Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle., a critically endangered medicinal plant of Kashmir Himalayas

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(MSF-2019-107)

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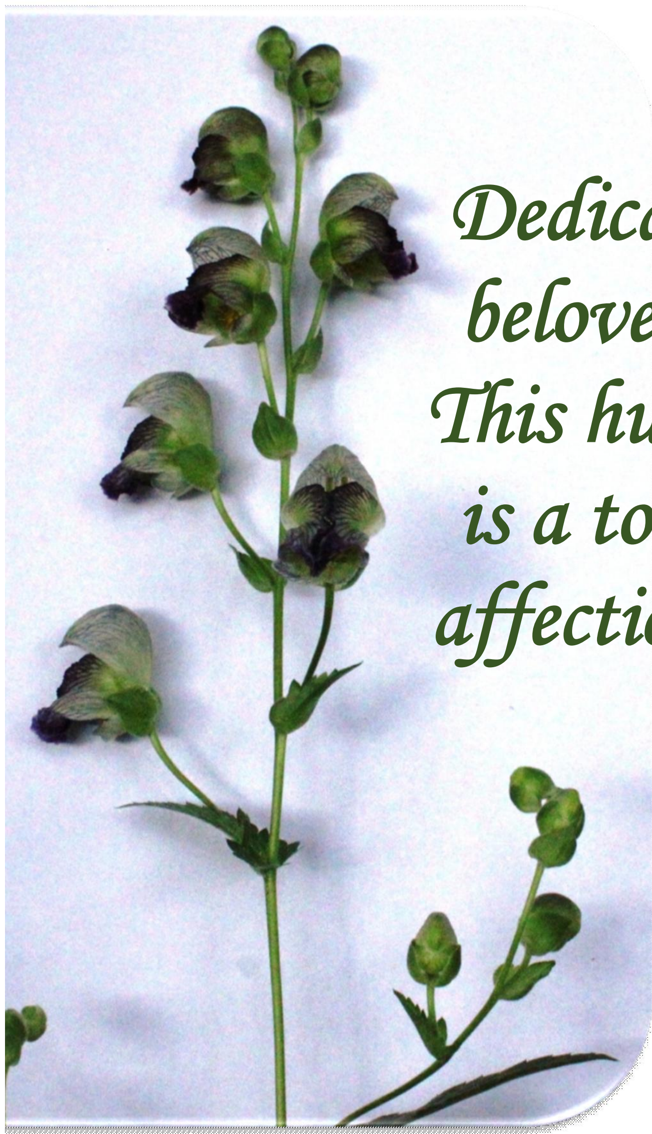

Dedicated to my beloved Parents
This humble work is a token of my affection for you!

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Forestry, Division of Forest Products and Utilization,
Benhama, Ganderbal

Certificate – I

This is to certify that the thesis entitled, **"Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle., a critically endangered medicinal plant of Kashmir Himalayas"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Forestry (Forest Products and Utilization)**, to the **Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Mir Muskan Un Nisa (Regd. No. MSF-2019-107)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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We, the members of the Advisory Committee of **Ms. Mir Muskan Un Nisa (Regd. No. MSF-2019-107)** a candidate for the degree of **Master of Science in Forestry (Forest Products and Utilization)** have gone through the manuscript of the thesis entitled, **"Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle., a critically endangered medicinal plant of Kashmir Himalayas"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of the degree.

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Certificate – III

This is to certify that the thesis entitled, **"Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle., a critically endangered medicinal plant of Kashmir Himalayas"** submitted by **Ms. Mir Muskan Un Nisa (Regd. No. MSF-2019-107)** to the **Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfilment of the requirements for the award of the degree of **Master of Science in Forestry (Forest Products and Utilization)** was examined and approved by the Advisory Committee and External Examiner on …………….. .

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ABSTRACT

The main purpose of this investigation was the **"Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle., a critically endangered medicinal plant of Kashmir Himalayas"**. The study was conducted in three forest divisions of north Kashmir i.e. Kamraj, Langate and Jehlum Valley falling in the districts Kupwara and Baramulla. This species is locally known as *'patris' i*n Haril and Boniyar, and 'mohra' in the Machil area. The natural populations of *Aconitum heterophyllum* were studied for morphological characters from selected sites in each forest division. Rhizopheric soil was collected from five randomly sampled plants for analysis of Physico-chemical properties, whereas, roots of the *Aconitum heterophyllum* plants were collected for the extraction and phytochemical characterization from each site. The naturally growing populations of *Aconitum heterophyllum* were found to be growing between 3200 to 3700 metres elevation across all sites in three forest divisions of North Kashmir. *Aconitum heterophyllum* is one of the most exploited species from the forests for trade and domestic use. The local communities across all the study sites use the tuberous root of the species for treating various ailments like stomach issues, arthritis, toothaches etc. The results of this study revealed that there is a significant intra-population variation in morphological characteristics such as plant height, leaf, flower, and tuber characteristics of this species. The plant height was found maximum (94.60 cm) in the Haril site of Langate Forest Division, followed by (28.5 cm) at JVFD. Leaf area was highest at LFD with (78.02) cm^2 followed by JVFD with (18.4) cm^2 and lowest was observed at KFD with (16.58) cm^2. Length of Floral axis was highest at LFD with (20.96) cm followed by (9.5) cm at JVFD and lowest at KFD with a mean value of (7.78) cm. Rhizospheric soil analysis revealed that the KFD has the maximum electrical conductivity (0.45 ds/m) and phosphorus (21.8 kg/ha), whereas LFD has the maximum organic carbon (2.3 per cent), nitrogen (473.81 Kg/ha), and potassium (487.46 kg/ha) with pH value (5.8). Methanol proved to be the most effective extraction solvent, followed by aqueous and petroleum ether respectively. The highest extraction yield was obtained from the tubers collected from LFD, followed by JVFD and KFD respectively. Alkaloids and flavonoids were found in the petroleum ether extract, whereas carbohydrates and saponins were found in the aqueous and methanol extracts of specimens collected from all the sites. GC-MS chromatogram of petroleum ether extract of the tubers of *Aconitum heterophyllum* (LFD) exhibited 10 peaks indicating the presence of a good number of compounds. 10 compounds matched the library and were identified accordingly. The results revealed that Ethoxyisoxazol-4-carboxylic acid (19.24 %) was a major compound. GC-MS chromatogram of methanol extract of JVFD reported 5 peaks specifying the presence of substantial compounds. About 5 compounds matched with the library and were recognised accordingly. The results revealed that 9,12-Octadecadienoic acid (Z, Z) (alpha-linoleic acid) (28.81 %) was the paramount compound. GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (LFD) exhibited 4 peaks. 1,2-Benzene dicarboxylic acid, bis (2-methyl propyl)ester was a major compound and GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (KFD) exhibited 1 peak only. 1,2-Benzenedicarboxylicacid, bis (2-methyl propyl) ester was the major compound. All compounds are known to possess a therapeutic potential and can be used by the drug industry. Hence, *Aconitum heterophyllum* naturally growing in the Haril area of Langate Forest Division (LFD) has a good quality of bioactive compounds and is the best germplasm for commercial cultivation as well as for conservation of this valuable species.

Keywords: *Aconitum heterophyllum*, morphology, phytochemistry, characterisation, GC-MS, bioactive compounds, Kashmir Himalayas

Signature of Student

Dated____________

Signature of Major Advisor

Dated _________________

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Mir Muskan Un Nisa

Place: Benhama, Ganderbal
Dated: ________________

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LIST OF ABBREVIATIONS

NMPB	:	National Medicinal Plants Board
WHO	:	World Health Organisation
LFD	:	Langate Forest Division
JVFD	:	Jhelum Valley Forest division
KFD	:	Kamraj Forest Division
GAPS	:	Good Agricultural Practices
GMPS	:	Good Manufacturing Practices
NPK	:	Nitrogen, Potassium, Phosphorous
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
FRAP	:	Fluorescence recovery after photobleaching
GC-MS	:	Gas chromatography–mass spectrometry
NMDS	:	Non-metric multidimensional scaling
SMs	:	Secondary metabolites
DCM	:	Dichloro methane
NLRP	:	Nucleotide-binding oligomerization domain
MIC	:	Minimum inhibitory concentration
OC	:	Organic carbon
HPLC	:	High Profile Liquid Chromatography
EC	:	Electrical conductivity
GAM	:	Generalized Additive Model
IHR	:	Indian Himalayan Region
MLPs	:	Malaysia's Permissible Limits
SOM	:	Soil Organic Matter
SR	:	Solar Radiation
TLC	:	Thin Layer Chromatography
NRLP3	:	NLR family pyrin domain containing 3

Chapter-1

INTRODUCTION

Plant-based medicine has played a significant role in maintaining human health since the dawn of civilization. The practice of an indigenous system of medicine is principally based on the preparations of herbal formulations used for therapeutic benefits from primitive times. The number of archaeological evidence supports the fact that plants were used by ancient people for treating different human as well as animal ailments. Plants found in the grave of Neanderthal man buried 60,000 years ago in Iraq and 'Ice Man' whose body was frozen in the Swiss Alps for more than 5,300 years were all of medicinal value. Herbal medicines were used mostly in their crude form as infusion, tincture and decoction or applied externally as a balm. However, from the late 19^{th} century onwards, scientists started isolation, purification and identification of bioactive compounds from medicinal plants, which led to the discovery of several valuable drugs that are being extensively used in the modern system of medicine. To name a few; morphine isolated from *Papaver somniferum* (opium poppy) is a powerful pain reliever and narcotic, taxol isolated from *Taxus brevifolius* (Yew) and vincristine isolated from *Catharanthus roseus* (Periwinkle or Sadabahar) are highly effective against certain cancers (Veeresham 2012; Koparde *et al.,* 2019). Even though there was a decline in the direct use of raw drugs during the late 19^{th} and early 20^{th} centuries, medicinal plants continue to play a pivotal role in the rural health care system of the developing world. World Health Organization reports that 60% population of the world and around 80% of the population of the developing countries depend entirely on herbal medicine for their primary health care needs.

Moreover, medicinal plants received worldwide attention due to their immense contribution to both regional and national economies. Subsequently, these products found a noteworthy place in the global development agenda of international organizations like the *Food and Agriculture Organisation* (FAO) and *World Bank* (WB) among others. Significant international commitments like *Millennium Development Goals* (MDG) and *Convention on Biological Diversity* (CBD) also recognized the potential of medicinal plants commercialization to achieve the dual goal of conservation and development. China and India are the principal producers, processors and consumers of medicinal plants in the world (Anonymous, 2002). The global herbal medicine market size is estimated at US$71.19 billion (Anonymous, 2017). With an annual growth rate of 15% to 25%, the demand for medicinal plants is likely to increase by more than US$5 trillion by 2050 (Anonymous, 2002). In India, about 1178 medicinal plant species are in trade, out of which 242 species have a demand of more than 100 metric tons/year. The consumption of the herbal raw drugs in India has been estimated at 5, 12,000 MT (during the year 2014-15) with a corresponding trade value of ₹ 5,500 crores. The export value has shown a record increase from ₹ 345.80 crores in 2005-06 to ₹ 3211 crores in 2014-15, registering a nine-fold increase during the last decade (Anonymous, 2018).

Despite such a glorious history and ever-increasing market demand, herbal medicine could not integrate fully into the modern health care system due to increasing concerns about poor quality control, sustainability of resources, lack of standardization, lack of pharmacological and clinical data on the majority of herbal products to ascertain their efficacy and concerns about toxicity. This sector has also suffered due to a lack of proper R&D on the authenticity of traditional healing practices and the efficacy of medicinal plants supplied through shady marketing channels. Nevertheless, the prospect of advancing research in herbal medicine has renewed the interest in the medicinal plant sector. The progress in quality control, regulatory and bioprospecting processes in many countries have revitalized the probability of integrating herbal medicine into the modern health care system. So many technologies have been developed to reap the magnanimous products that plants produce, like phytomedicine, natural dyes, biofertilizers, biopesticides and biofuel among others. Integrating traditional knowledge with modern scientific advancements may lead to standard clinical herbal therapy. Therefore, the medicinal plant's sector offers an exceptional investment opportunity for rapid and sustainable growth universally.

However, there are certain issues with regulation, standardization and quality assurance in the manufacturing of herbal medicine across the world, due to the complexity of their diverse secondary metabolites, which principally depend on genetic factors, age and geographical location of the plant species. The variability in phytochemical contents, as well as occurrences of adulteration, has an abysmal impact on uniform standards of herbal medicines. However, with careful implementation of Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) quality herbal medicines may be achieved. Moreover, for better healthcare and commercial benefits, these concerns have to be allayed by carrying out cutting edge research on the apparent shortcomings of herbal medications. There is an immense necessity for robust research programmes on the identification of quality germplasm and characterization of new chemical constituents, phytochemistry and pharmacological aspects of medicinal plants, to substantiate the claims of their efficacy and safety. Innovative approaches and insights into herbal medicine through cutting edge research and development will lead to the development of numerous traditional remedies and novel drug discovery systems. This will have an impact on modern medical sciences and shall help in informed decision making in the herbal medicine system.

The escalating commercial demand for medicinal plants for their therapeutic uses has led to the over-exploitation of many species from the wild, which has resulted in the loss of their natural populations. Consequently, several valuable medicinal plants from North India have been put under different categories of threat due to their over-exploitation and habitat degradation. Prominent medicinal plants from Kashmir, which are under certain categories of threat are: *Aconitum heterophyllum* Wallich., *Arnebia benthamii* Wallich., *Saussurea costus* (Falc.) Lipsch. and *Rheum emodi* Wall among others.

Therefore, there is an immense need for holistic research on medicinal plants, to rehabilitate these species and to identify high yielding Germplasm for mass cultivation. To initiate systematic cultivation of quality, germplasm has to be identified. In the case of wild plants, cultivation of medicinal plants and eventual returns from such cultivation is largely dependent upon the quality of planting material used. However, as of now, there is no such mechanism of providing authentic Germplasm or producing quality planting stock on large scale. Besides, there is an immense necessity for robust research programmes on the characterization of new chemical constituents and pharmacological aspects of medicinal plants, to substantiate the claims of their efficacy and safety. Innovative approaches and insights into herbal medicine through cutting edge research and development will lead to the development of numerous traditional remedies and novel drug discovery systems. This will have an impeccable impact on the modern medical sciences and shall help in informed decision making in the herbal medicine system.

Kashmir often referred to as a terrestrial valley, has nurtured various medicinal plants in the traditional healthcare system for thousands of years and is endowed with captivating biodiversity landscapes that contribute to its global reputation. Located phytogeographically at the roundabout of Paleotropical and Holarctic Floristic Domains and going down within the North-Western Himalaya, the province is endowed with an enormous diversity of medicinal plants (Rather and Baba, 2015; Peerzada *et al.,* 2021). Among which *Aconitum heterophyllum* has a prominent place, due to which its demand has escalated in local, national and international markets (Srivastava *et al.,* 2010, Peerzada *et al.,* 2021).

Aconitum is a large genus in the Ranunculaceae family with over 250 species, among which around 33 species are found in the Himalayas, from Afghanistan in the west to Myanmar (Burma) in the east. Aconites are also found in Europe and Asia, where they are employed in traditional and indigenous medical systems. Aconitum plants are rich in structurally complicated diterpenoid alkaloids with a wide range of therapeutic effects (Shen *et al.,* 2018). Since they are synthesized by amination of natural tetracyclic diterpenes, diterpenoid alkaloids are usually referred to as crypto/pseudo alkaloids (Wang *et al.,* 2002). One of the prominent species of aconites is *Aconitum heterophyllum.*

Aconitum heterophyllum Wall ex Royle is commonly called aconite, ativisha, atis, patrees, wolf's bane, devil's helmet, monkshood, women's bane and leopard's bane (krrtikar and Basu, 1975; Konda *et al.,* 2013 and Paramanick, 2017; Peerzada and Sofi, 2018). This species is distributed between 2400 to 4200 m altitude (Krrtikar and Basu, 1997) in the sub-alpine and alpine Himalayan zone. In India, the plant is generally found in Jammu and Kashmir, Himachal Pradesh and Uttarakhand (Nagaranjan *et al.,* 2015). Owing to significant pharmacological properties, overexploitation from forests due to increasing market demand, this species has been categorised as a critically endangered medicinal plant in the north-western Himalayas and is the sole non-poisonous member of the Ranunculaceae family (Wani *et al.,* 2021).

Aconitum heterophyllum is a perennial erect herb with about 30-90 cm height. The shoot is annual, while the root is biennial (Srivastava *et al.,* 2010). The stems are simple or branched from base, glabrous and puberulous above, broad, ovate or orbicular or 5-lobbed and toothed, above 3-fid or entire. The branches are absent or rarely one or two in number. This species flowers in the second year and the tuberous roots develop after two years of growth. Inflorescence terminal but sometimes axial. Flowers in racemes, 2.5 cm long, blue or greenish-blue with purple veins and helmet-shaped. Tuberous roots, in pairs, whitish or light grey up to 3 cm or more long, 0.5-1.2 cm thick with conical ends, break easily and taste bitter (Paramanick, 2017). The mother and daughter tubers occur in pairs. The flowering and fruiting in this species take place between July-October (Kirtikar and Basu, 1975). The seeds are pyramidal; 3-4 mm long, blackish brown with angles more or less winged (Kumar, 2018) and collected during October-November.

This species is considered the most important medicinal plant due to its high pharmacological value (Anonymous, 1989; Paramanick *et al.,* 2017). The identified phytochemicals in this plant comprise alkaloids, carbohydrates, proteins, saponins, amino acids, flavonoids, quinones, glycosides, terpenoids, etc (Balaramnavar, 2021). Tuberous roots contain a non-crystalline, non-toxic alkaloid called Atisine. Other alkaloids in this species are heteratisine, histine, heterophyllisine, heterophylline, heterophyllidine, atidine, F-dihydroatisine and hitidine. Aconitic acid, tannic acid, palmitic, stearic glycerides and vegetable mucilage are also present in addition to starch and sugars (Pelletier *et al.,*1968; Malhotra *et al.,* 2014; Rajakrishnan *et al.,* 2016).

The tuberous roots are used as antipyretic, analgesic, antiperiodic, aphrodisiac, astringent, anti-venom, anti-inflammatory, anti-rheumatic and vermifuge etc. It is reported to be useful in helminthiasis, haemorrhoids, haemorrhages, internal inflammatory conditions, malarial fever, dysentery, digestive, piles and reproductive disorders (Rastogi and Mehrotra, 1991; Sojitra *et al.,* 2013). The aqueous extract of the root induces hypertension through action on the sympathetic nervous system and in higher doses it becomes lethal (Rastogi and Mehrotra, 1991; Verma *et al.,* 2010; Sojitra *et al.,* 2013; Munir *et al.,* 2014; Kumar *et al.,* 2016).

In Kashmir, this species is mostly distributed across Langate, Kehmil, Jehlum Valley, Pirpanjal, Lidder and Bandipora forest divisions and is being extracted by local people for traditional use and income generation. The per kg (dry weight) cost of tuberous roots in Kashmir ranges between Rs. 4000- 6000/ kg (Peerzada *et al.,* 2018) with an annual demand of 200 to 500 MT (Anonymous, 2020) at the national level. The ever increasing demand for this species has led to its over-exploitation from the wild. Owing to its increasing demand, decreasing natural populations from forests due to anthropogenic pressures and seed dormancy (Beigh and Iqbal, 2008; Srivastava *et al.,*2010) the National Medicinal Plants Board (NMPB) has enlisted *Aconitum heterophyllum* as a priority species for the promotion of cultivation with 75% subsidy. Therefore, there is a great need for domestication and commercial cultivation of this species after proper morpho-chemical evaluation. The research on these aspects has not been considered well in Kashmir so far, due to limited focus on certain groups or regions (Rather and Baba, 2015). Given the above-mentioned facts, this study was designed to investigate the "Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle, a critically endangered medicinal plant of Kashmir Himalayas" with the following objectives:

Objectives:

1. Morphological Evaluation of *Aconitum heterophyllum*
2. Extraction and Phytochemical screening of the specimens

Chapter-2

REVIEW OF LITERATURE

The review of literature on this research work entitled "Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle, a critically endangered medicinal plant of Kashmir Himalayas" is presented in this chapter under the following headings:

2.1 Morphological evaluation of *Aconitum heterophyllum*

Orneno and Fernandez (2012) reported that terpenoid production (emanation and capacity) inside foliage plays immediate and indirect defensive capacities for the plant, intercedes complex trophic connections and controls the oxidation limit of the environment. They revealed that both biotic and abiotic conditions modify terpenoid content, with herbivory, light and temperature impacts being reasonable. They emphasized isoprene outflows and mono and sesquiterpenoid emanations and fixations as per preparing medicines and their possible connection with other ecological elements, natural, physiological, biochemical and biophysical theory formed over research examinations.

Aina *et al.* (2019) pre-treated various soil additives cow dung, chicken manure and (40 g / kg n: 60 g / kg n: 40 g / kg n) nitrogen phosphorus potassium in a shaded house on tomato seedlings. The results of their study showed that there is a strong relationship between the levels of major nutrients such as nitrogen, phosphorus and potassium in the soil and the levels of phenol, flavonoids, beta-carotene and lycopene in tomatoes, beta-carotene levels were significantly higher in fruits grown in soil supplemented with NPK ($p < 0.05$), significantly different from the results of other bioactive compounds such as phenol, flavonoids and lycopene. They revealed that tomato fruits from cow dung showed the highest levels of radical scavenging activity.

Zhou *et al.* (2021) reported that *Agriophyllums quarrosum* a pioneer plant native to Asia's temperate deserts has the potential to be domesticated into an excellent crop with superior ecological and therapeutic properties. They performed flavonoid-targeted metabolic profiling across 14 populations of *A. squarrosum* gathered from different elevations based on a common garden experiment to see if this accumulation was determined by environmental or genetic variables. They reported that Isorhamnetin (48.40 per cent, 557.45 g/g to be the most abundant flavonoid in *A. squarrosum*, followed by quercetin (13.04 per cent, 150.15 g/g), tricin (11.17 per cent, 128.70 g/g), isoquercitrin (7.59 per cent, 87.42 g/g), isovitexin (7.20 per cent. They revealed that based on a typical garden at a medium-altitude, nearly none of the flavonoids were shown to be more abundant in high-altitude communities, whereas other flavonoids, such as quercetin, tricin and rutin, were found to be much more abundant in low-altitude groups. According to them, the build up of flavonoids indicates that it was not due to local adaptation to high altitudes. Furthermore, in situ environmental variables revealed that the contents of quercetin, tricin and rutin were highly positively connected with latitude, longitude and precipitation gradients and negatively correlated with temperature gradients, according to association analysis. They disclosed that accumulations of flavonoids in *A. squarrosum* were more likely due to local adaptation to environmental heterogeneity paired with precipitation and temperature rather than high altitude

Ramaiya *et al.* (2021) reported that *Passiflora quadrangularis L.* is a member of the Passifloraceae family, which produces bigger fruits with an edible juicy mesocarp and pulp that is high in phytochemicals. They revealed that phytochemical contents of agricultural produce are known to be influenced by cultivation and plant management strategies. They reported antioxidant capabilities and secondary metabolite profiles of organically treated *P. quadrangularis* plants. In all experiments studied by them, leaves of *P. quadrangularis* grown organically had stronger antioxidant activity than those grown conventionally. They unveiled that organic gardening has also increased the antioxidant activity of the edible sections of the *P. quadrangularis* fruit. They demonstrated that the mesocarp had significantly higher total phenolic content and DPPH, while the pulp had significantly higher total flavonoid content, DPPH and FRAP. They revealed the presence of more vitamins and secondary metabolites in the samples. The secondary metabolites profile revealed by them shows that the mesocarps were high in phenolics, the pulps were high in flavonoids and the leaves had a good mix of phenolics, flavonoids and terpenoids with high antioxidant activity. 2-isopropyl-3-methoxy cinnamic acid (mesocarp and pulp), myricetin isomers (pulp and leaves) and malvidin-3-O-arabinoside isomers are all prevalent secondary metabolites for organically generated P. quadrangularis in various plant parts (pulp and leaves). They found that organically grown P. quadrangularis has increased antioxidant activity due to vitamins and secondary metabolites in the plant.

Hassan (2012) reported that plant metabolite biosynthesis (e.g. alkaloids, terpenoids, phenolic compounds, etc.) are largely controlled by genetics, environmental factors like stressors and nutritional elements which have an impact on metabolite production in plants. They unveiled that these metabolites are linked to a variety of biological functions and act as chemical defence agents against bacteria and herbivores, in addition to their medicinal properties. They revealed that the chemical characteristics of medicinal plants are linked to their therapeutic benefits and the chemical characteristics of these plants are an important determinant of their species uniqueness and pharmacological capabilities, allowing for their widespread usage in medicine and other applications.

Aye *et al.* (2019) studied the effects of soil and leaf nutrients on the compositions of *Siparuna muricata* essential oils (EOs) in four different Ecuadorian locations were studied for the first time. Gas chromatography/mass spectrometry (GC/MS) and a gas chromatography/flame ionisation detector (GC/FID) were used to evaluate the EOs obtained during hydrodistillation. They reported that by using GC/MS, the enantiomeric distribution of -pinene, limonene, element, bourbonene, cis-casino-1 (6), 4-diene and atractylone was found, with the enantiomeric separation of -pinene, limonene, limonene, limonene, limonene, limonene, Limon. They discovered a total of 44 chemicals. Guaiol, acetylone and 4-diene were the most representative for L1; cis-casino-1(6),4-diene and myrcene were the most representative for L2; myrcene and germacrene B were the most representative for L3; and germacrene B, myrcene and cis-casino-1(6),4-diene were the most representative for L4 germacrene B, myrcene and cis-cadina-1(6),4-diene. They discovered that the chemical composition of the essential oil of *Siparuna muricata* was significantly correlated with soil-leaf chemical elements such as Al, Ca, Fe, Mg, Mn, N and Si at different sites; however, correlations between soil and leaf K, P and Na were not significant. Cluster and NMDS analyses revealed substantial secondary metabolite dissimilarity values between four locations linked to variations in soil-leaf nutrients.

Kliemiene *et al.* (2021) reported how soil pH, humus, total nitrogen (Total), plant-available/mobile phosphorus (P2O5), potassium (K2O) and magnesium (Mg) concentrations affected total phenolic, flavonoid and extractive content in *Origanum vulgare L.* extracts. They unveiled that the extractive content was connected with the soil humus, N total and Na; the flavonoid content was correlated with soil P2O5, K2O, Mg and Na.

Pant *et al.* (2021) reported that secondary metabolites (SMS) help in the assessment of the quality of therapeutic ingredients and are today used as important natural medicines such as immunosuppressants, antibiotics, anti-diabetic and anti-cancer drugs. They revealed that plants can synthesize a variety of secondary metabolites to cope with the adverse effects of stress and individual environment variables like temperature, carbon dioxide, lighting, ozone, soil water, soil salt and soil fertility which are significant for the physiological and biochemical reactions of medicinal plants, as well as secondary metabolic activities.

Nautiyal *et al.* (2009) studied floral biology of *Aconitum heterophyllum*. They studied the implication of different breeding systems for its successful domestication and improvement in cultivation practices on floral biology, pollen germination, pollination, fruit and seed. It was reported that the plants grown in hothouse conditions showed considerable variation in the production of flowers and seeds. Controlled pollination in this species was reported to be self-incompatible, although few fruits developed from selfing. Such fruits were reported to be smaller than the fruits produced by open-pollinated and from hand-crossed flowers and most aborted early in development.

Pandey *et al.* (2005) studied ex-situ conservation of *Aconitum heterophyllum* Wall. A Himalayan medicinal plant is in danger of extinction due to its massive spread and its effects on growth and alkaloid content. They revealed that when endangered alpine plant *Aconitum heterophyllum* was conserved under ex-situ conditions (greenhouse and open) of Palampur by overcoming seed dormancy and plant establishment through hot water treatment (40–60 °C for 30–120 s) of seeds high seed germination (88 and 76%) was obtained when seeds were treated with hot water at 45 and 50 °C for 90 s. They reported that correlation studies of vegetative growth and reproductive yield of the plants were treatment enhanced. The roots and rhizomes of the plants growing under ex-situ conditions was having total alkaloid content lower than the ones collected from the natural habitat in the 1-year-old plants as compared to 2-year-old plants was almost at par with the ones collected from nature as reported by them. As they demonstrated, the life cycle of plants growing in ex-situ conditions was similar to that of plants growing in situ conditions. Their research revealed that *A. heterophyllum* plants can successfully adapt to settings outside of their normal environment, suggesting that it has the potential for long-term commercialization.

Guleria *et al.* (2013) studied habitat protection of 4 important medicinal plants including *Aconitum heterophyllum* by screening and cultivation of promising germplasm. It was reported that the conservation, habitat protection, cultivation is optimizing yield parameters for increased productivity and demonstrated to be the long term plan of conserving and protecting the important high-value drug plants such as *Valeriana jatamansi, Pricrorhiza kurrooa* species and *Aconitum heterophyllum.* The survival percentage of *Valeriana jatamansi* species was reported to range from 80-90 per cent followed by the survival percentage of *Picrorhiza kurrooa* species which ranged from 71.33-80.00% and the survival percentage of *Aconitum heterophyllum* ranged from 67-68%.

Jeelani *et al.* (2016) studied cytology, morphology and ecology from 21 natural populations of *Aconitum heterophyllum* in Kashmir and Ladakh Himalayas. They reported a high variability among various morphological characters including plant height, foliage, floral and tuber attributes at intra-population levels. They revealed that the number of flowers varied from 9 to 11 and was directly linked with altitude. All the populations were diploid (2n = 16) chromosomes their study depicted.

Gaira *et al.* (2011) studied the phenological pattern of *Aconitum heterophyllum*, a high-value medicinal plant native to the Indian Himalayan Region (IHR), a worldwide hotspot that is known to be vulnerable to climate change. 117 herbarium specimens of the species were gathered from three provinces in the region (Western Himalaya, North-West Himalaya and Trans Himalaya) by them. Because of the intricacy of the herbarium-based data structure, historic herbarium records (1848–2003) were studied by them to forecast blooming patterns using the Generalized Additive Model (GAM). The blossoming time responded strongly, according to GAM, with 26 days sooner per 1,000m (P\0.02). Similarly, throughout the previous 100 years, the model predicted much early blooming (17–25 days) (P \ 0.01). Furthermore, they reported that the greatest winter temperature (December–February) explains the rise and increase of 1°C in the mean winter temperature influenced the early blooming period (19–27 days) at both elevations (lower and mid) and the highest winter temperature (December–February) described increasing trends at both elevations (lower and mid). They revealed that in general early flowering of *A. heterophyllum* could be interpreted as a climate change indication; however, more herbarium records are needed to confirm this hypothesis.

Bhatt *et al.* (2014) studied phytosociological features and threat categorization of *A. heterophyllum* Wall. ex Royle and *A. ferox* Wall. in Kumaun Himalaya. It was reported that poor availability of the species in the study area indicates low population density across the surveyed populations and restricted distribution to specific pockets, there is a threat to the existence of this species due to exploitation. Their study clarified the population structure of identified aconite species in Kumaun Himalaya is endangered.

Bahuguna *et al.* (2013) studied quantitative enhancement of active content and biomass of two Aconitum species through suitable cultivation technology They reported that High altitude medicinal plants are facing problems due to their unsustainable utilization. They reported that the cultivation of these plants with appropriate technology may fulfil the demand of the pharmaceutical industry and may also promote the conservation of their natural habitat. They observed very positive results under greenhouse conditions yield enhancement was 12 to 9 times high, Pseudoaconitine and aconitine were also believed to be high (0.51% and 0.42% respectively) than naturally grown plants (0.49% and 0.40% respectively) similarly quantity of atisine and aconitine was also found high (0.35% & 0.27% respectively) in the greenhouse than naturally grown plants (0.19% & 0.16% respectively), leaf number, plant height and the average length of tubers were high in plants grown inside a greenhouse in comparison to natural habitat.

Ali *et al.* (2021) employed an ethnobotany-directed approach to study the conservation status of three Aconitum species. Their phytochemical profiles and biological properties were screened under in vitro conditions by them. Folin ciocalteu and Aluminium chloride assays were procured to measure their total phenolic and total flavonoid contents, respectively. Plant extracts were assessed for antioxidant, antimicrobial and anti-inflammatory activities. They looked at the conservation status of three Aconitum species: *Aconitum heterophyllum* Wall. ex Royle, *Aconitum violaceum, Jacquem. ex Stapf and Aconitum chasmanthum, Stapf.* ex Holmes in Kashmir Himalaya. The total phenolic and flavonoid content of aconitum extracts varied significantly. The antioxidant activity of *Aconitum chasmanthum* methanolic extract was comparatively higher (80.115%). *Aconitum chasmanthum* DCM & methanolic extracts showed a good MIC value of 0.125 mg/ml against Candida albicans and Streptococcus pyogenes, respectively. The per cent inhibition of NLRP3 inflammasome was found significant in *Aconitum violaceum* ethyl acetate extract (74.61%). They revealed that Aconitum species are constantly declining at least in investigated habitats of Kashmir Himalaya and hence need strategic conservation planning. Their findings also highlighted the Aconitum species potential as an antioxidant, antibacterial and anti-inflammatory agent that might be utilised to treat a variety of ailments.

Haruna and Yahya (2020) revealed that bioactive chemicals produced from plants and microbial sources are essential for human existence and ground breaking research in this area must continue. They reported that plants and microorganisms are the primary sources of naturally occurring bioactive compounds, which are used in a variety of biotechnological applications. They unveiled recent advances in the disciplines of bioactive chemicals and soil chemistry in agriculture which have paved the way for the development of effective medications to treat both human and plant ailments. They reported that soil offers a growing substrate for medicinal plants, but pollution has a significant impact on the quality of pharmaceuticals, food crops and other critical ingredients contained in plants that provide body power. They revealed that scientists and the pharmaceutical sector have been drawn to this field to generate stronger medications from medicinal plants growing in various soils. They have shown the influence of many parameters and soil characteristics, such as heavy metals, pH, soil organic matter and the phytoremediation process, which have provided a measure of some quality dependency of soil generating secondary metabolites and soil containing bacteria. They provided the data which will be important in determining the activity of bacteria and their interactions with soil and all real plants that produce medications. Their study discusses several active chemicals found in plants and microorganisms, as well as their features and uses. The implications of soil particle size, dispersibility and stability on soil microorganisms, their activities and their relationships The impact of soil particle size, dispersibility and stability of microorganisms in the soil and the prospects for the production of new active chemicals in the soil have all been described by them.

Zahidah *et al.,* 2021 worked on *Phyllanthus niruri* biomass output, bioactive chemicals and heavy metal concentration. They studied different rates (0, 100, 200, 300 and 400 kg N/ha) of two types of organic fertilisers (animal-based and plant-based). They reported that Plant-based fertiliser produced the highest fresh biomass and was under Malaysia's Permissible Limits (MPLs) and because of the lower heavy metal content in plants and increased Phyllanthus and hypophyllanthin output, animal-based fertiliser at 400 kg N ha-1 was chosen as the best rate by them.

Gairola *et al.* (2012) studied the chemical characteristics of soils and the structure and composition of forests. Twelve forest types were chosen based on height, slope aspect and species composition by them. Chemical properties of the soil, such as total nitrogen (N), available phosphorus (P), organic carbon (C) and soil organic matter (SOM), were investigated by them. They reported that C and K had a positive relationship with N and P had a positive relationship with C and a negative relationship with altitude. Also, P levels were greater in the soil of all forest types' lower horizons. K was discovered to have a positive relationship with altitude, C and N. they revealed that all of the forest types had an acidic pH. C was found in greater concentrations in mixed broad-leaved forest types by them. As reported by them there was no correlation between altitude and C, which might be attributable to the variable composition of forest types along the altitudinal gradient, as well as their varying decomposition rates. The amount of carbon in the soil reduced as it was dug deeper. C had a positive relationship with N, P and K. N had a positive association with Growth per hectare, K had a negative relationship with SR and H had no interaction with the phytosociological parameters. Their research also compares the results of the present study's chemical analysis to several other earlier investigations conducted in Uttarakhand's temperate Himalayan area. They unveiled that in the majority of the forest types studied, the current study's soil chemical characteristics are greater than those previously observed for other comparable forests in the region.

2.2 Extraction and phytochemical screening of plant specimens

Hashim *et al.* (2015) studied preliminary phytochemical studies which were performed on *Strychnosnux vomica* seeds and the existence of the species was verified using the thin layer chromatography (TLC) technique. By Using the solvent system toluene:chloroform: methanol in the ratio 8:2:1, the TLC profile of the methanolic extract of seeds of *S. nuxvomica* was established by them. After spraying with Dragendorff's reagent, the plate was examined by them under visible light (specific method). The reported proportion of strychnine in the seeds seems to be 0.36 per cent (w/w) according to a high-performance liquid chromatography (HPLC) profile of the methanolic extracts of *S. nuxvomica*. The TLC and HPLC profiles created are quite useful for distinguishing genuine medication from its adulterants. They unveiled the presence of strychnine in the plant material is determined by the TLC profile. As revealed by the method for determining the amount of strychnine in the seeds can be utilised for quality standards of the raw drug.

Umorya *et al.* (2017) determined the phytochemical potential of Aconitum ferox roots. with a voucher Specimen no. 316/Bot/Safia/12, the plant was collected, identified and validated by botanist Dr Zia Ul Hasan at the Department of Botany, Safia Science College, Bhopal (MP). They used the soxhlet apparatus to extract it using a variety of solvents, including water and ethanol. They performed chemical assays for their ability to identify a variety of components, including alkaloids, glycosides, tannins, resins, steroids, carbohydrates, flavonoids, proteins and amino acids are just some of the compounds found in plants. The outcome of their research confirmed the existence of the aforesaid elements. They proposed that more study is being done to separate the chemical elements that are responsible for the desired action.

Nengroo *et al.* (2021) reported analgesic, antipyretic, diuretic, carminative and other therapeutic activities. They revealed the fatty acid content, antibacterial and antioxidant capabilities of *A. heterophyllum* seed and root extracts were investigated in this work. The fatty acid content of petroleum ether extracts of seed (PPS) and root (PPR) in the form of FAMEs was determined by using gas chromatography-mass spectroscopy. They revealed that each PPS and PPR had five different fatty acids and linoleic acid was identified in abundance in both PPS and PPR, accounting for 74.28 per cent in PPS and 33.60 per cent in PPR. They unveiled the antioxidant activity of the extracts which was tested using the radical scavengers 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). As reported by them all of the extracts with methanol had good antioxidant activities in the radical scavenging assay and with methanol seed, PMS and root PMR extracts having the maximum activity at 200 g/mL concentration. The extracts were further tested by them for antimicrobial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* and all of them showed outstanding antibacterial activity. Based on their findings, this plant might be employed in the food, pharmaceutical and nutraceutical sectors after further modification.

Ukani *et al.* (1996) studied the pharmacology of *Aconitum heterophyllum* used as an ayurvedic herb known for its medicinal properties. It was revealed that the roots of the plant are found to be used in one form or the other in various ayurvedic preparations. They disclosed that it possess activities like antimalarial, anti-inflammatory, antidiabetic, diuretic etc.

Singh *et al.* (2015) investigated the presence of phytochemicals such as alkaloids, carbohydrates, protein, amino acid, cardiac glycosides, phenols, flavonoids, saponins, terpenoids and quinones in extracts from the leaves, roots and stem of *Aconitum heterophyllum*, a rare medicinal plant from the Himalayan region. They claim that methanol extracts of leaves, roots and stems do not demonstrate the presence of Glycosides. They also demonstrated that TLC profiling of all plant extracts gave them an idea regarding the existence of these phytochemicals. Rf (Retention factor), as described by them, depicts the worth of various phytochemicals and guides their polarity and solvent selection for phytochemical separation.

Pellitier *et al.* (1968) isolated seven new diterpene alkaloids from *Aconitum heterophile*. Apart from heteratisine 3 weak bases, they yielded compounds namely heterophyllisine and heterophyllidine these compounds resembled lactone alkaloids of heteratisine. Their study on strong base fraction resulted in two new reduction production of cuisine and isoatisine namely atidine and dihydroatisine. Very strong base fraction yielded transformation products of hetisine namely hetidine and hetisinone.

Aneja *et al.* (1973) reported that heteratisine is a diterpene lactone alkaloid occurring as mono benzyl ester in roots of *Aconitum heterophyllum* whose structure is highly related to highly toxic and polyoxygenated alkaloid of lycoctonine aconitine rather than its companion bases atisine, atidine and hetisine.

Mathew *et al.* (2021) studied pharmacognostic and phytochemical properties of *Aconitum heterophyllum* root by Macroscopy, microscopy, powder analysis, physicochemical standardisation and preliminary phytochemical screening of *Aconitum heterophyllum* roots. Anatomical examinations of the tuberous roots of *Aconitum heterophyllum* revealed a prominent endodermis, a large zone of secondary phloem and a ring of 4 to 9 vascular threads, according to them. Powder analysis revealed the existence of suberized metaderm cells, reticulate xylem arteries and glucose grains in their research. *Aconitum* *heterophylluym* physicochemical standards were determined, including moisture content, ash levels and extractive values.

Verma *et al.* (2010) reported that *Aconitum heterophyllum* can reduce the weight of cotton pellet in rats thereby showing anti-inflammatory effects and proving that effects are the same as that of diclofenac sodium, a standard anti-inflammatory drug.

Prasad *et al.* (2012) undertook physicochemical standardisation and assessment of *Aconitum heterophyllum* in-vitro antioxidant activity. According to their quantitative findings, the root is abundant in alkaloids, whereas phenols, tannins, flavonoids and saponins are detected in fewer amounts. All of the models in the in-vitro antioxidant research showed moderate to low activity, which might be related to poor phenolic and flavonoid content.

Jabeen *et al.* (2011) used a simple reversed-phase HPLC-UV-DAD approach to estimate the bioactive component aconitine in two aconitum species (*Aconitum chasmanthum, Aconitum heterophyllum*). Peak heights were shown to be linearly related to aconitine concentration, with a correlation value of >0.999. This test is both quick and repeatable. The extraction process presented yields a high yield of aconitine, a marker chemical that is otherwise difficult to extract.

Jaiswal *et al.* (2013) studied the traditional Indian Shodana and Chinese processing methods of aconite roots by characterization and determination of components, using performance liquid chromatography and quadrupole time flight mass spectrometry. Results of their study revealed that all three methods used in Ayurveda and TCM can productively yield the diester alkaloids and can lead to their conversion into monoester diterpenoid alkaloids hence proving that this method can be used to detoxify aconite roots TCM being the most effective method.

Adams *et al.* (2013) compared *Aconitum het*erophyllum to its alternatives in research that included the creation of pharmacognostic and phytochemical features. They used conventional techniques to conduct histology and phytochemical studies. Histochemical investigations revealed the presence of alkaloids, terpenoid-alkaloid complexes, lipids and calcium.

Konda *et al.* (2013) tested the hepatoprotective effect in Wistar albino rats, of an ethanolic extract of *Aconitum heterophyllum* root in paracetamol-induced liver injury. They assessed the hepatoprotective effect of an ethanolic extract of *Aconitum heterophyllum* root using biochemical markers such as SGOT, SGPT, ALP, total bilirubin, serum protein and liver histological examinations. The Ethanolic extract of the *Aconitum heterophyllum* root effectively decreased liver damage and all biochemical markers, according to the research..

Malhotra *et al.* (2016) discovered probable genes involved in tuberous root formation. GDP-MANNOSE, pro-phosphorylase shaggy, expansin, ring box protein, SRF receptor kinase beta-amylase, ADP glucose phosphorylase and oxen responsive factor 2 are eight of the eighteen genes. These have been found in greater quantities in the root system than in the shoot system. The transcript has been shown to have 11 to 97 folds in different phases of root growth.

Munir *et al.* (2014) investigated *Aconitum heterophyllum's* antifungal and antioxidant properties The extract's in-vivo antifungal activity was established by measuring inhibitory zones against *Aspergillus niger*, a fungus that causes black mould and *Alternaria solania,* a fungal pathogen that causes illness in potatoes and tomatoes. In the case of both species examined, the methanolic extract of *Aconitum heterophyllum* was found to exhibit significant antifungal activity. The antioxidant activity of the extract was evaluated using a radical scavenging technique.

Sinam *et al.* (2014) confirmed that alkaloid extract had antibacterial action against several bacterial species, including *S. aureus, B. bronchiseptica, B. subtilis, P. putida and X. campestris.* According to them, the antibacterial action of all alkaloids from the root was due to a synergistic impact of diverse alkaloids.

Khurshid *et al.* (2015) discovered the clinical and therapeutic potential of *Aconitum heterophyllum*. They uncovered alkaloids, flavonoids, diterpenoid and non-diterpenoid substances in *Aconitum heterophyllum* and described them using chromatographic separation techniques. The structure was investigated using the nuclear magnetic resonance method. Cotton pellet produced granuloma in rats triggered the anti-inflammatory effect of ethanolic root extract of *Aconitum heterophyllum.*

Rajakrishnan *et al.* (2016) used physio-chemical screening, HPTLC fingerprinting and GC-MS analysis to standardise root tubers of *Aconitum heterophyllum*. They discovered the presence of alkaloids, sugars, flavonoids, steroids, quinones and tannins in the initial phytochemical test using a pharmacopeial testing methodology that included powder microscopy. The GC-MS analysis of the diethyl ether fraction revealed the presence of 39 chemicals, 21 of which they identified.

Nagarajan *et al.* (2015) examined the pharmacology of *Aconitum heterophyllum* as well as the other three species. According to the ayurvedic categorization of dravyaguna, Ativisha and Musta have similar biological qualities. Modern pharmacological research support this, demonstrating that both *Aconitum heterophyllum* and *C.rotundus* exhibit, anti-pyretic, anti-diarrheal, anti hyperlipidemic, anti-inflammatory and hypolipidemic effects.

Budhadev *et al.* (2017) characterized phytochemical constituents of *Aconitum heterophyllum* with chromatographic separation technique and their structures were described by using nuclear magnetic resonance techniques. The plant possessed anti-inflammatory activity which was assessed by using cotton pellet granuloma.

Paramanick *et al.* (2017) studied phytochemistry and pharmacognosy as well as the medicinal properties of *Aconitum heterophyllum*. They reported that *Aconitum heterophyllum* has been utilized in the traditional healing system of

India (Ayurveda). It was reported to have been used in treating patients with urinary infection, diarrhoea and inflammation. The plant has been also used as an expectorant for the treatment of hepatoprotective activity. The chemical studies of plants bring to light that they contain alkaloids, saponins etc.

Chapter- 3

MATERIAL AND METHODS

The details of study sites and material and methods adopted during this study are provided in this chapter under separate headings.

3.1 Study sites:

The field data and species specimen for experimenting as per the approved technical programme were collected from three different sites i.e. Machil area of Kamraj Forest Division (KFD), Haril area of Langate Forest Division (LFD) and Boniyar area of Jhelum Valley Forest Division (JVFD) of the Kashmir, India (Figure 1).

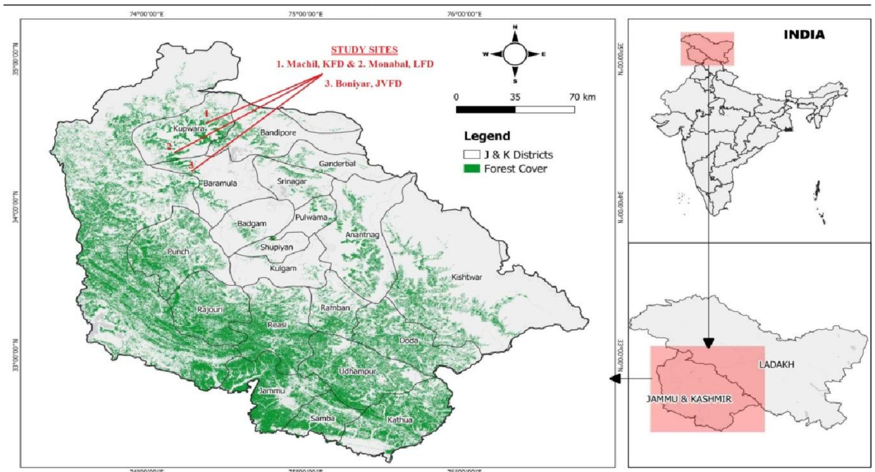


Figure 1: Location map of study sites

Morphological evaluation of *Aconitum heterophyllum*

3.2 Field investigation

To study the morphological characteristics of the species, field surveys were conducted across three different sites Machil, Haril, Boniyar, falling in the selected forest divisions Kamraj, Langate and Jhelum Valley respectively. The salient features of each study site were recorded (Table 1) following Aslam *et al.,* 2015.

Plate 1: Visit to sites and interaction with forest officials, tribals

Table 1: Salient features of study sites and investigated populations of *Aconitum heterophyllum*

Source	Collection date	Common Name	Ethnobotanical Information	Latitude	Longitude	Altitude (m)	Aspect	Habitat
Machil, KFD	27/07/21	Mohra	Used to treat gastric disorders	34°34’26”N	74°17’55”E	3778	Nort-East	Open slopes
Haril, LFD	05/08/21	Patris	Paste of dried roots in mustard oil is applied to joints to cure arthritis	34°15’22”N	74°07’52”E	3291	East	Open slopes
Boniyar, JVFD	13/08/21	Patris	Used to treat tooth aches and Body vigour	34°06’19”N	74°12’41”E	3500	East-West	Rocky open slope

3.3 Experimental design

One-way ANNOVA analysis has been used to know the effect of sources and for comparing the statistical differences among means of three different sites, R software was used for analysis.

3.4 Morphological evaluation

Following types of equipment were used for the onsite recording of the data on morphological characters:

1. Measuring scale
2. Measuring tape
3. Graph paper
4. Colour chart
5. Pocket Lens
6. GPS

The qualitative and quantitative data of morphological characters were recorded from five randomly selected plants at each site, following Jeelani *et al.* (2016).

3.4.1 Plant height (cm):

Plant height was measured with the help of a measuring scale. Plant height was measured by placing a measuring scale at the base up to the top of the plant.

3.4.2 Leaf length (cm):

By using a ruler the length of each leaf was measured from the point at one end to the point where the leaf joins the stalk

3.4.3 Leaf width (cm):

Leaf width was measured by using a ruler.

3.4.4 Leaf area (cm^2):

Leaf area was measured by using leaf area meter and graph paper method.

3.4.5 Length of the floral axis (cm):

The length of the floral axis was measured by using a ruler.

3.4.6 Flower colour:

Flower colour was observed by using a colour chart.

3.4.7 Sex of flower:

The sex of the flower was determined by using the pocket lens.

3.4.8 Tuber biomass (g):

Root biomass was measured by using the weighing machine.

3.4.9 Tuber length (cm):

Tuber length was measured by using the ruler.

3.4.10 Tuber thickness (cm):

Tuber length was measured by using the vernier clippers.

3.5 Soil analysis

The rhizospheric soil samples were collected from the five randomly selected plants for the recording of morphological characters at each site, following the methodology used by (Gairola *et al.,* 2012). Chemical properties of soil i.e; available nitrogen, available phosphorous, available potassium, organic carbon, pH and Electrical conductivity were determined as under.

3.5.1 pH:

The pH of the soil samples collected from the root zone of plants was measured by following the water suspension method (Rayment, 1992).

Apparatus used

1. pH meter, pH electrode and mechanical stirrer

Reagents used

1. pH buffer solution

Procedure

1. A suspension of soil and water was prepared at a ratio of 1:5 and shook for 1 hour at 15 rpm mechanically.
2. The pH meter was calibrated by using a buffer solution.
3. The electrode was submerged in the soil suspension and stirred with a mechanical stirrer; pH value was obtained when the equilibrium was reached.

3.5.2 Electrical conductivity

A soil and water suspension of 1:5 ratio was measured with a conductivity cell to determine the electrical conductivity (EC), following Rayment (1992).

Reagents used

1. Water that has been distilled or deionized
2. Reference solution

Apparatus used

1. Conductivity meter and cell.
2. Shaking bottles.

Procedure

1. By weighing 10 g air-dry soil, 1:5 soil: water suspension was made.
2. To acquire the cell constant, calibrate the conductivity metre using the KCl reference solution.

3. The cell was thoroughly rinsed. At the same temperature as the soil suspensions, the electrical conductivity of KCl was measured
4. The soil suspension was rinsed using a conductivity cell. The conductivity cell was refilled while being careful not to disrupt the settled dirt. The value displayed on the conductivity metre was noted down. Between samples, the cell was rinsed with deionized water.

3.5.3 Organic Carbon

The Walkley-Black chromic acid wet oxidation method was used to determine soil organic carbon.

Apparatus used

1. A pipette (or syringe pipette) with a 10 mL capacity.
2. For dispensing concentrated sulphuric acid, a measuring cylinder or syringe pipette with a capacity of 20 mL.
3. Erlenmeyer flasks with a capacity of 250 mL
4. A thermometer set to 200 degrees Celsius.
5. A titration unit with a 50-mL burette
6. A volumetric flask with a 1000 mL capacity.
7. A volumetric flask with a 100 mL capacity

Reagents used

1. Potassium Dichromate
2. Orthophosphoric acid
3. Diphenylamine
4. Ammonium ferrous sulphate

Procedure

1. 5 g of soil was put in a beaker
2. 10 ml potassium dichromate was added to this soil sample
3. Then 20 ml of H_2SO_4 was added to the sample.
4. 10 ml distilled water was added to each sample
5. 5-6 drops of diphenylamine was added as an indicator
6. Then titration process was done

The carbon percentage was calculated as follows:

$$0.003\ g \times N \times 10\ mL \times (1-T/S) \times 100\ /ODW$$

$$= 3(1-T/S)\ /W$$

Where:

N = $K_2Cr_2O_7$ solution normality

T is the volume of diphenylamine utilised in the titration of the sample (mL)

S refers to volume of H_2SO_4 utilised in the blank titration (mL)

ODW stands for Oven-Dried Sample Weight (g)

Available nitrogen, phosphorus and potassium were measured by using Mridaparishak (mini lab) following the lab manual.

3.6 Extraction and Phytochemical Screening

Three different solvents in increasing order of polarity were used for the extraction of collected plant material from three different sites.

3.6.1 Collection of sample

Materials used

1. Khurpi
2. Cutters
3. Herbarium sheets

Procedure

Tuberous roots of *Aconitum heterophyllum* were collected from three different forest divisions viz; Langate, Kamraj and Jhelum valley During August-September. The mature plants of this species along with tuberous roots were collected from the wild for conducting experiments in the laboratory on the extraction and chemical characterisation. Herbarium sheets were also prepared following Jain and Rao (1985) to ensure proper identification and authentication of the species before conducting laboratory experiments. The herbarium sheets were submitted to the Botany Department of Kashmir University and voucher specimen numbers 3742-KASH for Langate Forest Division, 3743-KASH for Kamraj Forest Division and 3744-KASH for Jhelum Valley Forest Division were collected.

3.6.2 Sterilization of plant specimens

The tubers collected from each site was cleaned, washed with water and air-dried properly for about 5 days. The tuberous roots were then sterilized with Mercuric Chloride ($HgCl_2$) @ 0.1 % concentration for 4 minutes following Srivastava *et al.* (2010).

3.6.3 Extraction

Types of equipment and chemicals used

1. Mortar pestle
2. Grinder

3. Weighing machine
4. Soxhlet apparatus
5. Petroleum ether
6. Methanol
7. Distilled water
8. Vacuum evaporator
9. Thimble
10. Whatman’s filter paper

Procedure:

Using a mortar pestle and grinder, the prewashed, dried and sterilized sample material was coarsely grounded. The powdered material was weighed in total at 180g and 20g was put in a soxhlet apparatus at 40-60°C and extracted with 250 mL petroleum ether (60°-80°C), methanol and macerated for 3 days in 250 mL water to make an aqueous extract. Following that, different extracts were filtered using Whatman's filter paper and the filtrate (petroleum ether and methanol) was evaporated using a vacuum evaporator under decreased pressure and regulated temperature, while the aqueous extract filtrate was evaporated under natural circumstances. After evaporation, the crude extract was preserved in desiccators. The leftover root residue was removed after extraction and the extract was weighed following Rasve *et al.* (2018). The physical properties of several extracts, as well as their percentage yield, were documented. Several extract percentage yields were determined by using the formula as under:

% Yield= Weight of extract (g)/ Weight of dry powder (g) ×100

3.7 Phytochemical Screening

The phytochemical screening of the species was conducted using the methodologies of Silva *et al.* (2017), Hashim *et al.* (2015) and Udeozo *et al.* (2013)

Table 2: Various chemical tests for preliminary phytochemical screening

Chemical test(s)
Alkaloids
Mayer's test
Wagner's test
Dragendroff's test
Flavonoids
Alkaline reagent test
Lead acetate test
Carbohydrate
Molish's test
Fehling's test
Saponins
Foam test

Reagents used

1. Dragendroffs reagent
2. Wagners reagent
3. Mayers reagent
4. Molichs reagent
5. Fehling solution A and B
6. Distilled water

3.7.1 Determination of alkaloids

1. Dragendroffs Experiment

A little drop of the extract was splattered on a pre-coated TLC plate, that was then sprinkled with a modified Dragendorff's reagent (bismuth nitrate+ potassium iodide). The presence of alkaloids was indicated by the orange colour.

2. Mayers Test

The reagent was prepared by weighing 1.3 g of mercuric iodide and 5.0 g of KI (Potassium Iodide) on a weighing balance and dissolving them in 100 ml of distilled water. A white creamy precipitate was formed when two drops of Mayer's reagent were added to a small amount of plant extract, indicating the presence of alkaloids

3. Wagners Test

The reagent was prepared by weighing 2 g of iodine and 6 g of KI (Potassium Iodide), then dissolving them in distilled water and diluting them to 100 ml. The presence of alkaloids was determined by adding a few drops of Wagner's reagent to a small volume of extract. The presence of alkaloids was indicated by a reddish-brown precipitate.

3.7.2 Determination of flavonoids

1. Test with Alkaline Reagent

The presence of yellow colour on the TLC plate after a small amount of the extract was treated with a few drops of sodium hydroxide indicated the presence of flavonoids.

2. Test with Lead Acetate

The extract was treated with a few drops of lead acetate solution and the presence of flavonoids was determined by the formation of a yellow colour solution.

3.7.3 Determination of Carbohydrates

The 100 ml of extract was dissolved in distilled water and filtered for conducting the below-mentioned tests:

1. Molisch's test

Two drops of alpha –naphthol alcoholic solution were added to 2 ml filtrate and 1 ml concentrated sulphuric acid was progressively applied along the test tube's sides and formation of the violet ring between the two solutions indicated the presence of carbohydrates.

2. Fehling's test

In a water bath, an equal volume of Fehling solution A and B with an equal volume of the filtrate was brought to a boil and the formation of red precipitate indicated the presence of sugar.

3.7.4 Determination of Saponins

1. Foam Test

In a graduated cylinder, the extract (50 mg) was dissolved in distilled water approximately 20 ml and agitated for 15 minutes and the presence of saponins was indicated by the formation of a 2 cm thick foam.

3.8 GC-MS Analysis

3.8.1 Gas chromatograph

Chemical characterization of *Aconitum heterophyllum* was carried out using GC-MS analysis. The chemical components of extracts were analysed using a Perkin Elmer (USA) gas chromatograph type auto system XL. The GC used a flame ionisation detector (FID) and a Restek fused silica capillary column (30 m 0.25 mm ID, 0.25 m film thickness) coated with dimethylpolysiloxane (Bellefonte, PA, USA) (RTX-1).

3.8.2 Gas chromatograph- Mass spectrometer

A Varian GC MS 4000 equipped with a Varian Factor Four VF-5 MS fused silica capillary column (30 m 0.25 mm id, film thickness 0.25 um) was used for GC-MS analysis.

3.8.3 Analysis of GC

The temperature of the column oven was automatically adjusted from 50 to 240 degrees celsius at a rate of 5 degrees Celsius per minute. Temperatures were set at 250 ° c and 270ºC for the injector and detector, respectively. During the current investigation, nitrogen gas was used as the mobile phase at a flow rate of 1mL/min and an injector split ratio of 1:80 GC-MS analysis.

3.8.4 GC-MS Analysis

The oven temperature was adjusted to rise at a rate of 5ºC/min from 50 to 240ºC. Helium was employed as the carrier gas in this study, with a flow rate of 1 mL/min. Mass spectra in the 50-300 amu region were recorded at 1 scan per second with an E.I. of 70 eV. The retention duration of generated peaks was compared to reference materials injected under comparable chromatographic conditions to identify them. The mass spectra were compared to those in the NIST and WILEY computer libraries, as well as those in the literature.

Chapter-4

EXPERIMENTAL FINDINGS

The results of the present investigation entitled "Morpho-chemical characterisation" of *Aconitum heterophyllum* Wall. Ex Royle., a critically endangered medicinal plant of Kashmir Himalayas" is presented in this chapter.

4.1 Morphological Evaluation

The results of quantitative and qualitative morphological characters are presented as under:

4.1.1 Plant height:

The plant height was recorded maximum (94.60 cm) at LFD followed by (28.5 cm) at JVFD and (24.50 cm) at KFD respectively (Table 3).

4.1.2 No. of leaves/plant:

The number of leaves/plant were recorded highest (10.20 leaves/plant) at LFD followed by (4.6 leaves/plant) at JVFD and (3 leaves/plant) KFD with respectively (Table 3).

4.1.3 Leaf length:

The mean leaf length was recorded maximum (8.42 cm) at LFD, followed by (4.60 cm) at JVFD and (4.15 cm) at KFD respectively (Table 3).

4.1.4 Leaf width:

The mean leaf width was recorded maximum (7.96 cm) at LFD, followed by (3.90 cm) at KFD and (3.76 cm) at JVFD respectively (Table 4).

4.1.5 Leaf area:

The leaf area was observed maximum (78.02 cm^2) at LFD, followed by (18.4 cm^2) JVFD and (16.58 cm^2) at KFD respectively (Table 4).

4.1.6 Length of floral axis:

Length of Floral axis was found highest (20.96 cm) at LFD followed by (9.5 cm) at JVFD and (7.78 cm) at respectively (Table 4).

Plate 2: Height measurement at three different sites

Table 3: Intra-population variation in plant height, number of leaves/plant and leaf length at three different sites

Characters		Plant Height (cm)			Number of Leaves/Plant			Leaf Length (cm)		
Sources / Samples		KFD	LFD	JVFD	KFD	LFD	JVFD	KFD	LFD	JVFD
P1		35.00	87.00	35.00	3.00	11.00	7.00	6.00	5.81	5.00
P2		18.00	90.00	27.00	4.00	8.00	10.00	4.30	7.70	5.20
P3		16.50	32.00	25.00	2.00	7.00	8.00	3.25	9.70	5.80
P4		33.00	113.00	21.00	3.00	9.00	5.00	4.00	9.00	3.10
P5		20.00	151.00	33.00	3.00	16.00	5.00	3.20	9.90	3.90
Mean		24.50	94.60	28.20	3.00	10.20	7.00	4.15	8.42	4.60
SE(Mean)		3.93	19.39	2.58	0.32	1.59	0.95	0.51	0.76	0.48
CI (95%)	LL	13.59	40.77	21.05	2.12	5.78	4.37	2.74	6.32	3.25
	UL	35.41	148.43	35.35	3.88	14.62	9.63	5.56	10.53	5.95
CV (%)		35.87	45.83	20.43	23.57	34.94	30.30	27.42	20.13	23.56

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, P = Plant specimens

Table 4: Intra-population variation in leaf width, leaf area and length of the floral axis at three different sites

Characters		Leaf Width (cm)			Leaf Area(cm^2)			Length of Floral axis (cm)		
Samples \ Sources		KFD	LFD	JVFD	KFD	LFD	JVFD	KFD	LFD	JVFD
P1		5.20	9.30	2.90	30.50	94.90	18.40	11.50	20.91	11.20
P2		4.70	7.10	3.60	19.80	57.30	18.20	6.00	22.60	9.70
P3		4.70	7.70	3.90	15.60	69.00	22.80	4.30	11.40	8.70
P4		3.00	7.80	2.40	11.30	75.50	11.00	11.00	24.70	6.00
P5		1.90	7.90	6.00	5.70	93.40	21.60	6.10	25.20	11.90
Mean		3.90	7.96	3.76	16.58	78.02	18.40	7.78	20.96	9.50
SE(Mean)		0.62	0.36	0.62	4.19	7.21	2.05	1.45	2.5	1.03
CI (95%)	LL	2.17	6.95	2.04	4.94	58.01	12.70	4.30	11.40	6
	UL	5.63	8.97	5.48	28.22	98.03	24.10	11.50	25.20	11.90
CV (%)		35.76	10.19	36.78	56.53	20.65	24.96	41.1	26.7	24.2

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, P = plant specimens

4.1.7 Number of flowers/plant:

The number of flowers were observed highest (11) at LFD, followed by (10) at JVFD and (7) at KFD respectively (Table 5).

4.1.8 Tuber length:

The tuber length was found highest (6.04 cm) at LFD, followed by (5.90 cm) at KFD and (4.52 cm) at JVFD respectively (Table 5).

4.1.9 Number of root hairs per plant:

The number of root hairs per plant were observed highest (18.2) at LFD followed by (1.26) KFD and (13.8) at JVFD respectively (Table 5).

4.1.10 Tuber Biomass

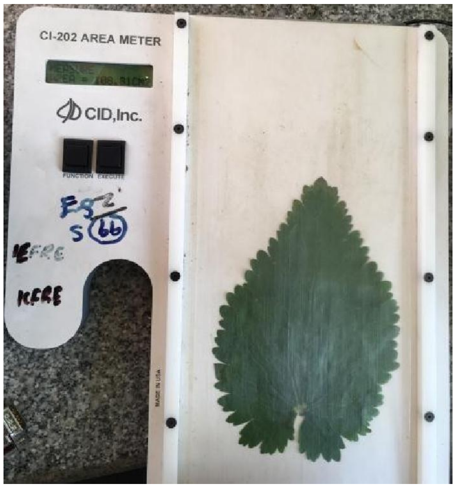
The tuber biomass was recorded highest (1.86 g) at LFD followed by (1.58 g) at JVFD and (1.26 g) at KFD respectively (Table 6).

4.1.11 Tuber thickness:

The mean tuber thickness was recorded maximum (2.1 cm) at LFD followed by (1.34 cm) at JVFD and (1.1 cm) at KFD respectively (Table 6).

4.1.12 Number of daughter tubers:

The number of daughter tubers were at par in all three sites (Table 6).



a) measurement of leaf area by using leaf area meter

b) Spiral arrangement of leaves

c) Measurement of leaf length

d) Measurement of leaf width

e) measurement of leaf area by using graph paper

Plate 3: Measurement of Leaf characteristics

Table 5: Intrapopulation variation in the number of flowers/plant, number of root hairs/plant and tuber length at three different sites

Characters		Number of Flowers/Plant			Number of Root Hairs/Plant			Tuber Length (cm)		
Sources / Samples		KFD	LFD	JVFD	KFD	LFD	JVFD	KFD	LFD	JVFD
P1		9	10	9	16	19	15	7.00	6.50	4.00
P2		7	11	10	10	20	12	4.50	6.00	3.40
P3		5	10	12	11	18	13	7.00	5.00	5.00
P4		8	11	11	18	16	14	6.50	5.70	5.50
P5		6	13	8	16	18	15	4.50	7.00	4.70
Mean		7	11	10	14.2	18.2	13.8	5.90	6.04	4.52
SE(Mean)		0.70	0.54	0.70	1.56	0.66	0.58	0.58	0.34	0.37
CI (95%)	LL	5	10	4	10	16	13	4.29	5.09	3.49
	UL	9	13	12	18	20	15	7.51	6.99	5.55
CV (%)		22.5	10.9	15.00	24.5	3.6	9.4	21.94	12.64	18.34

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, P = plant specimens

a) counting of flowers

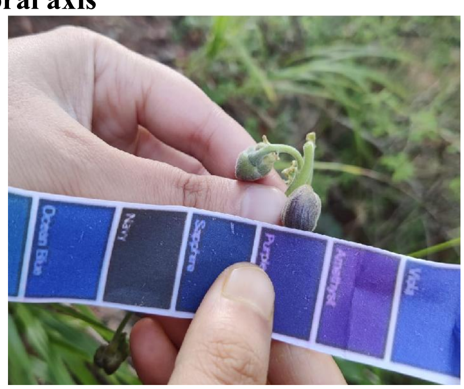
b) Androecium and gynoecium

c) fruit

d) Whole flower

e) Measurement of length of the floral axis

f) Seeds



g) Flower colour

Plate 4: Flower characteristics

Table 6: Intrapopulation variation in root biomass, tuber thickness and number of daughter tubers at three different sites

Characters		Tuber Biomass (g)			Tuber Thickness (cm)			Number of Daughter Tubers		
Sources / Samples		KFD	LFD	JVFD	KFD	LFD	JVFD	KFD	LFD	JVFD
P1		1.30	2.06	1.49	1	3	1.2	1	1	1
P2		1.20	2.68	1.26	1.5	2	1	1	1	1
P3		1.00	1.20	1.98	1	2.5	1.7	1	1	1
P4		1.50	2.21	1.21	1	1	1.8	1	1	1
P5		1.30	1.16	1.97	1	1	1	1	1	1
Mean		1.26	1.86	1.58	1.1	2.1	1.34	1	1	1
SE(Mean)		0.08	0.29	0.16	0.1	0.4	0.1	-	-	-
CI (95%)	LL	1	1.16	1.21	1	1	1	-	-	-
	UL	1.5	2.68	1.98	1.5	2.5	1.8	-	-	-
CV (%)		14.2	36.2	24.6	20	46.8	28.3	-	-	-

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, P = Plant specimens

a) Tuber colour **b) daughter and mother tubers**

c) measurement of tuber biomass **d) Measurement of tuber length**

e) counting of root hairs

Plate 5: Tuber characteristics

The data on qualitative morphological parameters revealed that the species flowered between August to September at LFD and JVFD, whereas, the flowering took place between July to September at KFD. The sex of the flower bisexual, flower colour purple and tuber colour was observed light brownish white across all the three studied sites. (Table 7).

Table 7: Variation in qualitative morphology of *Aconitum heterophyllum* at different sites

Characters / Source	Observed flowering time	Sex of flower	Flower colour	Tuber colour
KFD	July-Sep	Bisexual	Purple	Light brownish white
LFD	Aug-Sep	Bisexual	Purple	Light brownish white
JVFD	Aug-Sep	Bisexual	Purple	Light brownish white

4.2 Rhizopheric soil analysis:

The soil analysis was carried out to determine the following parameters:

4.2.1 Electrical conductivity:

The mean electrical conductivity was found highest (0.45 ds/m) at KFD followed by (0.33 ds/m) at JVFD and (0.30 ds/m) at LFD Respectively (Table 8).

4.2.2 Organic Carbon status:

The mean value of organic carbon was found highest (2.3 %) at LFD followed by (1.7%) at KFD and (1.5%) at JVFD respectively (Table 8).

Table 8: Electrical conductivity and distribution of available OC in rhizospheric soil of *Aconitum heterophyllum*

Characters		Electrical Conductivity ds/m			Organic Carbon %		
Sources / Samples		KFD	LFD	JVFD	KFD	LFD	JVFD
S1		0.41	0.45	0.25	1.90	2.60	1.30
S2		0.37	0.32	0.36	1.70	2.30	1.10
S3		0.39	0.29	0.30	1.30	2.70	1.60
S4		0.43	0.40	0.34	1.70	1.90	1.80
S5		0.45	0.34	0.40	1.90	2.00	1.70
Mean		0.41	0.36	0.33	1.7	2.3	1.5
SE(Mean)		0.01	0.03	0.03	0.10	0.15	0.13
CI (95%)	LL	0.37	0.28	0.26	1.3	1.9	1.1
	UL	0.45	0.44	0.40	1.9	2.7	1.8
CV (%)		7.71	17.89	17.41	14.11	15.2	19.3

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, S= Soil samples

4.2.3 pH

The pH level was found highest (5.8) at LFD followed by (5.4) at KFD and (4.90) at JVFD respectively (Table 9).

4.2.4 Phosphorus

The phosphorus was found highest (21.8 kg/ha) at KFD followed by (16.16 kg/ha) at LFD and (13.09 kg/ha) at JVFD respectively (Table 9).

4.2.5 Available Nitrogen:

The available nitrogen was found highest (473.81 Kg/ha) at JVFD followed by (461 kg/ha) at KFD and (441.702 kg/ha) at LFD respectively (Table 10).

4.2.6 Available Potassium:

The available potassium was found highest (487.46 kg/ha) at LFD followed by (480 kg/ha) at JVFD and (389.58 kg/ha.) at KFD respectively (Table 10).

Plate 6. Measurement of soil parameters

Table 9: pH and distribution of available phosphorus in rhizo-spheric soil of *Aconitum heterophyllum*

Characters	Phosphorus kg/ha			pH		
Sources / Samples	KFD	LFD	JVFD	KFD	LFD	JVFD
S1	17.60	13.60	10.20	5.20	5.60	4.60
S2	16.20	14.20	11.55	5.40	5.80	4.80
S3	20.80	17.60	13.40	5.80	5.80	5.00
S4	25.00	18.32	15.60	5.10	5.90	5.20
S5	27.80	17.10	14.70	5.50	5.90	5.90
Mean	21.48	16.16	13.09	5.40	5.80	4.90
SE(Mean)	2.19	0.95	0.99	0.12	0.05	0.10
CI (95%) LL	15.41	13.53	10.33	5.06	5.65	4.62
CI (95%) UL	27.55	18.80	15.85	5.74	5.95	5.18
CV (%)	22.77	13.13	16.96	5.07	2.11	4.56

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, S= Soil samples

Table 10: Available nitrogen and potassium in rhizospheric soil of *Aconitum heterophyllum* at three different sites

Characters		**Nitrogen kg/ha**			**Potassium kg/ha**		
Sources / Samples		**KFD**	**LFD**	**JVFD**	**KFD**	**LFD**	**JVFD**
S1		367.2	469.29	456.00	382.33	485.30	481.20
S2		390.54	432.7	467.00	396.40	490.00	484.60
S3		414.12	489.76	397.00	387.11	483.00	476.73
S4		412.8	499.07	498.00	396.60	487.00	468.40
S5		395.85	478.23	487.00	385.50	492.00	489.30
Mean		396.10	473.81	461	389.588	487.46	480.0
SE(Mean)		8.57	11.4	17.6	2.9	1.6	3.56
CI (95%)	LL	367.2	432.7	397	382.33	483	468.4
	UL	414.12	499	498	396.6	492	489.3
CV (%)		4.8	5.4	8.5	1.03	1.92	1.74

CI = Confidence Interval at 95%, LL = Lower Limit, UL = Upper Limit, CV = Coefficient of Variation, S= Soil samples

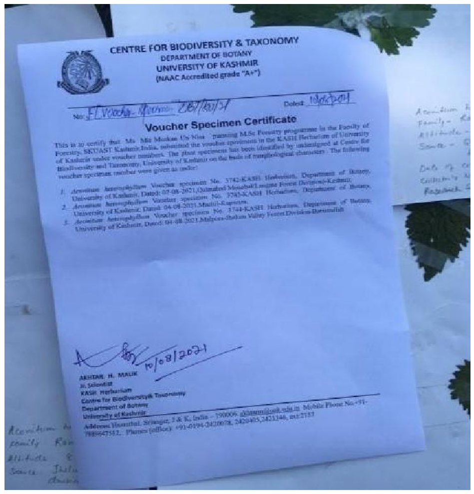


Plate 7: Herbarium preparations, collection and authentication of plant specimens

4.3 Extraction and Phytochemical Screening

Methanol was found to be the best solvent for extraction followed by aqueous. The highest quantity (3.3 g) was recovered with methanol extract from the LFD followed by (1.7 g) from the JVFD and (1.4 g) from KFD respectively. Similarly the percentage yield was found maximum (16.5%) in LFD followed by (8.5%) in JVFD and (7%) in KFD respectively (Table 11).

The physical nature of extracts showed no significant difference with respect to the sample source. The pungent odour, viscous with crystal texture and honey brown color was observed in the methnol extracts from different sources i.e. LFD, JVFD and KFD (Table 12).

Alkaloids and flavonoids were found in the petroleum ether extract, whereas carbohydrates and saponins were found in the aqueous and methanol extracts (Table 13).

a) Removal of dirt

b) Drying

c) Grinding

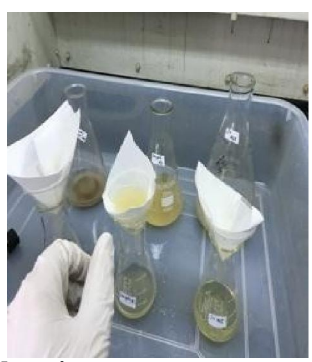
d) powder

e) adding of solvent to round bottom flask

f) adding of powder to thimble

g) Soxhlet extraction

h) extract



i) Filtration

j) evaporation by using Vaccum evaporator

Plate 8: Stepwise extraction by using Soxhlet apparatus

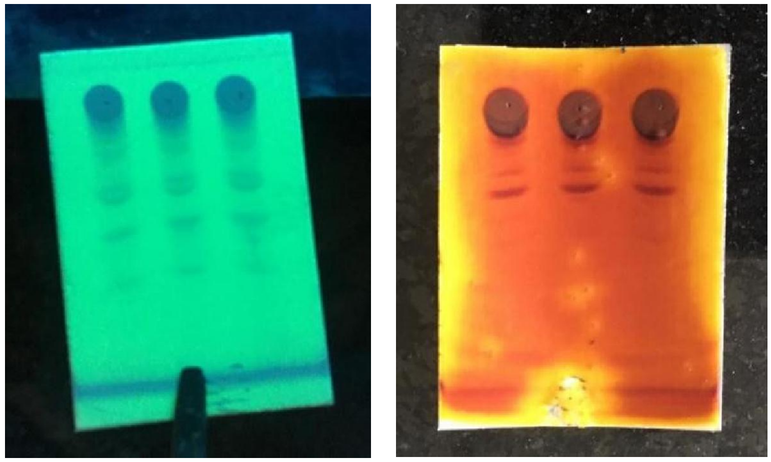
Plate 9: Various extracts

Table 11: Quantity and percentage yield of successive extracts of *Aconitum heterophyllum* tubers

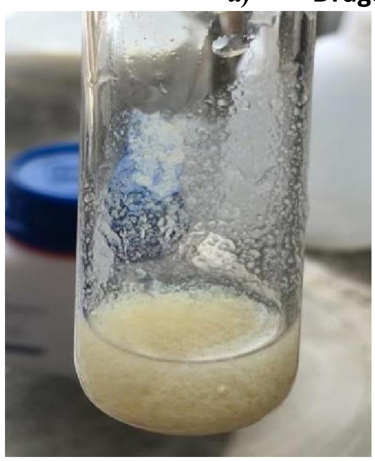
	Sample weight (g)			Quantity recovered (g)			Yield %		
Extract(s) / Source	Petroleum Ether	Methanol	Aqueous	Petroleum Ether	Methanol	Aqueous	Petroleum Ether	Methanol	Aqueous
KFD	20	20	20	0.5	1.4	1.1	2.5	7	5.5
LFD	20	20	20	1.3	3.3	2.1	6.5	16.5	10.5
JVFD	20	20	20	1.1	1.7	1.5	5.5	8.5	7.5

Table 12: Physical nature of successive extracts from tubers of *Aconitum heterophyllum*

	Odour			Texture			Color		
Extract(s) / Source	Petroleum Ether	Methanol	Aqueous	Petroleum ether	Methanol	Aqueous	Petroleum ether	Methanol	Aqueous
KFD	Chemical like	Pungent	Pungent	Sticky	Viscous with crystals	Viscous	Colorless	Honey brown	Brown
LFD	Chemical like	Pungent	Pungent	Sticky	Viscous with crystals	Viscous	Colorless	Honey brown	Brown
JVFD	Chemical like	Pungent	Pungent	Sticky	Viscous with crystals	Viscous	Colorless	Honey Brown	Brown



a) **Dragendroffs test**



b) **Mayers test**

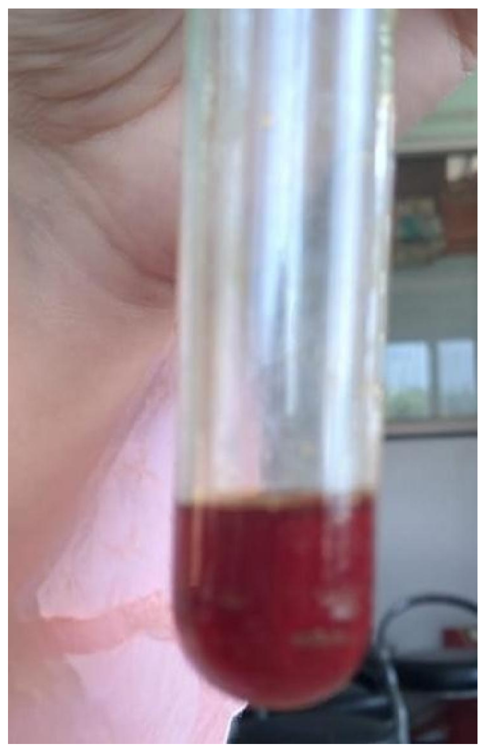
c) Wagners test

d) Foam test

Plate 10: Phytochemical screening for Alkaloids and Saponins

a) Alkaline reagent test b) Lead acetate test

Plate 11: Phytochemical screening for flavonoids



c) Molichs test d) Fehlings test

Plate 12: Phytochemical screening for carbohydrates

Table 13: Phytochemical screening of petroleum ether extract, methanol extract and aqueous extract of *Aconitum heterophyllum*

Extract (s) Tests	**Petroleum ether**	**Methanol**	**Aqueous**
	Alkaloids		
Mayer's test	+	-	-
Wagner's test	+	-	-
Dragendroff's test	+	-	-
	Flavonoids		
Alkaline reagent test	+	-	-
Lead acetate test	+	-	-
	Carbohydrates		
Fehling's test	-	+	+
Molisch's test	-	+	+
	Saponins		
Foam test	-	+	+

(+) signifies presence and (_) signifies absence

4.4 GC-MS Analysis

GC-MS chromatogram of petroleum ether extract of the tubers of *Aconitum heterophyllum* (LFD) exhibited 10 peaks indicating the presence of good number of compounds. The 10 compounds were identified after matching with the library. The results further revealed that Ethoxyisoxazol-4-carboxylic acid was major compound followed by ethyl ester, Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester,Hexa (methoxymethyl) melamine, gamma.-Aminobutyric acid, N-heptafluorobutyryl-,1,1,1-trifluoro-2-propylester, Carbamic acid, N-(4-amino-6-ethylaminopyrimidin-5-yl), ethyl ester, 1,3,5,7,9,11-Hexa (trimethylene)-1,3,5,7,9,11-hexasila-2,4,6,8,10,12-hexaoxacyclododecane, 1-[2-[2H,4H-1,2,4-Triazin-3,5-dithione]]-1-deoxy-.beta.-d-ribose, 5-Methyl-1R,3-trans-cyclohexanediol, Barban, Pyridine, 3-nitro-2-(2H-1,2,3-triazol-4-yl)thio (Table 14).

Table 14: Phytochemicals identified by GC-MS analysis of petroleum ether extract of *Aconitum heterophyllum* sourced from LFD

Peak Name	Retention time	Area %	Name of compound
1	4.405	19.24	Ethoxyisoxazol-4-carboxylic acid
2	6.562	10.97	gamma.-Aminobutyric acid, N-heptafluorobutyryl-, 1,1,1-trifluoro-2-propylester
3	12.625	13.72	Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester
4	15.270	10.01	1,3,5,7,9,11-Hexa(trimethylene)-1,3,5,7,9,11-hexasila-2,4,6,8,10,12-hexaoxacyclododecane
5	16.454	4.16	Pyridine, 3-nitro-2-(2H-1,2,3-triazol-4-yl)thio-
6	16.535	6.51	5-Methyl-1R,3-trans-cyclohexanediol
7	19.455	4.81	Barban
8	23.970	8.88	1,2,3-Tri-O-acetyl-5-deoxy-b-D-ribofuranose
9	30.404	10.38	Carbamic acid, N-(4-amino-6-ethylaminopyrimidin-5-yl)-, ethyl ester
10	30.687	11.32	Hexa(methoxymethyl)melamine
Total		**100.00**	

GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (JVFD) exhibited 5 peaks, specifying the presence of substantial compounds. Around 5 compounds were recognised through the match with the library. The results further revealed that 9,12-Octadecadienoic acid (Z,Z) was the paramount compound followed by 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Hexadecanoic acid, methyl ester and cis,cis,cis-7,10,13-Hexadecatrienal (Table 15).

Table 15: Phytochemicals identified by GC-MS analysis of a methanolic extract of *Aconitum heterophyllum* sourced from JVFD

Peak Name	Retention Time	Area %	Name
1	31.024	19.74	Hexadecanoic acid, methyl ester
2	31.592	12.13	n-Hexadecanoic acid
3	33.600	28.13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
4	34.155	28.81	9,12-Octadecadienoic acid (Z,Z)
5	34.245	11.19	cis,cis,cis-7,10,13-Hexadecatrienal
Total		**100**	

GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* sourced from LFD exhibited 4 peaks.1,2-Benzenedicarboxylicacid,bis (2-methylpropyl) ester is major compound followed by Cyclooctasiloxane, hexadecamethyl-, Cycloheptasiloxane, tetradecamethyl, Silane, [bicyclo[4.2.0]octa-3,7-diene-7,8-diylbis(oxy)]bis[trimethyl respectively. (Table 16).

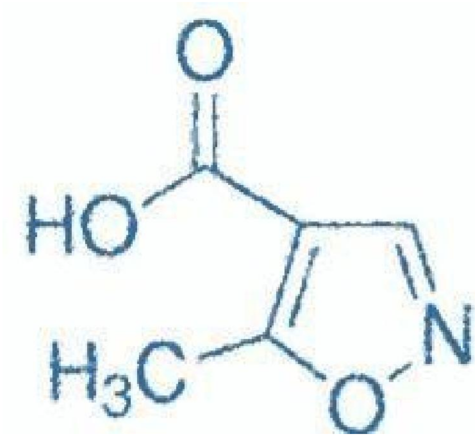
Table 16: Phytochemicals identified by GC-MS analysis of methanolic extract of *Aconitum heterophyllum* sourced from LFD

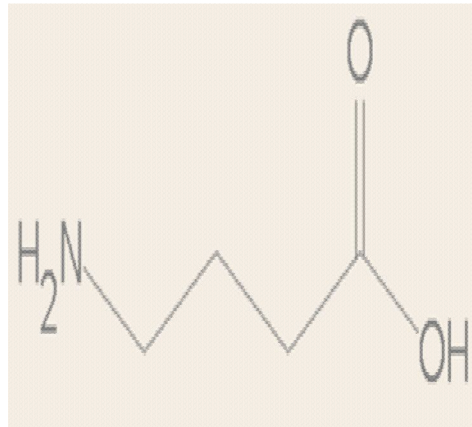
Peak	R time	Area %	Name of compound
1	21.291	1.81	Cycloheptasiloxane, tetradecamethyl-
2	21.305	1.36	Silane, [bicyclo[4.2.0]octa-3,7-diene-7,8-diylbis(oxy)]bis[trimethyl-
3	26.071	12.63	Cyclooctasiloxane,hexadecamethyl-
4	30.076	84.20	1,2-Benzenedicarboxylicacid,bis(2-methylpropyl)ester
Total		**100**	

GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* sourced from KFD exhibited 1 peak only, 1,2-Benzenedicarboxylicacid,bis(2-methylpropyl) ester was the major compound. (Table 17).

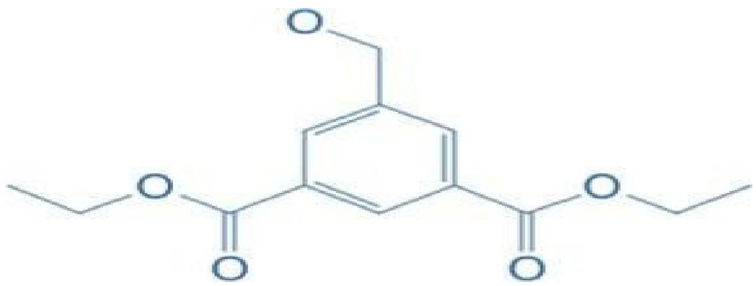
Table 17: Phytochemicals identified by GC-MS analysis of methanolic extract of *Aconitum heterophyllum* sourced from KFD

Peak	R time	Area %	Name of compound
1	30.081	100.00	1,2-Benzenedicarboxylicacid,bis(2-methylpropyl)ester
Total		**100**	

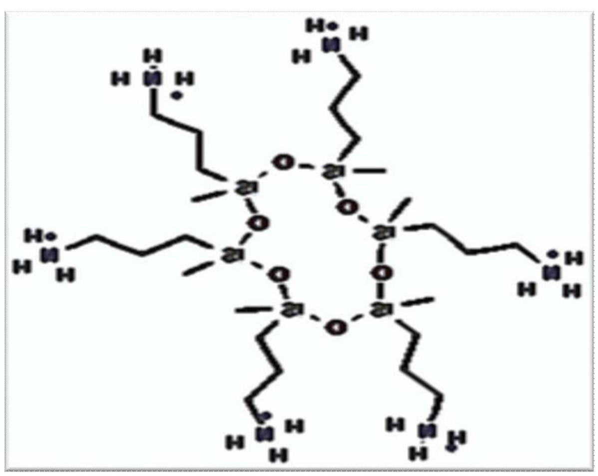




Ethoxyisoxazol-4-carboxylic acid **gamma.-Aminobutyric acid**

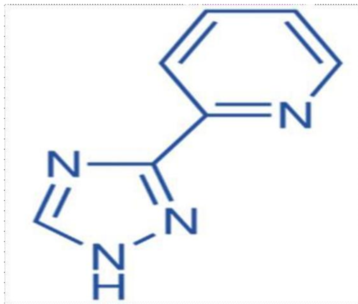


Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester

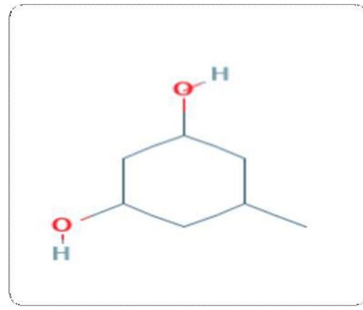


1,3,5,7,9,11-Hexa(trimethylene)-1,3,5,7,9,11-hexasila-2,4,6,8,10,12-hexaoxacyclododecane

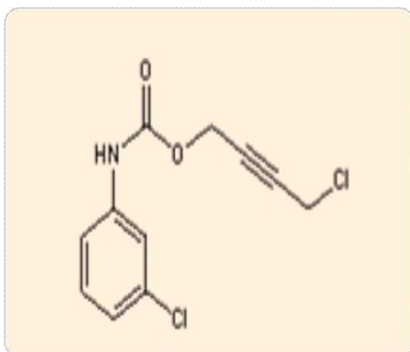
Plate 13: Structure of a chemical compounds



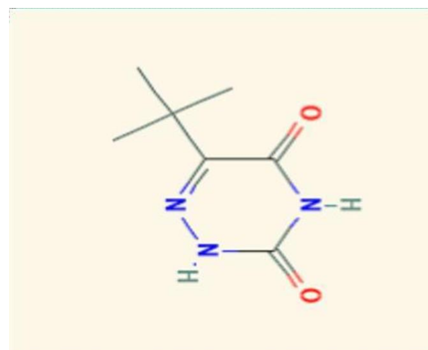
Pyridine, 3-nitro-2-(2H-1,2,3-triazol-4-yl)thio



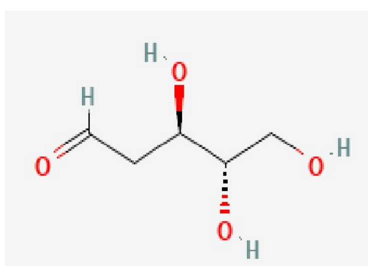
5-Methyl-1R,3-trans-cyclohexanediol



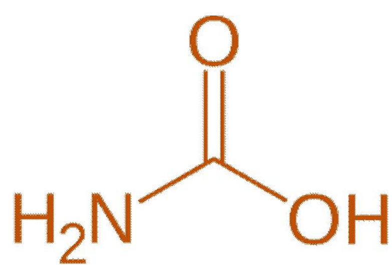
Barban



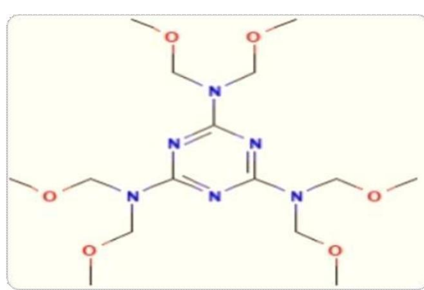
1-[2-[2H,4H-1,2,4-Triazin



3,5-dithione]]-1-deoxy-.beta.-d-ribose

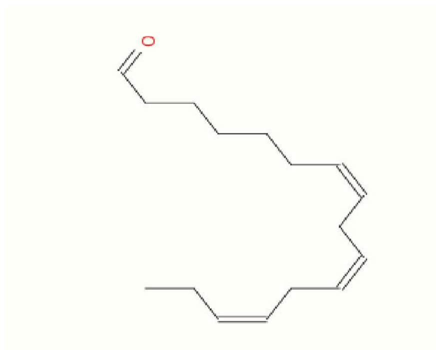


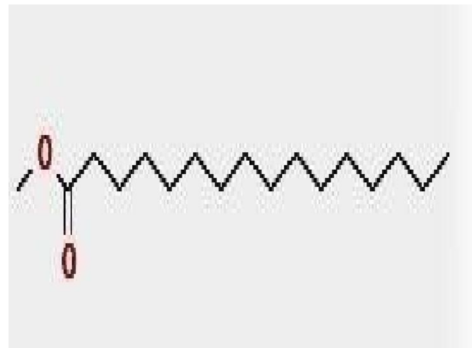
carbamic acid



Hexa(methoxymethyl)melamine

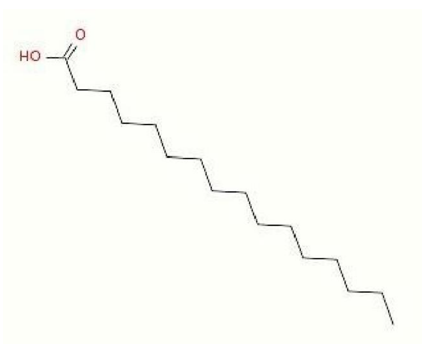
Plate 14: Structure of chemical compounds

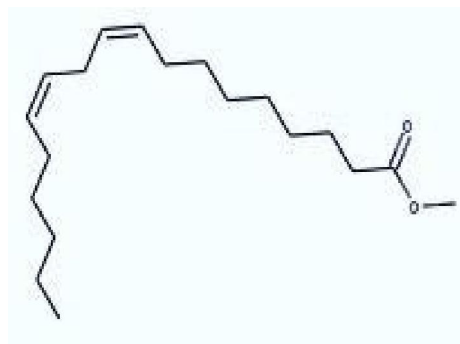




cis,cis,cis-7,10,13-Hexadecatrienal

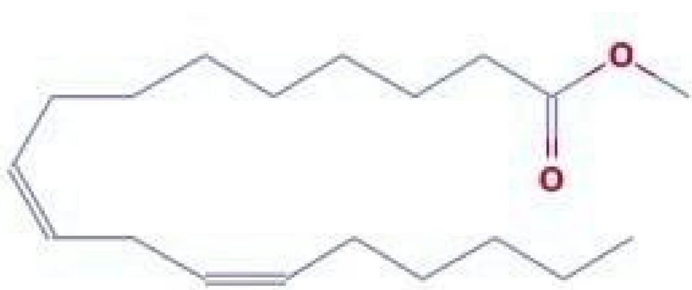
Hexadecanoic acid, methyl ester





n-Hexadecanoic acid

9 ,12-Octadecadienoic acid (Z,Z)



9 ,12-Octadecadienoic acid (Z,Z)-, methyl ester

Plate 15: Structure of chemical compounds

Table 18: List of already identified chemicals and our new findings

Name of bioactive compounds	Previous references	Our new findings	Methodology used
Linoelaidic acid	Nengroo *et al.,* 2021	-	GC-MS
Isophthalic acid	Nengroo *et al.,* 2021	-	GC-MS
Palmitic acid	Nengroo *et al.* 2021	-	GC-MS
Pyridine	Rajakrishnan *et al.* 2016	-	GC-MS
Alpha-Linoleic acid	Rajakrishnan *et al.* 2016	-	GC-MS
Benzene	Rajakrishnan *et al.* 2016	-	GC-MS
Bis(2-methyl propyl) ester	Tomar *et al.,* 2018	-	GC-MS
Cycloheptasiloxane	Tomar *et al.,* 2018	-	GC-MS
Barban	-	New	GC-MS
gamma.-Aminobutyric acid (GABA)	-	New	GC-MS
(6-Aminohexyl)carbamic acid	-	New	GC-MS
Methenamine hydrochloride	-	New	GC-MS
7,8-Bistrimethylsiloxybicyclo[4.2.0]octadiene-3,7	-	New	GC-MS
N-heptafluorobutyryl-,	-	New	GC-MS
Propyl triflate	-	New	GC-MS
Diisobutyl phthalate	-	New	GC-MS
Methyl palmitate	-	New	GC-MS
1,2,3-Tri-O-acetyl-5-deoxy-b-D-ribofuranose	-	New	GC-MS
1,3,5,7,9,11-Hexa(trimethylene)-1,3,5,7,9,11-hexasila-2,4,6,8,10,12-hexaoxacyclododecane	-	New	GC-MS
5-Methyl-1R,3-trans-cyclohexanediol	-	New	GC-MS
Ethoxyisoxazol-4-carboxylic acid	-	New	GC-MS

4.5 Weather parameters

The data on weather parameters for the three years i.e. 2019, 2020, 2021 (gestation period of the species) were obtained from the Agromet Unit of SKUAST-Kashmir, to analyse the findings of this research in light of the climatic conditions in the study sites.

By comparing mean weather of three years solar radiation (18.37) lux units, temperature with mean value of 25.91 °C and rainfall with mean value of 3.07 mm was found maximum in LFD (Figure 2,3,4).

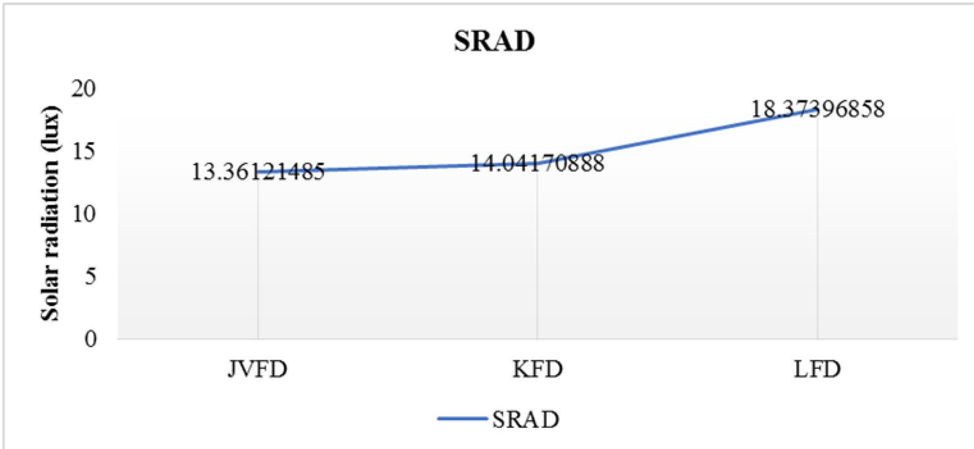


Figure 2: Variation in solar radiation (lux) at three different sites

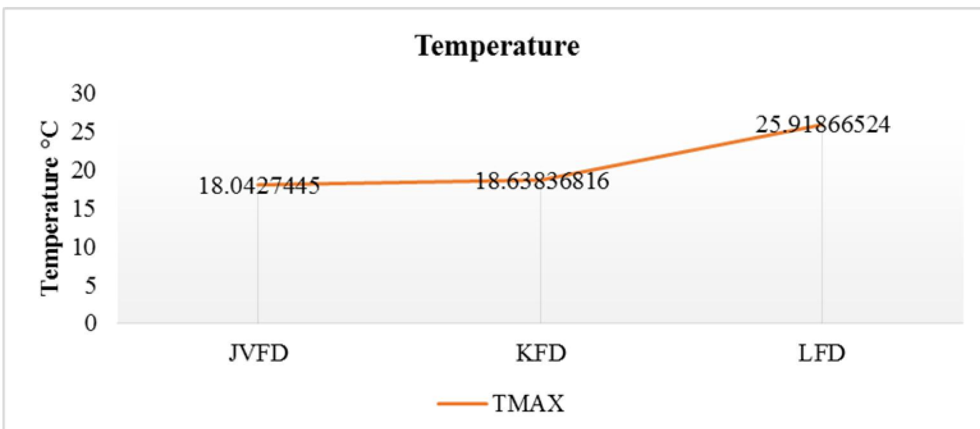


Figure 3: Variation in temperature at three sites

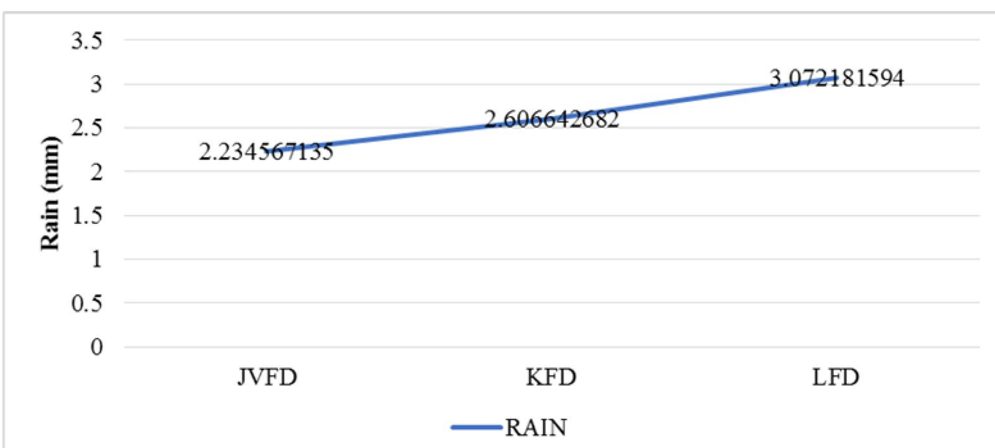


Figure 4: Variation in rainfall (mm) at three different sites

Chapter-5

DISCUSSION

The present study revealed that morphological traits of *Aconitum heterophyllum* vary substantially with habitat because of weather, soil and topography at three different study sites of Kashmir i.e. Kamraj Forest division, Langate Forest Division and Jhelum Valley Forest Division. The results of this study is supported by the finding of Talebi *et al.* (2014), who discovered that numerous populations of the same species developing in different environmental situations can display alteration in morphology. The results of this study revealed that the quantitative morphological parameters observed at LFD proved this site to be best for growth and development of *Aconitum heterophyllum* possibly due to adaptation of species to various edaphic, topographic and climatic conditions of the area. It was also observed that plants growing along medium elevation had better growth and had a better accumulation of secondary metabolites, than on higher elevation, these findings are also supported by the findings of other authors (Khan *et al.,* 2017). Plants at KFD were stunted however had a good quantity of flowers because of the pressure of high altitude (Kuniyal *et al.,* 2003). Plant growth and productiveness are negatively affected through temperature extremes and the pace of phenological development will be accelerated by warmer temperatures (Hatfield and Pruegar, 2015).The fundamental elements related to radiation encompass photoperiod, intensity, direction and quality. In response to light, plants are capable of alteration through the increase of numerous secondary metabolites. Rainfall performs a key function in biomass production (Zhang *et al.,* 2020).

According to Pigliucci *et al.,* 1997 soil nutrients additionally play a critical function in the morphology of plant life. The acid/alkaline response of soil is important for mineralization of organic matter, the solubility of substances, microbiological approaches and for an increase of plant life, pH of soil at LFD was slightly alkaline. Nitrogen is a requirement for all plant boom processes. In case of insufficient nitrogen content in soil vegetation period is shortened, LFD had excessive nitrogen in comparison to other two sites, phosphorous is vital for plenty important processes in case of inadequate phosphorus in the soil, the plant develops poorly and their leaves curl, phosphorous was found in a true amount at 3 unique sites, potassium is critical for maximum plant boom, an activator of enzymes, photosynthesis and metabolism in plants which was observed maximum in LFD. Soil organic carbon and pH are widely recognized to be signs of the fertility of the soil (Lauchli and Grattan, 2012). Organic carbon of LFD was present in an excessive amount which enables in retention of soil nutrients as those plants are the handiest in regions liable to soil erosion. It's been hypothesised that nitrogen increases the creation of additional roots, which improves plant growth and productivity (Sultana *et al.,*2012; Rai *et al.,* 2013). The study performed by Mani *et al.* (2002) proved that greater plant height and yield in plants using NPK fertilizer was ascribed to higher N and P content. Chemical fertilizer like NPK has been acclaimed to increase plant biomass and crop yield due to its abundance in N and P contents which are reported to assist growth in the plant (Ekbic *et al.,* 2010; Zeng *et al.,* 2011; Stephen *et al.,* 2014; Mishra and Singh, 2006). Figure (5,6,7)

A high advantageous correlation was observed among potassium and plant height, the number of flowers per plant, length of floral axis and tuber length. Similarly, the advantageous correlation was observed among nitrogen and tuber thickness, as well as among soil pH and leaf length, the robust advantageous correlation between organic carbon and tuber thickness and excessive advantageous correlation of Electrical conductivity with leaf width and leaf area at LFD. (Figure 8).

Potassium was shown to have a positive relationship with plant height, leaf width, leaf area, and floral axis length. Nitrogen was shown to have a strong positive correlation with tuber length and thickness. There is a link between pH and leaf width and the number of root hairs per plant. At JVFD, phosphorus and

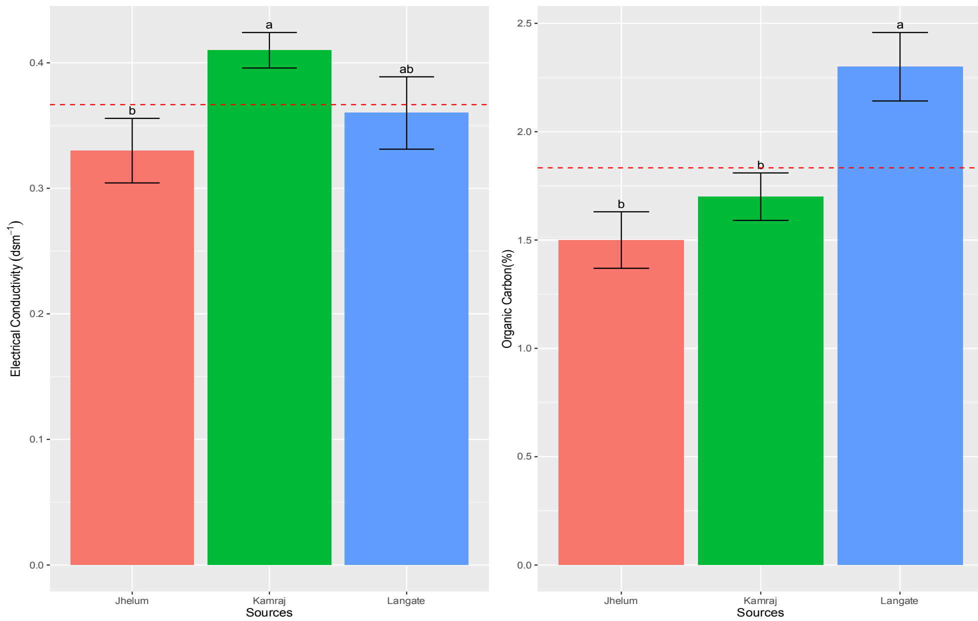


Figure 5: **Electrical conductivity and distribution of available OC in rhizo-spheric soil of *Aconitum heterophyllum* collected from three different sites**

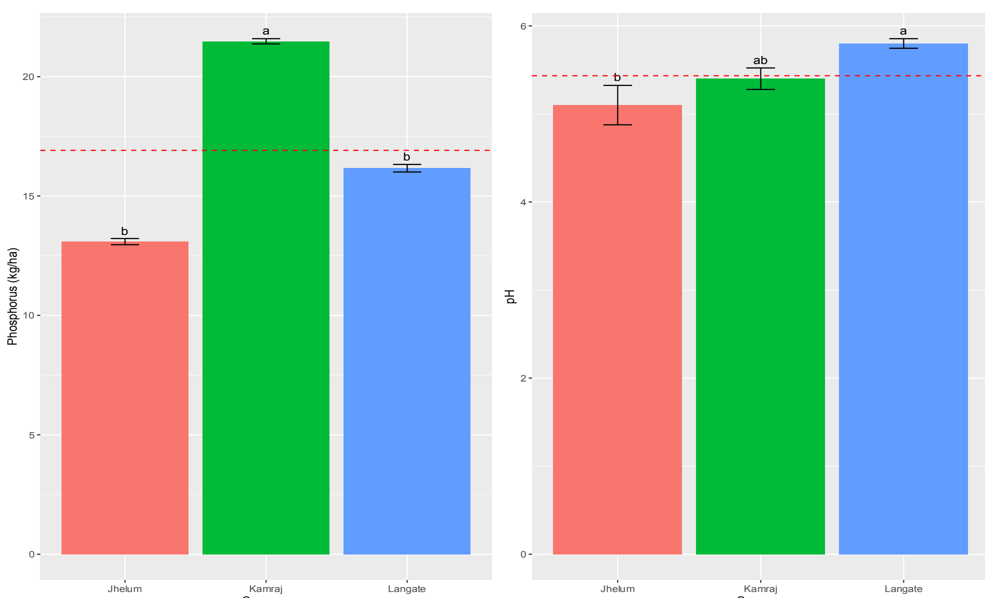


Figure 6: **pH and distribution of Available Phosphorus in rhizo-spheric soil of *Aconitum heterophyllum* collected from three different sites**

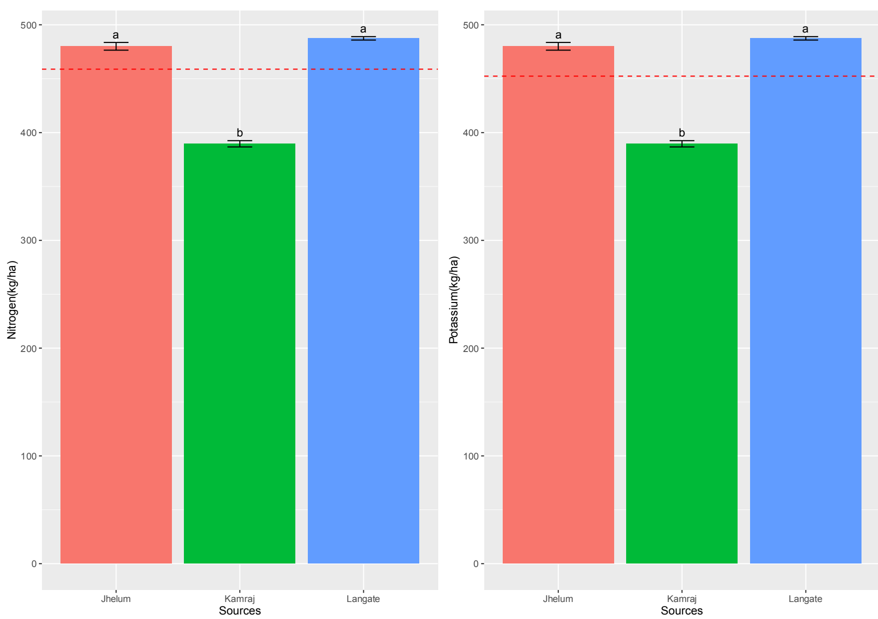


Figure 7: Distribution of available nitrogen and potassium in rhizo-spheric soil of *Aconitum heterophyllum*collected from three different sites

tuber length, organic carbon and tuber length, electrical conductivity and leaf breadth all have a strong positive correlation. (Figure 9).

At KFD the number of leaves per plant and tuber thickness has a positive association with potassium. Plant height, number of leaves per plant, number of flowers per plant, and number of root hairs per plant all have a positive association with nitrogen, as does tuber biomass. Phosphorus has a positive correlation with the number of root hairs per plant, organic carbon has a positive correlation with the number of leaves per plant, length of floral axis, tuber biomass, number of flowers per plant, and root hairs per plant. Electrical conductivity and the number of root hairs per plant have a close relationship. (Figure 10).

The broad abuse of plants in different clinical practices lies in the guideline of dynamic fixings contained in their concentrates. Plants without a doubt give these medical advantages to people generally in view of their inborn capacity to give a boundless pool of synthetic mixtures, with different scopes of restorative properties. Since restorative plant separates get their remedial impacts from auxiliary metabolites, the main thrusts behind the creation and aggregation of these important mixtures in plants will surely have "the most intense" voice in directing the nature of phytomedicines. Given the need and demand for a ceaseless inventory of therapeutic plants with uniform quality ascribes, expanding the number of restorative plants through cultivation would give off an impression of being a significant methodology for fulfilling a developing need (Uniyal *et al.,* 2000). The development of therapeutic plants is that as it may, has not been broadly embraced as a preservation methodology in many societies. The complete number of therapeutic plants developed on any scale is not many (Hamilton, 2004). Extraction is the process of drawing out important compounds from plants when a new drug is to be discovered extraction is the separation of medicinally important bio-actives and leaves residue from various parts of plants by using different solvents and methods (Handa, Khamuja, Longo and Rakesh,2008).

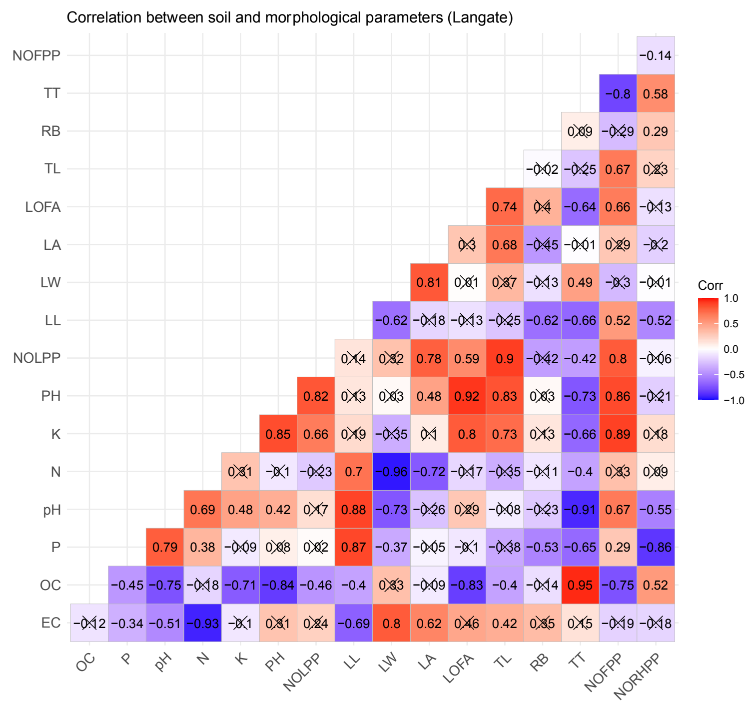


Figure 8: Correlation between soil and morphological parameters of LFD

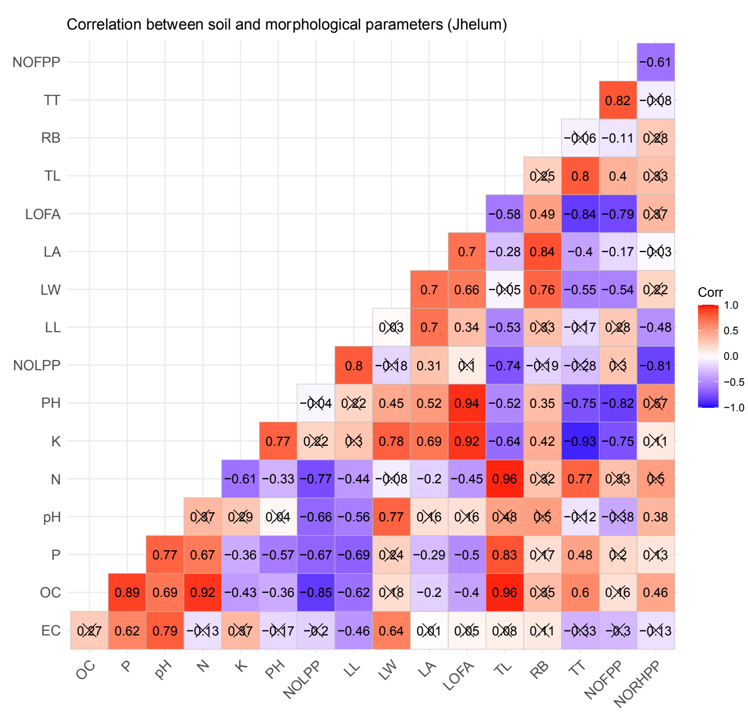


Figure 9: Correlation between soil and morphological parameters of JVFD

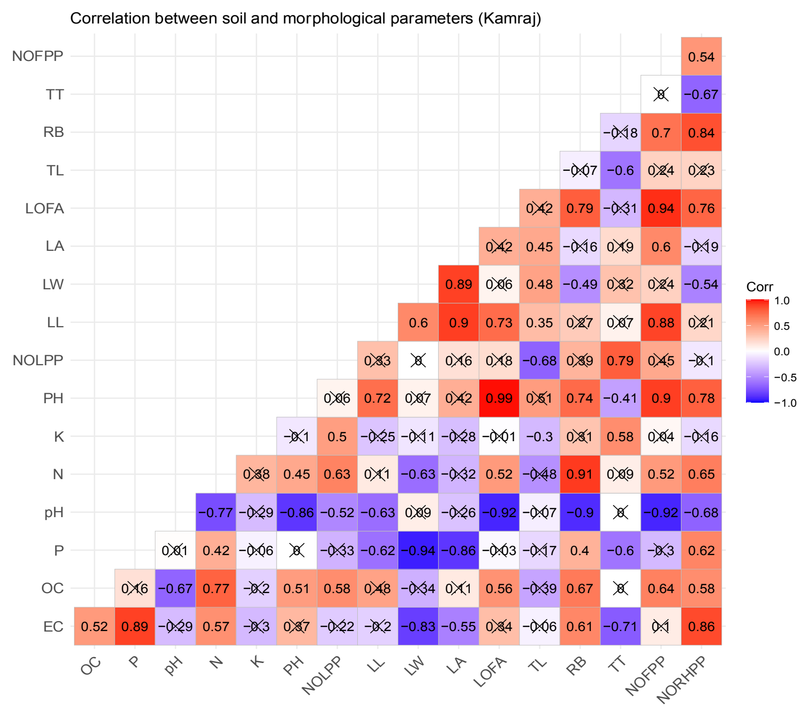


Figure 10: Correlation between soil and morphological parameters of KFD

There are various extraction methods but soxhlet is simple, easy (Nengroo *et al.,* 2021). The capability of extraction solvents could be ranked in this way Methanol> Aqueous>Petroleum ether i.e. methanol had the highest extraction potential with petroleum ether as lowest. Similarly, the potential of sites concerning extracting yield could be ranked like this LFD>JVFD>KFD i.e. LFD had the highest yield with the lowest percentage of yield at Kamraj which might be due to high temperature in LFD high temperature is preferable for an increase in alkaloid content. Solar radiation energy is one of the most significant environmental elements needed for plant development and advancement. The formative pliancy of plants in any climate is accomplished, to some extent part of the way by consistent checking of the quality (wavelength), term and amount of solar radiation. The endurance of plants pivots much on their capacity to complete photosynthetic carbon obsession and biomass accumulation and have accordingly grown very sensitively with precise abilities to detect diverse light spectra and bright (UV) light present in the solar radiation (Kazan and Manners, 2011). To guarantee ideal advantage from light, plants have developed biochemical defensive components against potentially harming raised dosages of UV radiation and outrageous light intensities. Likewise, relating systems have been created in plants as a way to improve the assimilation of valuable light spectra. Among these, UV-B (280-315 nm) has been perceived by plants as a natural stressor that advances UV acclimation and endurance in daylight (Rozema *et al.,* 1997). High temperature (35 °C) treatment expanded the leaf total peroxidase action along with an increment in hypericin, pseudo hypericin and hyperforin focus in the shoot tissues of St. John's Wort (Zobayed *et al.,* 2005). Likewise, a dramatic expansion in a variety of volatile compounds, with a linear expansion in temperature has been depicted in the scope of plant groups (Parker, 1977; Sharkey and Loreto, 1993; Sharkey and Yeh, 2001). Plant supplement balance in the soil is thought to impact the creation of secondary metabolites at the degree of metabolic guideline in plants (Herms and Mattson, 1992). Yan *et al.* (2004) observed that rich organic matter, low pH and undeniable degrees of exchangeable nitrogen and total nitrogen in the soil are crucial for a significant level of salidroside. Simon *et al.* (2010) tracked down proof that, for each N invested into cyanogenic glycosides, extra N is added to the leaf in Eucalyptus clado calyx.

The subjective phytochemical investigation is essential to get information about the presence and absence of any essential or auxiliary metabolites and assuming whether any critical bioactive compound is available or not, it is important to isolate that compound with the assistance of GC-MS. In this study phytochemical screening has been used with the assistance of different subjective tests and for characterization, GC-MS has been utilized. Subjective screening demonstrated the presence of alkaloids, flavonoids, sugars and saponins. With the assistance of GC-MS, bioactive compounds got secluded and identified with a library. GC-MS chromatogram of petroleum ether extract of the tubers of *Aconitum heterophyllum* (LFD) exhibited 10 peaks indicating the presence of a good number of compounds.10 compounds got matched with the library and were identified. The results revealed that Ethoxyisoxazol-4-carboxylic acid (19.24 %) was a major compound followed by ethyl ester, Benzene-1,3-dicarboxylic acid, 5-hydroxymethyl-, diethyl ester, Hexa(methoxymethyl)melamine, gamma.-Aminobutyric acid, N-heptafluorobutyryl-, 1,1,1-trifluoro-2-propyl ester, Carbamic acid, N-(4-amino-6-ethylaminopyrimidin-5-yl)-, ethyl ester, 1,3,5,7,9,11-Hexa (trimethylene) - 1,3,5,7,9,11 – hexasila - 2,4,6,8,10,12 hexaoxacyclododecane, 1-[2-[2H,4H-1,2,4-Triazin-3,5-dithione]]-1-deoxy-.beta.-d-ribose,5-Methyl-1R,3-trans-cyclohexane diol, Barban, Pyridine, 3-nitro-2-(2H-1,2,3-triazole-4-yl)thio. Similarly, a GC-MS chromatogram of methanol extracts of *Aconitum heterophyllum* (JVFD) tuber tubers reported 5 peaks specifying the presence of substantial compounds. 5 compounds got matched with the library and were recognised. The results revealed that 9,12-Octadecadienoic acid (Z, Z) (alpha-linoleic acid) (28.81 %) was the paramount compound followed by 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Hexadecanoic acid, and cis,cis,cis-7,10,13-Hexadecatrienal. All compounds are known to possess a therapeutic potential and can be used by the drug industry. GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (LFD) exhibited 4 peaks.1,2-Benzenedicarboxylicacid,bis(2-methyl propyl) ester is a major compound followed by Cyclooctasiloxane, hexadecamethyl, Cycloheptasiloxane, tetradecamethyl, Silane, [bicyclo[4.2.0] octa-3,7-diene-7,8-diylbis (oxy)]bis[trimethyl respectively and GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (KFD) exhibited 1 peak only.1,2-Benzenedicarboxylicacid, bis(2-methyl propyl) ester was the major compound. (Figure 11,12,13,14)

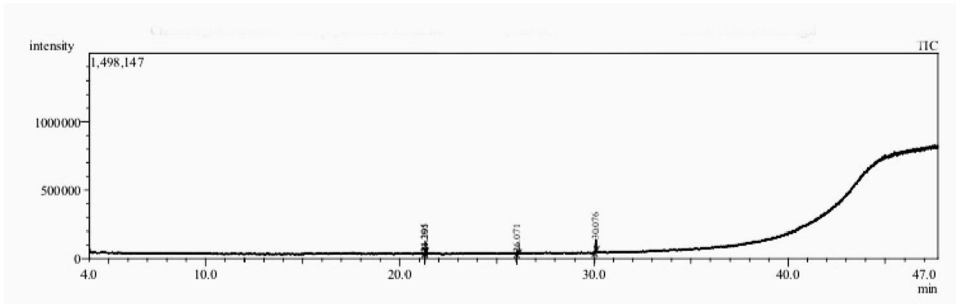


Figure 11: GC-MS chromatogram of methanol extract LFD

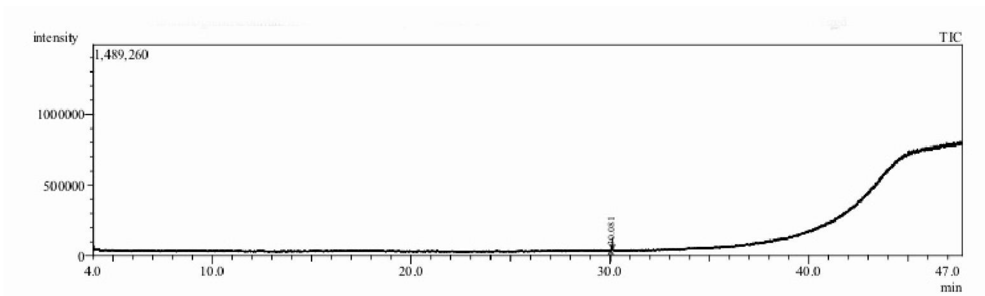


Figure 12: GC-MS chromatogram of methanol extract KFD

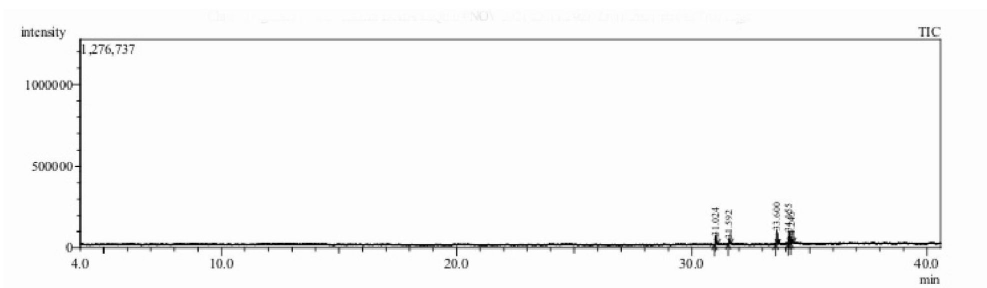


Figure 13: GC-MS chromatogram of methanol extract JVFD

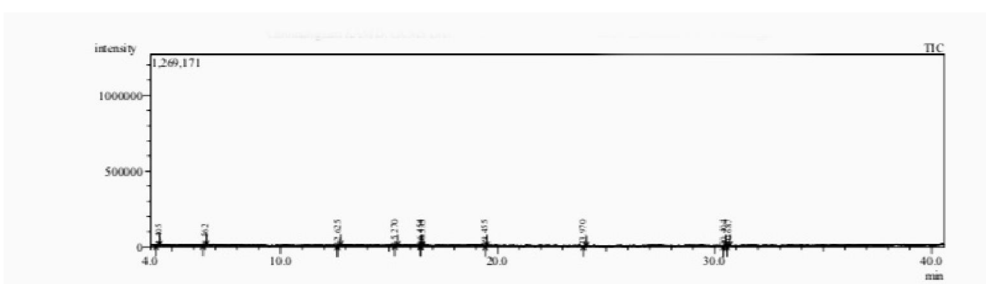


Figure 14: GC-MS chromatogram of petroleum ether extract LFD

Chapter-6

SUMMARY AND CONCLUSION

The summary and conclusion of this study are presented in the following headings.

6.1 Morphological characteristics

The plant height was recorded maximum (94.60 cm) at LFD, followed by (28.5 cm) at JVFD and (24.5 cm) at KFD. Leaf width was found maximum (7.96 cm) at LFD and minimum (3.76 cm) at JVFD. Leaf area was noticed maximum (78.02 cm^2) at LFD and minimum (16.58 cm^2) at KFD. Tuber length was recorded maximum (6.04 cm) at LFD and minimum (4.52 cm) at JVFD. The maximum number of flowers (11) were found at LFD and the minimum (7) at KFD. The tuber thickness was found maximum (2.1 cm) at LFD and minimum (1.1 cm) at KFD. The tuber biomass was recorded maximum (1.86 g) at LFD and minimum (1.26 g) at KFD.

6.2 Soil characteristics

The study revealed that the electrical conductivity was highest in KFD with a mean value of 0.45 ds/m having LL of 0.37 ds/m and UL of 0.45 ds/m followed by JVFD with a mean value of 0.33 ds/m and lowest in LFD with 0.30 ds/m.

It is obvious from the data that rhizospheric soil at KFD had the greatest phosphorus content, with a mean value of 21.8 indicating a medium level of phosphorus, followed by LFD with a mean value of 16.16 kg/ha and JVFD with a mean value of 13.09 kg/ha, indicating a low level of phosphorus. The pH level was highest at LFD, which was somewhat alkaline with a mean value of 5.8, followed by KFD with a mean value of 5.4 and lowest at JVFD with a value of 4.90. JVFD had the greatest nitrogen level with a mean of 473.81 kg/ha. Potassium level was highest at LFD with 487.46 kg/ha with LL 483 kg/ha and UL

492 Kg/ha as high in status followed by JVFD with 480 kg/ha and minimum in KFD with 389.588 kg/ha.

6.3 Extraction and Phytochemical screening

Methanol had the maximum extraction capacity and petroleum ether had the lowest. Similarly, prospectuses of sites about extract yield might be categorised as LFD>JVFD>KFD, with LFD having the greatest extract yield and KFD having the lowest percentage of yield. Petroleum ether extracts of three sites had chemical odour while methanol and aqueous extracts from three sites had a pungent smell. Petroleum ether extract of all the three sites was colourless while methanol and aqueous extract had honey brown and brown colour respectively. The GC-MS chromatogram of a petroleum ether extract of *Aconitum heterophyllum* tubers revealed ten peaks, suggesting the presence of a large number of chemicals Ethoxyisoxazol-4-carboxylic acid was found to be the most common chemical while as GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* revealed 5 peaks specifying the presence of substantial compounds 9,12-Octadecadienoic acid (Z, Z) was the paramount compound.

6.4 Conclusion

Humans have traditionally relied on plants as key sources of medicine all around the world. The rise of modern western medicine was initially accompanied by a decline in herbal medicine in many cultures and we came to assume that synthetic chemicals were the best treatments for treating illness and curing disease. Humans have been using plant-based cures since the dawn of time. For the treatment of various disorders, the people of Jammu and Kashmir (India) rely on a variety of medicinal plants. People in this area utilize extracts or parts of these plants to treat a variety of ailments. Morphological features assist to link the distribution of plants in space and time by indicating their distinct environments. For phylogeny, morphological traits are also important. Quantitative data on a species is essential for developing a conservation strategy and comprehending the ecology of the species; it aids in identifying the state of the species and may be used in conservation initiatives. Of all the qualities analysed, morphological characteristics were determined to be the most prevalent in the Haril area Langate Forest Division (LFD). The fact that some populations have a greater frequency of occurrence suggests that these species have the potential to perform better in particular places (habitats) in the region and maybe exploited for mass propagation/cultivation. Plants create a wealth of biogenic resources to create phytomedicines. However, among a variety of factors, the most important is the environment, which impacts the quality and amount of these chemical metabolites in plants. The methanol extract is reported to be effective in the current study because methanol had the maximum extraction capacity. From the present investigation, the petroleum ether extract included alkaloids and flavonoids, whereas the aqueous and methanol extracts contained carbohydrates and saponins. A petroleum ether extract of *Aconitum heterophyllum* tubers (LFD) yielded 10 peaks in a GC-MS chromatogram, indicating the presence of a high number of compounds. The most prevalent component was discovered to be ethoxy isoxazole-4-carboxylic acid, while as GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (LFD) exhibited 4 peaks.1,2-Benzenedicarboxylicacid,bis(2-methyl propyl) ester is a major compound. GC-MS chromatogram of a methanolic extract of *Aconitum heterophyllum* tubers (JVFD) revealed 5 peaks indicating the presence of significant chemicals. The most important component was 9,12-octadecadienoic acid (Z, Z)(alpha-linolenic acid), GC-MS chromatogram of methanolic extract of the tubers of *Aconitum heterophyllum* (KFD) exhibited 1 peak only.1,2-Benzenedicarboxylicacid, bis(2-methyl propyl) ester was the major compound. Ethoxy isoxazole-4-carboxylic acid is known to possess anti-inflammatory and antiarthritic effects while as by assisting in the maintenance of proper heart rhythm and pumping, alpha-linolenic acid is considered to lower the risk of heart disease. It may also help to prevent blood clots. 1,2-Benzenedicarboxylicacid, bis(2-methyl propyl) ester is found to be used in flooring, adhesives, lacquers, inks, hydraulic fluids, and lubricants. It is utilized in the manufacturing of titanium catalysers as well as a tax marker in fuels. In addition to these bioactive compounds Linoleic acid, one of the dominant fatty acids has been found. A fatty acid is required for the production of several hormones. Prostaglandins, thromboxanes, and leukotrienes are the hormones involved. Hormones of these three types are involved in the control of a wide range of physiological activities. This research supports isolating compounds from *Aconitum heterophyllum* tuberous roots for use in the treatment of a variety of ailments/diseases, with the added benefit of being readily available and cost-effective. It is, therefore, established by this study that the population growth in the Haril area of Langate Forest Division, has great potential in the pharmaceutical industry, hence must be used for large scale commercial cultivation to meet the demand.

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Certificate

Certified that all the corrections/amendments as suggested by External Examiner **Dr Javid Iqbal Mir,** Senior scientist ICAR-CITH during Viva-Voce examination held on 15-03-2022 have been incorporated in the manuscript entitled **"Morpho-chemical characterisation of *Aconitum heterophyllum* Wall. ex Royle., a critically endangered medicinal plant of Kashmir Himalayas"** submitted by **Ms. Mir Muskan Un Nisa (Regd. No. MSF-2019-107).**

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