, H.T., Amoo, S.O., Mokgehle, S.N., Pofu, K.M. et al. 2022. Medicinal plant cultivation for sustainable use and commercialisation. *South African Journal of Science*, 118(7/8). https://doi.org/10.17159/sajs.2022/12190

Chen, S.L., Yu, H., Luo, H.M., Wu, Q., Li, C.F. and Steinmetz, A. 2016. Conservation and sustainable use of medicinal plants:

problems, progress and prospects. *Chinese Medicine*, 11: 37. https://doi.org/10.1186/s13020-016-0108-7

Shukla, S.K. 2023. Conservation of medicinal plants: challenges and opportunities. *Journal of Medicinal Botany*, 7. https://doi.org/10.25081/jmb.2023.v7.8437

Ali, S., Chouhan, R., Sultan, P., Hassan, Q. and Gandhi, S. 2021. A comprehensive review of phytochemistry, pharmacology and toxicology of the genus *Aconitum*. *Advances in Traditional Medicine*, 23: 299–320. https://doi.org/10.1007/s13596-021-00565-8

Ganie, A.H., Tali, B.A., Khuroo, A.A., Reshi, Z.A. and Nawchoo, I.A. 2019. Anthropogenic threats to high-valued medicinal plants of Kashmir Himalaya. *Journal of Nature Conservation*. https://doi.org/10.1016/j.jnc.2019.125715

Jeelani, S., Siddique, M. and Rani, S. 2015. Morphology, ecology and chromosome variation of *Aconitum heterophyllum*. *Caryologia*, 68: 294–305. https://doi.org/10.1080/00087114.2015.1109933

Tomar, S., Thakur, S., Kanwal, K., Bhatt, I. and Puri, S. 2025. Population assessment and habitat suitability modelling of *Aconitum heterophyllum*. *Scientific Reports*, 15. https://doi.org/10.1038/s41598-025-03324-w

Dad, J. and Rashid, I. 2022. Differential responses of Kashmir Himalayan threatened medicinal plants to anticipated climate change. *Environmental Conservation*, 49: 33–41. https://doi.org/10.1017/S0376892922000030

Chauhan, J., Purohit, V., Patni, B., Charles, B. and Prasad, P. 2025. Impact of climate change on distribution and niche dynamics of Himalayan aconites. *Environmental Monitoring and Assessment*, 197. https://doi.org/10.1007/s10661-025-13848-3

Nisa, M.M.U., Sofi, P.A., Ahmad, P.I., Masoodi, K.Z., Pala, N.A., Bhat, G.M. et al. 2025. Phytochemical profiling and ethnobotanical study of *Podophyllum hexandrum* Royle: insights from Langate Forest Division, Jammu and Kashmir. *International Journal of Environment and Climate Change*, 15(4): 91–103. https://doi.org/10.9734/ijecc/2025/v15i44795

Jeelani, S., Siddique, M. and Rani, S. 2015. Morphology, ecology and chromosome variation of *Aconitum heterophyllum*. *Caryologia*, 68: 294–305. https://doi.org/10.1080/00087114.2015.1109933

Jain, S.K. and Rao, R.R. 1977. *A Handbook of Field and Herbarium Methods*. Today and Tomorrow Printers and Publishers, New Delhi, India.

Rasve, V., Paithankar, V.V., Shirsat, M.K. and Dhobale, A.V. 2017. Evaluation of the antiulcer activity of *Aconitum heterophyllum*. *World Journal of Pharmacy and Pharmaceutical Sciences*, pp. 819–839.

Silva, G.O., Abeysundara, A.T. and Aponso, M.M. 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.

Uarrota, V.G., Hernández, I., Ponce, E., Bauer, C.M., Maraschin, M. and Pedreschi, R. 2022. Metabolic profiling and biochemical analysis of stored Hass avocado fruit by GC-MS and UHPLC-UV-VIS revealed oxidative stress as the main driver of 'blackspot' physiological disorder. *International Journal of Food Science and Technology*, 57(12): 7896–7916.

Shaikh, J. and Patil, M. 2020. Qualitative tests for preliminary phytochemical screening: an overview. *International Journal of Chemical Studies*. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Maheshwaran, L., Nadarajah, L., Senadeera, S., Ranaweera, C., Chandana, A. and Pathirana, R. 2024. Phytochemical testing methodologies and principles for preliminary screening. *Asian Plant Research Journal*. https://doi.org/10.9734/aprj/2024/v12i5267

Stephen, M., Igbokwe, N., Idowu, A. and Ezeobiora, C. 2025. Preliminary phytochemical and GC–MS screening of medicinal plants. *Tropical Journal of Phytochemistry and Pharmaceutical Sciences*. https://doi.org/10.26538/tjpps/v4i4.4

Atlı, B., Ghahramani, S., Ak-Sakallı, E., Öztinen, N. and Koşar, M. 2025. Microscopic evaluation and qualitative phytochemical screening of medicinal plants. *EMU Journal of Pharmaceutical Sciences*. https://doi.org/10.54994/emujpharmsci.1686372

Ameera, O.H., Ghaidaa, J.M., Mohammed, Y.H. and Hadi, I.H. 2016. Pharmacognostic and phytotherapeutic evaluation of medicinal herbs. *Journal of Pharmacognosy and Phytotherapy*, 8(3): 49–59. https://doi.org/10.5897/JPP2015.0368

Mathew, A., Kishore, A., Pai, V. et al. 2021. Pharmacognostical and phytochemical evaluation of *Aconitum heterophyllum* root. *Research Journal of Pharmacy and Technology*. https://doi.org/10.52711/0974-360X.2021.00669

Wani, T., Kaloo, Z. and Dangroo, N. 2021. *Aconitum heterophyllum*: a critically endangered medicinal herb. *Journal of Integrative Medicine*. https://doi.org/10.1016/j.joim.2021.12.004

Padma, M., Ganesan, S., Jayaseelan, T. et al. 2019. Phytochemical screening and GC–MS analysis of medicinal plants. *Journal of Drug Delivery and Therapeutics*, 9: 85–89. https://doi.org/10.22270/jddt.v9i1.2174

Yang, B., Yang, H., Chen, F., Hua, Y. and Jiang, Y. 2013. Phytochemical analyses using UHPLC–MS and GC–MS. *Analyst*, 138: 6881–6890. https://doi.org/10.1039/C3AN01478A

Singh, K. and Saloni, S. 2015. Phytochemical screening and TLC profiling of *Aconitum heterophyllum*. *International Journal of Pharma and Bio Sciences*.

Tomar, K. and Shalini, S. 2018. GC–MS analysis of phytochemicals of *Aconitum heterophyllum*. *International Journal of Research in Ayurveda and Pharmacy*. https://doi.org/10.7897/2277-4343.094114

Omega, A., Ilyas, M., Ali, A., Villalta, P. and Thomas, W. 2024. Metabolite profiling and pharmacological investigation of *Aconitum heterophyllum*. *ACS Omega*, 9: 26922–26940. https://doi.org/10.1021/acsomega.3c09668

Ilyas, M., Shad, A., Orakzai, A., Shah, Z., Alotaibi, B. and Maashi, M. 2025. Novel targets for treating cancer and inflammatory disorders modulated by *Aconitum heterophyllum*. *Biomedicine and Pharmacotherapy*, 193: 118752. https://doi.org/10.1016/j.biopha.2025.118752

Sekar, K., Hari, R., Moorthy, D., Sampath, S. and Alagasen, S. 2023. GC–MS analysis and antioxidant evaluation of Ativisa root extract. *Research Journal of Pharmacy and Technology*. https://doi.org/10.52711/0974-360X.2023.00120

Nengroo, Z., Ganie, A. and Azeem, M. 2021. Fatty acid profile, antibacterial and antioxidant activities of *Aconitum heterophyllum*. *Carbohydrate Polymer Technologies and Applications*. https://doi.org/10.1016/j.carpta.2021.100105

Rajakrishnan, R., Lekshmi, R. and Samuel, P. 2016. Analytical standards for root tubers of Ativisha. *International Journal of Scientific Research Publications*, 6.

Chelladurai, G.R.M. and Chinnachamy, C. 2018. GC–MS analysis of bioactive phytochemicals. *Brazilian Journal of Pharmaceutical Sciences*, 54(1). https://doi.org/10.1590/S2175- 97902018000117151

Suleiman, R.K., Iali, W., El Ali, B. and Umoren, S.A. 2021. Fatty acid constituents of plant leaves. *Molecules*, 26: 4192. https://doi.org/10.3390/molecules26144192

Alam, M., Rony, S., Afroz, F., Mansur, A., Sharmin, S., Moni, F. et al. 2023. A new annulenol from *Aconitum heterophyllum* with bioactivity and cytotoxicity against Vero and lung cell lines. *Dhaka University Journal of Pharmaceutical Sciences*. https://doi.org/10.3329/dujps.v22i1.64141

Gad, H.A., Al-Sayed, E. and Ayoub, I.M. 2021. Phytochemical discrimination of medicinal species using GC–MS and ATR-IR analyses. *Phytochemical Analysis*, 32: 820–835. https://doi.org/10.1002/pca.3028

Ali, S., Kour, D., Ntemafack, A., Kapoor, N., Sultan, P., Kumar, A. et al. 2021. Assessment of threatened status, phytochemical composition and biological properties of three *Aconitum* species from Kashmir Himalaya. *Current Botany*. https://doi.org/10.25081/cb.2021.v12.6892

Anmol, K., Kumari, S., Kumar, R., Singh, R., Aggarwal, G., Agrawal, P. et al. 2021. Antiplasmodial diterpenoid alkaloid from *Aconitum heterophyllum*: isolation, characterization and UHPLC-DAD quantification. *Journal of Ethnopharmacology*: 114931. https://doi.org/10.1016/j.jep.2021.114931

Shen, T., Xu, J., Ahmed, W., Yu, X., He, Y., Li, G., Yang, H. and Liu, Q. 2025. Diterpenoid alkaloids of *Aconitum*: a comprehensive review of classification, bioactivities, and therapeutic potential. *ChemistrySelect*, 10(15). https://doi.org/10.1002/slct.202500176.