GC-MS Analysis and Antioxidant Activity of *Aconitum laeve* Royle, an Endangered species, Jammu & Kashmir, 1ndia

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Abstract

The chemical composition of *Aconitum laeve* Royle from chloroform extract was analyzed by GC-MS and in combination with retention indices, growing in the higher Himalayan region & its suburbs. There are 35 compounds had been identified, most of them was alkanes, olefins, esters and acids. The content of main components substantially is phthalic acid (21.51%), tetratetracontane (12.26%) deoxycelidoniol (11.53%), nonadecane (7.81), dibutylphthalate (6.05%), stigmast-4-en-3-one (4.01%). ethylhexylphthalate (3.24%), 2-methylphenylphthalate & cholest-4-en-3-one (1.49%) as the major constituents whereas the compounds namely, dexoycelidoniol, dibuytylphthalate, 2-methylphenylphthalate, nonadecane was first identified This was first report of chemical constituents from root tubers of *Aconitum laeve* from chloroform extract and deoxycelidoniol was first reported in present identification which provides further exploitation and uses of *A. laeve*. The DPPH assay was used to evaluate the antioxidant effects and their results showed better antioxidant activity of *Aconitum laeve* chloroform extract and a positive correlation showed against ascorbic acid & total phenolic contents of chloroform extract of *Aconitum laeve* were found to be 27.3 mg catechin/g and 22.5 GAE/g of dried weight of tubers. Thus, the present study showed that the tubers contain bioactive compounds with good antioxidant activity for pharmaceutical and nutraceutical applications.

Key words: *Aconitum*, phthalic acid, constituents, extract & antioxidant.

Introduction

The genus *Aconitum* is a perennial herbaceous plant native to mountainous parts of Northern Hemisphere in alpine and subalpine regions at an elevation of 2000-4300m commonly called aconite or mousebane belonging to family Ranunculaceae possessing 300 species[1]. *Aconitum* is used in Ayurvedha as well as of Chinese and Japanese traditional medicine. They have pharmacological properties like blood pressure elevation, cardio tonic effect, analgesic, anti-inflammatory and anesthetics[2]. *Aconitum heterophyllum* tubers are used to cure throat infection, abdominal pain, diabetes, diarrhea and dyspepsia. Ethanolic extract of *A.*

heterophyllum at dose of 50, 100, 200, mg/kg was evaluated using fecal excretion and castor oil induced diarrhea models [3]. Aconites are poisonous plants that require care and caution. It is contemporary to mention, prior to their use in traditional medicines including BTM, aconite components are detoxified using region-specific traditional approaches, all of which are highly effective at reducing the toxicity of the plant by converting its diester diterpene alkaloids to their less toxic monoester diterpene forms and several publications had suggested that carefully defined amounts of toxic aconite diterpenoid alkaloids contribute to a diverse range of desired clinical effects, and it is essential to define the dose range that distinguishes toxicity from therapeutic efficacy of the plant [4,5]Two new compounds were isolated from *Aconitum anthora* L. namely, quercetin-3-O-((*β*-D-glucopyranosyl-(1→3)-(4-O-(E-*p*-coumaryl))-α-L-rhamnopyrnosyl-(1→6)-*β*-D-galactopyranoside))-7-O-α-Lrhamnopyranoside & kaempferol-3-O-((*β*-D-glucopyranosyl-(1→3)-(4-O-(E-*p*-coumaryl))-α-L-rhamnopyrnosyl-(1→6)-*β*-D-galactopyranoside))-7-O-*α*-L-rhamnopyranoside were identified and their antioxidant activity was screened by *in-vitro* tests[6]. The methanolic extract was analyzed by GC-MS contains alkenes, fatty acid, esters and steroids of which mains chemical components were n-hexadecanoic acid (12.083%), stigmasteron-4-en-3-one (10.183%), 1-mono-linolein (8.96%) and 9,12(Z,Z)-octadecanoic acid (8.054%) were reported first time except alkaloid[7] where as the methanolic extract of flower, stem and leaf from this possessed nonacosane (13.057%), 19-methyl-18,21-hexatriacontanediether (8.80%), ethylene glycol monooctadecyl ether (7.851%), 3,7,11,15-tertamethyl-2-hexadecen-1-ol (7.805%) and methyl palmitate (6.676%) was also first report of constituents[8]. The ethanolic extract of *A. heterophyllum* Wall contain phenols, tannins, flavonoids, alkaloids and saponins screened for hemolytic activity was found to be 212.5 units/g of plant material[9]. Aerial parts of *Aconitum leave* Royle contain lycoctonine-type norditerpenoid alkaloids like swatinine, puberanine, lappaconitine, delphatine, and N-acetylsepaconitine were isolated and anti-inflammatory, tyrosine's inhibition and antioxidant studies of all these compounds was carried out[10]. The two new aconitine-type norditerpenoid alkaloids was isolated from roots of *A. heterophyllum* were 6-dehydroacetylaepaconitine and 13-hydroxyappaconitine along with lycoctonine, lappaconitine and delphatine exhibited antibacterial activity[11]. Five diterpenoid alkaloids viz. 14-O-acetylneoline, neoline, 14-O-vertroylpseudaconine, senbusine A and pseudaconitine were isolated from *A. laciniatum* first time and two of them had anti-inflammatory properties against TNBS-induced colitis model in mice. Thus, the alkaloid 14-O-acetylneoline is considered to be a novel anti-colitis drug lead compound[12]. The extracted polysaccharide from *Aconitum kusnezoffii* Reichb. had possessed *invitro*-

antioxidant testing for ion-chelating ability, reducing power, and DPPH radical, hydroxyl radical, superoxide anion, self oxidation of 1,2,3-phentriol and H_2O_2 suggested that it should be a natural antioxidant[13]. The roots of *A. carmichaelii* Debx contained salicylic acid, p-hydroxy-cinnamic acid, songorine, karakoline, honokiol, mesaconotine, hypaconitine & 5-hydroxy-2-benzoyl-aminomethyl ester was isolated and analyzed by spectroscopic studies[14].

2. Material and Methods

2.1 Plant Material

The fresh plant material was collected from mountainous region of Tehsil Bhalessa Distt. Doda, Jammu and Kashmir (India) at an elevated altitude of 3100-3300m in month of August 2017.The tubers were washed with cold water and their dead skin was skimmed off and were used for the extraction of oil. The preliminary plant identification was done by Prof. P. C. Pandey, Department of Botany, Kumaun University, Nainital. The plant was further confirmed by Botanical Survey of India, Dehradun, Voucher specimen *Aconitum laeve* Royle, Acc. No.118090 where herbarium of plant specimens has been deposited of dried whole plant.

2.2 Isolation of Chloroform Extract

The tubers were first dried in shadow at 25±3^{0}C in room temperature & then tubers were grinded into powdered sample with mechanical grinder. The powder was stored in airtight container for further use and allowed to put into extract bottle and extracted with chloroform, respectively. After complete extraction the solvent removed with Rotovap at moderate pressure and 38^{0}C temperature and stored at 4^{0}C for further analysis. The percentage yield was found to be 8.2%. All chemical and reagents of analytical grade and were obtained from Merk, Mumbai, India.

2.3 Chemical and Reagents

All chemicals and reagents used were of analytical grade. Chloroform, anhydrous sodium sulphate (Na_2SO_4), Dimethyl sulphoxide (DMSO), ethanol, and sodium hypochlorite (NaOCl) were obtained from Merk, Mumbai, India whereas potato dextrose agar (PDA), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ascorbic acid, α-tocopherol and dextrose (D-glucose) were obtained from Hi-media Pvt. Ltd, Mumbai India.

3. Analysis of Chloroform extract

3.1 GC-MS Analysis

The GC-MS analysis was carried out with GC-MS QP 2010 (Shimadzu) fitted with RTx-5 MS capillary column, 1009701(30.0m x 0.25mm, film thickness: 0.25µm). The oven temperature (50^0C-280^0C) was programmed at 50^0C for first 2 minutes, then 3^0C/min. to 200^0C and then 10^0C/min. to 280^0C. After which it was maintained isothermally at 280^0C for 8 min. N_2 was used as the carrier gas. The injection volume 0.5µL and split ratio was 1:90. The mass spectra were taken at 70eV. The percentage by peak area normalization was taken to express the relative percentage of the oil constituents.

3.1 Identification of Compounds

Identification of different chemical constituents of the chloroform extract was done by comparing their Retention Indices/Kovat indices in relation to a series of n-alkanes (C_6-C_{33}) indices on the RTx-5 MS capillary column, either with those of published data[15] or with authentic samples which were further supported by NIST and WILEY mass spectral library searches. There are total 35 chemical constituents was identified from chloroform extract of *Aconitum laeve* from Himalayan region and most of them are alkanes, olefins acid and esters of which esters predominant in compositions. The results are presented in **table 1.**

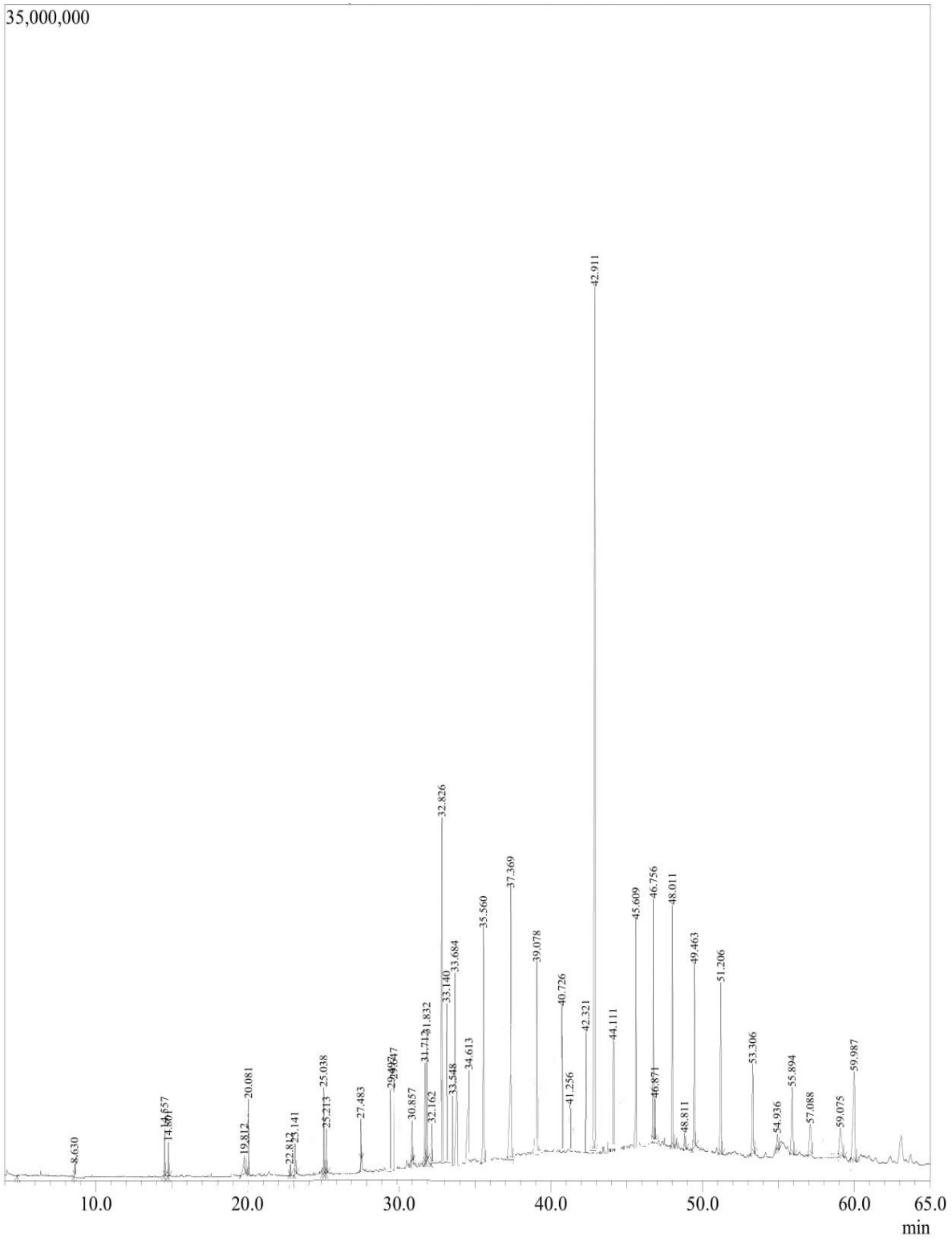

Figure 1: GC-MS Chromatogram from tubers of *Aconitum Laeve*

Table 1: Phytochemical composition from tubers of *Aconitum laeve*

S. No.	Compound	% in the oil	Method of identification	
			RI[a]	Others
1.	1-decene	0.12	994	b, c, d
2.	dodecane	0.40	1002	b, c, d
3.	Z-3-tetradecene	0.56	1392	c, d
4.	tetradecane	0.35	1405	b, c, d
5.	Z-3-hexadecene	0.91	1495	c, d
6.	pentadecane	1.17	1506	c, d,
7.	butyl phthalate	0.82	1546	b, c, d
8.	2-methylphenylphthalate	2.87	1566	c, d
9.	decanoic acid	0.61	1569	c, d
10.	diamylphthalate	0.11	1576	b, c, d
11.	butyloctylphthsalate	0.69	1585	c, d
12.	dodecanoic acid	0.99	1595	c, d
13.	2-methyl-heptadecane	0.78	1750	c, d
14.	3-octadecane	1.09	1804	c, d
15.	nonadecane	7.81	1960	b, c, d
16.	2-ethylhexylphthalate	3.24	1868	b, c, d
17.	dibutylphthalate	6.05	1968	c, d
18.	2-methyloctacosane	0.53	1974	c, d
19.	E-9-eicosene	1.05	1984	b, c, d
20.	1-nonadecene	0.66	1995	b, c, d
21.	9-methyl nonadecane	0.19	2010	c, d
22.	2,4-dimethyl ecosane	0.78	2016	b, c, d
23.	phenyl ethyl phenol	0.91	2058	c, d
24.	phthalic acid	21.51	2084	b, c, d
25.	henecosane	0.21	2105	c, d
26.	heneicosanol	0.53	2117	c, d
27.	docosane	0.31	2206	c, d
28.	eicosane	1.99	2211	c, d
29.	tricosane	0.17	2304	c, d
30.	heptacosanal	0.21	2321	b, c, d
31.	deoxycelidoniol	11.53	2370	c, d
32.	squalene	0.48	2410	b, c, d
33.	tertatetracontane	12.26	2434	c, d
34.	cholest-4-en-3-one	1.49	2560	c, d
35.	stigmast-4-en-3-one	4.01	2580	b, c, d
Total identified		**87.43%**		
Yield		**4.1gm (8.2% by weight)**		

[a] Retention index (RI) relative to homologous series of n-alkane (C_6 – C_{32}) on RTx-5MS Capillary Column.

[b] Compound checked by authentic standards compounds.
[c] Retention index (RI).
[d] MS, NIST08.LIB and WILEY8.LIB libraries spectra and the literature.

3.5 In-vitro Antioxidant Activity

The DPPH assay was done according to the method described by Brand William *et al* 1995[16]. The DPPH 0.0039gm was dissolved in ethanol and made up to 100ml with double distilled water. The ethanol (20%) 20ml and 80ml double distilled water was prepared. The 100µM DPPH (50µl) was added to equal volume of 20% ethanol to generate 400µl DPPH. The extract samples of different concentrations was taken in different test tubes and added DPPH 400µl and make volume up to 100µl with double distilled water. Then it was shaken vigorously and taken in dark for 20 minutes at room temperature. The reduction in absorbance was recorded at 520nm in UV-VIS spectrometer. Ascorbic acid and α-tocopherol was used as standard and controlled absorbance of DPPH was taken without adding oil sample and all the assays was carried out in triplicate. Scavenging effect (%) of free radical DPPH was calculated as:

$$\text{Scavenging effect } \% = = \frac{Absorbance\ of\ control - Absorbance\ of\ oil\ sample}{Absorbance\ of\ control} \times 100$$

IC_{50} assay was calculated graphically using curve by plotting antioxidant capacity or percentage inhibition versus corresponding sample concentrations.

3.6 Determination of Total Phenolic Content

The total phenolic contents were determined by Folin-Ciocalteau Reagent [17]. The sample were homogenized in 805aqueous ethanol at room temperature and centrifuged in cold at 10,000 for 15 minute &supernatant was saved. The residue was extracted twice with 80% ethanol and supernatant was pooled and put into evaporating dishes to dryness at room temperature. Residue was dissolved in 5ml of distilled water, 100µl of this extract was diluted to 3ml with distilled water & 0.5ml of Folin-ciocalteau reagent was added. After 3 minute 2ml mo0f 20% sodium carbonate was added thoroughly. The color was developed and absorbance was measured at 650nm in UV spectrometer after 60 minute after using catechin and Gallic acid as standard. The results were expressed as mg catechin/g and GAE/g of dry weight[18].

Table 2: Absorbance shown by the chloroform extract and control (Ascorbic acid & Vitamin E) at different concentrations.

Concentration (µg/ml)	Absorbance		
	chloroform extract	Ascorbic Acid	α-Tocopherol
100 µg/ml	0.23±0.006	0.236±0.022	0.921±0.021
200 µg/ml	0.26±0.021	0.280±0.032	1.320±0.004
300 µg/ml	0.39±0.001	0.520±0.002	1.890±0.002
400 µg/ml	0.69±0.012	0.826±0.001	2.348±0.006
500 µg/ml	0.82±0.002	1.890±0.043	2.613±0.013
600 µg/ml	0.96±0.011	2.520±0.001	3.124±0.042

Values in the column are men of three absorbance results ± SD

Table 3: Percentage scavenging activity of different concentration of chloroform extract and controls at various concentrations.

S.No.	Conc. (µg/ml)	%age of DPPH scavenged by chloroform extract	%age of DPPH scavenged by Ascorbic acid	%age of DPPH scavenged by α-Tocopherol
1	100	24.5	23.4	35.2
2	200	38.4	41.2	56.4
3	300	51.6	55.6	60.2
4	400	63.1	68.3	71.8
5	500	70.6	71.2	84.00
6	600	86.3	89.3	94.3

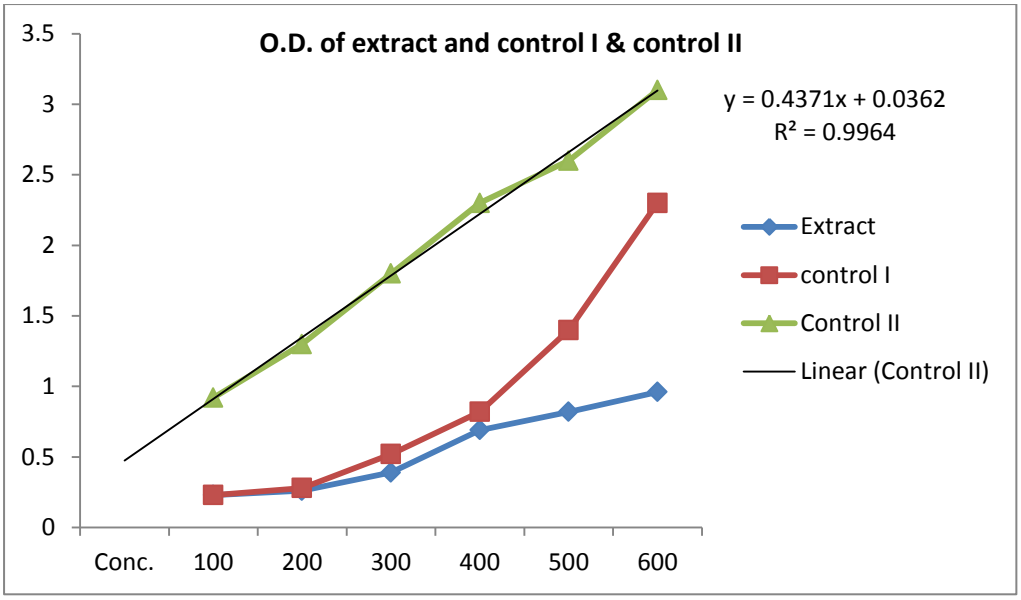

Figure 2: Absorbance graph of chloroform extract, Ascorbic acid and *α*-tocopherol

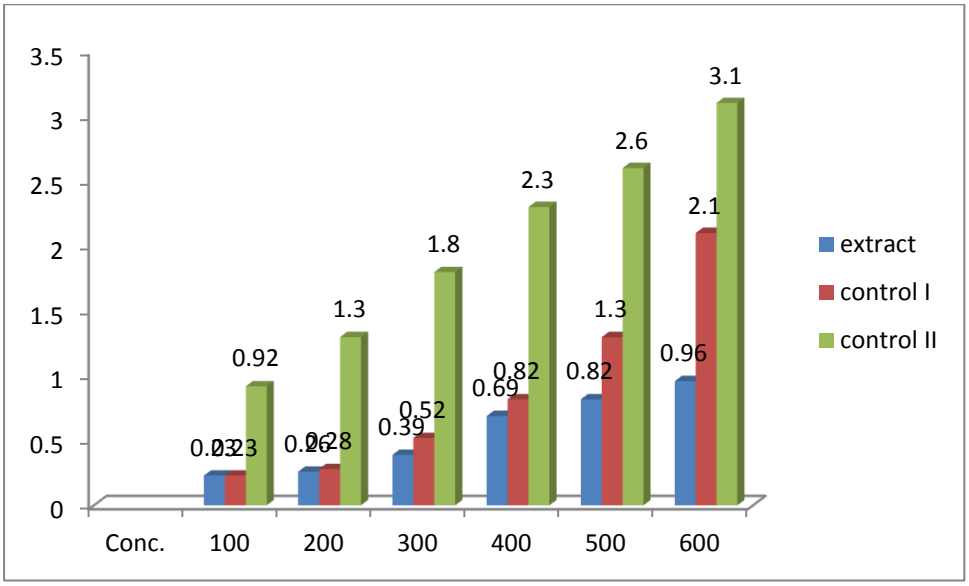

Figure 3: Effect of different concentrations of chloroform extract and controls on DPPH scavenging assay.

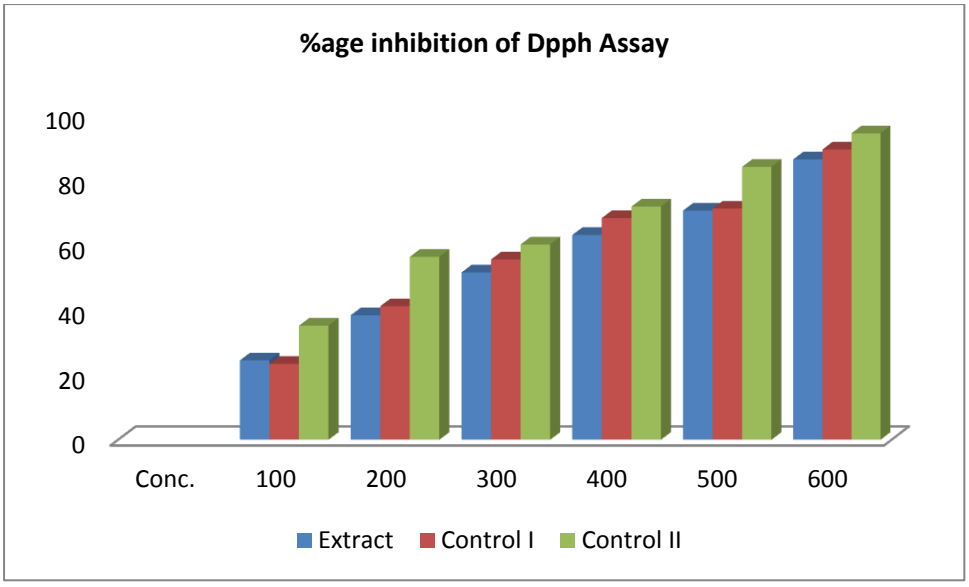

Figure 4: Histogram showing %age inhibition of DPPH assay of chloroform extract &controls at various concentrations.

3.6 Statistical Analysis

For all the tests, the mean values and standard deviations were calculated and data was analyzed using SPSS 16.0 statistical software. The one way analysis of variance (ANOVA) was applied for calculating the results. The means was compared by Duncan tests at level of significance of $P \leq 0.05$.

Result and Discussion

This study affirms that phthalic acid (21.51%), tetratetracontane (12.26%), dexoycelidoniol (11.53%), nonadecane (7.81%), dibutylphthalate (6.05%), stigmast-4-en-3-one (4.01%), 2-ethylhexylphthalate (3.24%), 2-methylphenylphthalate & cholest-4-en-3-one (1.49%) as the major constituents whereas the compounds dexoycelidoniol, dibuytylphthalate, 2-methylphenylphthalate, nonadecane was first identified in this extract which was absent in previous findings whereas methanolic extract of was analyzed by GC-MS contains n-hexadecanoic acid (12.083%), stigmasteron-4-en-3-one (10.183%), 1-mono-linolein (8.96%) and 9,12(Z,Z)-octadecanoic acid (8.054%) [7,8].

This study affirms that in-vitro antioxidant activity of chloroform extract of *Aconitum leave* had ability to reduce of stable radical of DPPH. The degree of decolouration indicates the scavenging potential of antioxidant as comparable to those of standard ascorbic acid and

vitamin E[19].Higher absorbance indicated higher reducing power. The present study indicated that the chloroform extract at concentration of 100µg/ml-600µg/ml ranges from 24.5% to 86.3% could play an important role in management of oxidative stress however, extracted polysaccharide from *Aconitum kusnezoffii* Reichb. had possessed *invitro*-antioxidant testing for ion-chelating ability, reducing power, and DPPH radical **(Table 3 & 4).** Thus, it was considered that extract of *A. laeve* had antioxidant activity against DPPH radical but low as compared to standard ascorbic acid and *α*-tocopherol.

The total phenolic contents of chloroform extract of *Aconitum laeve* were found to be 27.3 mg catechin/g and 22.5 GAE/g of dried weight of tubers. In plants the total phenolic compounds acts powerful chain breaking antioxidant due to their hydroxyl group present in them directly contribute towards antioxidant action. Thus, the tubers extract of *Aconitum laeve* could be useful and potent antioxidant in activity.

Conclusions

Aconites are poisonous plants that require care and caution. Its compulsory to mention, prior to their use in traditional medicines including BTM, aconite components are detoxified using region-specific traditional approaches, all of which are highly effective at reducing the toxicity of the plant by converting its diester diterpene alkaloids to their less toxic monoester diterpene forms[6]. This study showed that phthalic acid, tetratetracontane, dexoycelidoniol, nonadecane, dibutylphthalate, stigmast-4-en-3-one, 2-ethylhexylphthalate, 2-methylphenylphthalate & cholest-4-en-3-one as the major constituents whereas the compounds dexoycelidoniol, dibuytylphthalate, 2-methylphenylphthalate, nonadecane was first identified as the major components in this extract which was absent in previous findings of *Aconitum laeve*[7]. The DPPH scavenging effect at concentration of 100µg/ml-600µg/ml ranges from 24.5% to 86.3% and total phenolic contents of chloroform extract of *Aconitum laeve* were found to be 27.3 mg catechin/g and 22.5 GAE/g. Thus, we hope that the phytochemical investigation and antioxidant activity helps in coping management oxidative stress of our region.

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