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ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF THE METHANOLIC EXTRACTS OF LEAVES OF *ACONITUM HETROPHYLLUM* USING GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) TECHNIQUE

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ABSTRACT

Aconitum heterophyllum is herbal constituent of alpine pastures of Central Himalaya, has been used as a traditional medicine. The present study was conducted to check the GC-MS analysis of the *Aconitum heterophyllum* leaves methanolic extracts. The GC-MS analysis of the methanolic extract showed the presence of 60 compounds from *A. heterophyllum* by comparison of their retention indices and mass spectra fragmentation patterns with those stored on the GC-MS computer library. The major compound identified were 2-Butenedioic acid (2E)- (10.99%), Cholesterol, 2-fromyl-3-benzyl- (10.58%), Phytol isomer (10.06%) with retention time 28.45, 46.35 and 26.98 minute respectively. The minor compound identified were 2,5-Dihydroxy-2,5-Dimethyl-3-Hexyne (0.19%), Hexanoic acid, 2-Methyl- (0.17%), Razoxane (0.14%) with retention time 18.33, 18.07 and 17.56 minute respectively. The study summarizes the information concerning the phytochemical constituents present in methanolic leaves extracts. These constituents may be responsible for pharmacological activities.

KEYWORDS: *Aconitum heterophyllum*, Gas chromatography, Mass spectroscopy, Butenedioic acid, Hexanoic acid

INTRODUCTION

Plants, as the source of medicine, have been playing an important role in the health services around the globe³. About three quarters of the world's population relies on plant and their extracts for health care. A good number of our population particularly those living in rural areas depend largely on herbal remedies for the treatment of different types of diseases. It indicates the importance of the individual plants in the health care system¹.

Aconitum heterophyllum is herbal constituent of alpine pastures of Central Himalayas, ranging altitude, 3500 m to 4000 m. *Aconitum* is the botanical name of the genus commonly known as aconite, monkshood etc. The genus *Aconitum* belongs to the family Ranunculaceae and is widely distributed in the alpine and sub-alpine regions of the tropical parts of Northern hemisphere. The genus *Aconitum* has marked its key position in many research of research. The indiscriminate exploitation has reduced the species towards rarity, so endangering its survival. Many species of this genus have been listed in the Red Data Book, due to which many conservation programs came into existence these includes many *in-situ*, *ex-situ/in-vitro* mode of conservation. Phytochemical analysis as well as molecular facts of medicinal plant species of this genus has been and is being explored in many research institutes globally⁴. *Aconitum heterophyllum* wall. Ex Royle commonly known as *Atis* is one of the most important medicinal plants of family Ranunculaceae. The herb has been characterized with the presence of blue and yellow coloured spur bearing flowers and the height of the plant is up to 1m to 1.8 m. It has been used as traditional medicines to cure various ailments, stomach febrifuge, dyspepsia, abdominal pain, analgesic and diabetes⁷. Aconite, a diterpenoid alkaloid and a principle biologically active compound, is used as a life-saving drug in Ayurvedic and Unani system of medicines. The flavonoids a

major group of natural products from genus *Aconitum* following the alkaloids, have previously been isolated and characterized from its various species². It has been established that the curing of various disease from the extracts of traditional medicinal plants have been attributed to the presence of polyphenolic compounds. The flavonol glycosides have previously been identified from the antioxidant bioassay-guided fractionation of aqueous extracts of *Aconitum* herbs and it has also been established that some caffeoyl glycosides of quercetin, a catechol grouping polyphenolic, have been identified to have more antioxidant potential compared to quercetin and dihydroquercetin⁵.

Due to its high cost as well as availability, the chance for adulterating leaves of *Aconitum heterophyllum* with substandard products is high. Thus, to avoid adulteration, standardization of this valuable herbal drug is the need of the hour. In the present study an attempt has been made to standardize the original and authenticated leaves of *Aconitum heterophyllum* by phytochemical constituents and GC-MS analysis.

MATERIALS AND METHODS

Plant collection, identification and authentication

Vegetative plants of *Aconitum Heterophyllum* were collected from forest nursery Deoban chakrata (District Dehradun, Uttarakhand). One plant specimen of *Aconitum Heterophyllum* was submitted to Taxonomy Division, Botanical Survey of India Dehradun for authentication and accession No. 1155367 was obtained. A herbarium sample of the material is stored in the Herbarium, Taxonomy Division, Botanical Survey of India Dehradun Uttarakhand India.

Preparation of the extract

The collected leaves of *Aconitum heterophyllum* were dried in shade at room temperature and milled to a coarse powder. The obtained dried powder was subjected to continuous extraction with 100% methanol in a Soxhlet apparatus. The powdered plant material was packed in a tumble made of Whatman's filter paper.

It was extracted with methanol for 20 cycles. The extract thus obtained was concentrated in a flask evaporator under reduced pressure and controlled temperature. The yield of Methanolic extract of leaves of *A. heterophyllum* was 22%. The obtained residue was green and brown in colour, thick and sticky paste. The extract was stored in the refrigerator.

Figure 1: Map showing sample collection sites in Uttarakhand, India

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis was performed at University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi using a GC-MS Shimadzu QP 2010 ultra-system interfaced to a GC-MS equipped with a Elite-5MS {5% diphenyl/95% dimethyl polysiloxane} fused to capillary column $30 \times 0.25 \mu\text{m}$ ID (inner diameter of column) $\times 0.25 \mu\text{m}$ df (film thickness)}. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 μl was employed (a split pattern). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST (National Institute of Standards and Technology, U.S.A.) database.

RESULTS

GC-MS analysis

GC-MS chromatogram of the methanolic extract of the leaves *A. heterophyllum* showed 60 peaks indicating the presence of as much as compounds and were presented in (Table 1, Fig. 2,3). Of the 60 compounds, all compounds got matched with the library and were identified. The high percentage compound identified 2-

Butenedioic acid (2E)- (10.99%), Cholesterol, 2-fromyl-3-benzyl- (10.58%), Phytol isomer (10.06%), Hexadecadienoic acid, methyl ester (5.79%), 2,6,10-Trimethyl,14-ethylene-14-pentadecene (5.32%), Pentadecanoic acid, 14-methyl-, methyl ester (3.32%), Glutaric acid, di (dodec-9-ynyl) ester (3.14%) with retention time 28.45, 46.35, 26.98, 23.13, 24.42 and 42.12 minute respectively. Lower percentage compound were identified Eicocylbis (Trifluoromethyl) Phosphine Sulfide (0.28%), Ethanone, 1-(3,4-Dimethoxyphenyl)- (0.26%), 3,5- DI Tert butylphenol (0.25%), Decanoic acid (0.23%), Cyclohexanol, 3,5-Dimethoxy-, Stereoisomer (0.21%), 3,6-Dimethyl-4-octyn-3,6-diol (0.20%), 2,5-Dihydroxy-2,5-Dimethyl-3-Hexyne (0.19%), Hexanoic acid, 2-Methyl- (0.17%), Razoxane (0.14%) with retention time 39.87, 18.83, 17.92, 18.89, 26.89, 20.91, 18.33, 18.07 and 17.56 minute respectively.

DISCUSSION

This study highlights the presence of many secondary metabolites in the leaves of *Aconitum heterophyllum*; providing an overview of the different classes of molecules present that have led to their pharmacological activities. This study confirms that the *Aconitum heterophyllum* leaves extract could be used for the treatment of various diseases. The presence of various bio-active compounds detected in GC-MS analysis using the methanolic extracts justifies the use of plant for pharmacological action

Table 1: Chemical constituents identified by GC-MS analysis of methanolic extract of Leaves of *A. heterophyllum*

Sl. No.	Rt	Area %	Name of Compound	Molecular Formula	Molecular Weight
1	12.669	2.89%	2,3-DIHYDROBENZOFURAN	C ₈ H ₈ O	120
2	14.248	1.06%	2-METHOXY-4-VINYLPHENOL	C ₉ H ₁₀ O ₂	150
3	14.563	0.84%	FORMIC ACID, HEX-2-YL ESTER	C ₇ H ₁₄ O ₂	130
4	15.680	0.41%	3-HEXADECENE, (Z)-	C ₁₆ H ₃₂	224
5	17.564	0.14%	RAZOXANE	C ₁₁ H ₁₆ N ₄ O ₄	268
6	17.922	0.25%	3,5- DI-TERT-BUTYLPHENOL	C ₁₄ H ₂₀ O	206
7	18.071	0.17%	HEXANOIC ACID, 2-METHYL-	C ₇ H ₁₄ O ₂	130
8	18.333	0.19%	2,5-DIHYDROXY-2,5-DIMETHYL-3-HEXYNE	C ₈ H ₁₄ O ₂	142
9	18.831	0.26%	ETHANONE, 1-(3,4-DIMETHOXYPHENYL)-	C ₁₀ H ₁₂ O ₃	180
10	18.896	0.23%	DECANOIC ACID	C ₁₀ H ₂₀ O ₂	172
11	19.270	0.56%	1-TETRADECANOL	C ₁₄ H ₃₀ O	214
12	20.416	0.17%	SABINYL ACETATE	C ₁₂ H ₁₈ O ₂	194
13	20.913	0.20%	3,6-DIMETHYL-4-OCTYN-3,6-DIOL	C ₁₀ H ₁₈ O ₂	170
14	21.408	0.44%	HEXADECANOIC ACID, METHYL ESTER	C ₁₇ H ₃₄ O ₂	270
15	22.091	0.31%	HEXADECANOIC ACID	C ₁₆ H ₃₂ O ₂	256
16	22.333	0.66%	5-ISOPROPYL-6-METHYL-3,5-HEPTADIEN-2-OL	C ₁₁ H ₂₀ O	168
17	22.483	0.62%	1-HEXADECENE	C ₁₆ H ₃₂	224
18	23.131	5.32%	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECENE	C ₂₀ H ₃₈	278
19	23.218	1.85%	2-PENTADECANONE, 6,10,14-TRIMETHYL-	C ₁₈ H ₃₆ O	268
20	23.495	1.70%	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	C ₂₀ H ₄₀ O	296
21	23.767	2.12%	PHYTOL ACETATE	C ₂₂ H ₄₂ O ₂	338
22	24.345	0.26%	OXIRANE, HEXADECYL-	C ₁₈ H ₃₆ O	268
23	24.426	3.32%	PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER	C ₁₇ H ₃₄ O ₂	270
24	24.514	0.44%	BENZENEPROPANOIC ACID, 3,5-BIS(1,1-DIMETHYLETHYL)-4-HYDROX	C ₁₈ H ₂₈ O ₃	292
25	25.054	1.10%	9-OCTADECENOIC ACID (Z)-	C ₁₈ H ₃₄ O ₂	282
26	25.395	0.47%	PHOSPHONIC ACID, DIOCTADECYL ESTER	C ₃₆ H ₇₅ O ₃ P	586
27	26.658	0.90%	1-HEXADECANOL	C ₁₆ H ₃₄ O	242
28	26.738	2.77%	9,12-OCTADECADIENOIC ACID, METHYL ESTER	C ₁₉ H ₃₄ O ₂	294
29	26.828	5.79%	HEXADECADIENOIC ACID, METHYL ESTER	C ₁₇ H ₃₀ O ₂	266
30	26.897	0.21%	CYCLOHEXANOL, 3,5-DIMETHOXY-, STEREOISOMER	C ₈ H ₁₆ O ₃	160
31	26.980	10.06%	PHYTOL ISOMER	C ₂₀ H ₄₀ O	296
32	27.175	0.90%	OCTADECANOIC ACID, METHYL ESTER	C ₁₉ H ₃₈ O ₂	298
33	27.347	0.46%	BIS(2-ETHYLHEXYL) MALEATE	C ₂₀ H ₃₆ O ₄	340
34	28.126	0.59%	N-TRICOSANE	C ₂₃ H ₄₈	324
35	28.287	0.30%	PHYTOL, ACETATE	C ₂₂ H ₄₂ O ₂	338
36	28.451	10.99%	2-BUTENEDIOIC ACID (2E)-	C ₂₀ H ₃₆ O ₄	340
37	29.310	0.40%	9,10- DIAZATRICYCLO[4.4.0.0(2,8)] DECANE-9,10-DICARBOXIMIDE,N PHENYL-7	C ₁₆ H ₁₇ N ₃ O ₂	283
38	29.365	1.07%	OCTADECANE	C ₁₈ H ₃₈	254
39	29.613	0.45%	C16 FATTY ACID	C ₁₆ H ₃₂ O ₂	256
40	29.699	1.39%	METHYL N-EICOSANOATE	C ₂₁ H ₄₂ O ₂	326
41	29.758	0.60%	1,1'-BIPHENYL, 5,2',3',4'-TETRAMETHOXY-	C ₁₆ H ₁₈ O ₄	274
42	30.568	1.89%	2,6,10,15- TETRAMETHYLHEPTADECANE	C ₂₁ H ₄₄	296
43	31.149	0.61%	17-HYDROXY-17-.ALPHA.-PREGN-4-EN-2	C ₂₁ H ₂₈ O ₂	312
44	31.563	0.30%	9-OCTADECENOIC ACID	C ₃₉ H ₇₂ O ₅	620
45	31.935	2.78%	TETRA-TETRACONTANE	C ₄₄ H ₉₀	618
46	32.876	2.12%	3-AZIDOANDROST-16-ENE	C ₁₉ H ₂₉ N ₃	299
47	33.601	2.45%	2-METHYLOCTACOSANE	C ₂₉ H ₆₀	408
48	35.647	2.36%	2,6,10,15-TETRAMETHYLHEPTADECANE	C ₂₁ H ₄₄	296
49	36.164	0.39%	OCTADECANOIC ACID, METHYL ESTER	C ₁₉ H ₃₈ O ₂	298
50	37.127	1.32%	N-HEPTACOSANE	C ₂₇ H ₅₆	380
51	37.256	0.37%	SQUALENE	C ₃₀ H ₅₀	410
52	37.484	0.82%	3-ADAMANTAN-1-YL-6-AMINO-4-THIOPHEN-2-YL-1,4-DIHYDRO-PYR	C ₂₁ H ₂₂ N ₄ OS	378
53	38.126	1.49%	(-)-SINULARENE	C ₁₅ H ₂₄	204
54	38.407	0.98%	EICOSANE	C ₂₀ H ₄₂	282
55	39.416	0.33%	DES-N-26-METHYLENE-DIHYDROTOMATIDINE	C ₂₈ H ₄₈ O ₂	416
56	39.871	0.28%	EICOCYLBIS(TRIFLUOROMETHYL)PHOSPHINE SULFIDE	C ₂₂ H ₄₁ F ₆ PS	482
57	42.122	3.14%	GLUTARIC ACID, DI (DODEC-9-YNYL) ESTER	C ₂₉ H ₄₈ O ₄	460
58	44.156	1.46%	ERGOST-5-EN-3-OL	C ₂₈ H ₄₈ O	400
59	46.357	10.58%	CHOLESTANOL, 2-FROMYL-3-BENZYL-	C ₃₅ H ₅₄ O ₂	506
60	48.603	3.18%	BETA.-CARYOPHYLLENE	C ₁₅ H ₂₄	204

Figure 2: GC-MS chromatogram of methanolic extract of Leaves of *A. heterophyllum*

Compound name: 2-Butenedioic acid (2E)-, Molecular formula:- C₂₀H₃₆O₄, Molecular weight:-340

Compound name: Cholesterol, 2-benzyl-3-phenyl-, Molecular formula: $C_{35}H_{54}O_2$, Molecular weight: 506

Compound name: Phytol isomer, Molecular formula: $C_{20}H_{40}O$, Molecular weight: 296

Compound name: Hexadecadienoic acid, methyl ester, Molecular formula: $C_{17}H_{30}O_2$, Molecular weight: 266

Compound name: 2,6,10-Trimethyl,14-ethylene-14-pentadecene, Molecular formula: $C_{20}H_{38}$, Molecular weight: 278

Compound name: Pentadecanoic acid, 14-methyl-, methyl ester, Molecular formula: $C_{17}H_{34}O_2$, Molecular weight: 270

Compound name: Glutaric acid, di(dodec-9-ynyl) ester, Molecular formula: $C_{29}H_{48}O_4$, Molecular weight: 460

Compound name: Eicocylbis (trifluoromethyl) phosphine sulfide, Molecular formula: $C_{22}H_{41}F_6PS$, Molecular weight: 482

Compound name: Ethanone, 1-(3,4-Dimethoxyphenyl)-, Molecular formula:- C₁₀H₁₂O₃, Molecular weight: 180

Compound name: 3,5-Di-tert-butylphenol, Molecular formula: C₁₄H₂₂O, Molecular weight: 206

Compound name: Decanoic acid, Molecular formula: C₁₀H₂₀O₂, Molecular weight: 172

Compound name: Cyclohexanol, 3,5-Dimethoxy-, Stereoisomer, Molecular formula:- C₈H₁₆O₃, Molecular weight: 160

Compound name: 3,6-Dimethyl-4-octyn-3,6-diol, Molecular formula: C₁₀H₁₈O₂, Molecular weight: 170

Compound name: 2,5-Dihydroxy-2,5-Dimethyl-3-Hexyne, Molecular formula: C₈H₁₄O₂, Molecular weight: 142

Compound name: Hexanoic acid, 2-methyl-, Molecular formula: C₇H₁₄O₂, Molecular weight: 130

Compound name: Razoxane, Molecular formula: C₁₁H₁₆N₄O₄, Molecular weight: 268

Figure 3: Word mass spectra of the main bioactive molecules found in the methanolic extract of the plant Leaves

CONCLUSION

The results of the present study reported that *Aconitum heterophyllum* leaves have numerous bioactive compounds. The mass spectra are used for analysis of different chemical compounds. It can be used for various medicinal applications, and it will prove beneficial to the healthy human life.

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