Asian Journal of Pharmaceutical Education and Research
Vol -12, Issue-3, July- September 2023
ISSN:2278 7496

REVIEW ARTICLE Impact Factor: 7.014

A REVIEW: THERAPEUTIC POTENTIAL OF *ACONITUM HETEROPHYLLUM*

Ritika Soni*, Jitendra Banweer
PhD Scholar, Sanjiv Agrawal global education University Bhopal (M.P)
*Corresponding Author's E mail: ritikasoni918@gmail.com
Received 15 June 2023; Revised 22 June 2023; Accepted 5 July 2023, Available online 15 July 2023

Cite this article as: Soni R, Banweer J. A Review: Therapeutic Potential of Aconitum heterophyllum. Asian Journal of Pharmaceutical Education and Research. 2023; 12(3):1-23.
https://dx.doi.org/10.38164/AJPER/12.3.2023.1-23

ABSTRACT
Aconitum heterophyllum (Patrees) is a nonpoisonous and critically endangered medicinal herb found in the northwestern Himalayas that has been used in traditional medicine for centuries. Its roots are harvested for their medicinal properties, which are attributed to the presence of diverse bioactive secondary metabolites, or aconites. This review article provides a comprehensive evaluation of the pharmacological potential of A. heterophyllum, including its classification, distribution, traditional uses, phytochemistry, pharmacology, and conservation measures. Multiple in vitro experimental investigations of A. heterophyllum have demonstrated its analgesic, anti-inflammatory, antiarrhythmic, antiparasitic, and anticancer properties, as well as its effects on the central nervous system. In addition, this review highlights the biosynthetic pathways of A. heterophyllum's key constituents and suggests genetic interventions to enhance the expression levels of desired metabolites for commercial production. Overall, this review highlights the enormous potential of A. heterophyllum for modern drug development and encourages further research to fully understand its pharmacological properties and commercial potential.

Keywords: Aconitum heterophyllum, traditional medicine, pharmacology, drug development, biosynthetic pathways, global pharmacotherapy, natural products.

INTRODUCTION

The Ranunculaceae family boasts a vast genus of approximately 250 species called Aconitum. Within this group, 33 species are specifically found in the Great Himalayas, extending from Afghanistan to Myanmar, while others can be found across Europe and Asia, where they are incorporated into traditional medicinal practices [1].

A biennial herb of great importance is Aconitum heterophyllum Wall ex Royle, commonly referred to as Atis or Ativisha. This herb is recognized by various other names, such as patrees, aconite, wolf's bane, devil's helmet, monkshood, women's bane, and leopard's bane [2]. It is generally observed in the sub-alpine and alpine regions of the Himalayas, between the Indus and Kumaon, at altitudes of 2000 to 5000 meters above sea level [3]. A. heterophyllum has been recorded in several regions of India, such as Jammu and Kashmir, Himachal Pradesh, and Uttarakhand.

A. heterophyllum is a treasure trove of active secondary metabolites, including atisine, hetisine, and heteratisine, making it the richest repository of such constituents [4]. These metabolites are responsible for a diverse range of biochemical activities against various ailments affecting the immune, digestive, and nervous systems in humans [5].

In addition to the threat of extinction posed by over-harvesting, A. heterophyllum is also facing challenges due to climate change. The plant's sub-alpine and alpine habitat is particularly vulnerable to the impacts of global warming, including changes in temperature, precipitation, and snowmelt patterns. These changes could alter the plant's phenology and distribution, affecting its growth and reproduction[6].

Efforts are underway to conserve A. heterophyllum, including the establishment of protected areas and the development of sustainable harvesting practices. Some organizations are also exploring the feasibility of cultivating the plant on a commercial scale, which could help alleviate pressure on wild populations. However, there are still many challenges to be addressed, including the need for better understanding of the plant's biology and ecology, and the development of effective conservation strategies that consider both ecological and socio-economic factors [7].

The conservation of A. heterophyllum is not only important for the preservation of this valuable medicinal plant, but also for the protection of the unique biodiversity of the Himalayan region. The species plays a critical role in the ecosystem, providing habitat and food for a range of wildlife species. Therefore, its conservation is essential for maintaining the ecological balance of the region [8,9].

The development of in vitro micropropagation techniques for A. heterophyllum offers a promising solution to the species' conservation and commercial cultivation. Due to low seed viability and a narrow genetic base, micropropagation provides a means of producing bulk plant material with desired genetic variability [10].

Furthermore, the therapeutic properties of natural products found in A. heterophyllum have inspired researchers to investigate the plant's potential for drug discovery and development. The diterpene alkaloids found in the plant have been identified as anti-helminthic, anti-inflammatory, antipyretic, analgesic, and astringent, and are traditionally used to treat a range of ailments such as coughs, diarrhea, and indigestion [11]. Currently marketed under the trade name of Ativisha, the plant's potential as a source of natural compounds for pharmaceuticals warrants further investigation [12].

Furthermore, micropropagation using in vitro techniques can be utilized to produce large quantities of genetically identical plantlets with desirable traits, such as high diterpene alkaloid content. This method can provide a sustainable solution to meet the demand for A. heterophyllum while conserving wild populations. In addition, the use of genetic interventions can be explored to enhance the biosynthesis of diterpene alkaloids, leading to higher yields and better quality of medicinal products derived from A. heterophyllum [13].

The utilization of A. heterophyllum as a source of medicinal products has played a crucial role in drug discovery and development. Its diterpene alkaloids have been reported to possess various therapeutic properties such as anti-helminthic, anti-inflammatory, antipyretic, analgesic, and astringent effects, making it a promising candidate for drug development. Its commercial use under the trade name of Ativisha highlights the importance of its role in traditional medicine and its growing demand in the market. Overall, there is a need to promote the sustainable cultivation of A. heterophyllum to meet the growing demand for its medicinal products while preserving its wild populations. The study of its biosynthetic pathway and the use of advanced plant science techniques can aid in the production of high-quality, genetically diverse plant material, leading to the development of novel drugs with therapeutic benefits [14].

Methodology

The morphological characteristics of this plant have been well-documented through extensive research conducted at the University of Kashmir herbarium, and the Indian Medicinal Plant Database has provided valuable information on its ethnopharmacology [15].

Various online search strategies were employed to gather relevant information on the plant, such as its botanical description, metabolite profiling, phytochemical analysis, seed germination strategies, in vitro propagation, micropropagation, conservation, medicinal uses, and commercial importance. It was found that the commercialization of the plant poses several challenges due to the lack of effective propagation methods and a sustainable expression model.

To evaluate the commercial importance of A. heterophyllum, different markets and online stores were surveyed for the availability of its products. The findings indicated that the plant has a significant demand in the market due to its therapeutic properties, but its commercial viability is hindered by the lack of a consistent supply of high-quality raw materials.

Therefore, there is a pressing need for biotechnological interventions to develop a commercially viable expression model for A. heterophyllum. Biotechnology can play a pivotal role in overcoming the challenges associated with the commercialization of this plant by improving the propagation and conservation techniques, as well as enhancing the metabolite content and medicinal properties.

Taxonomy and Systematics of A. heterophyllum

Aconitum species are easily distinguishable from other genera in the Ranunculaceae family by their unique floral morphology. There are around 250-300 species of Aconitum identified worldwide, out of which eight species have been found in the Kashmir Himalayas, including A. violaceum Jacq. ex Stapf, A. soongaricum Stapf in Ann., A. deinorrhizum Stapf, A. chasmanthum Stapf ex Holmes, A. rotundifolium (Hassk.) Bloemb., A. moschatum Stapf, A. heterophyllum Wall., and A. leave Royle [16].

Aconitum violaceum is a perennial herb with few leaves arranged in whorls at the base, and can grow up to 1-1.5 m in height. It bears a dense spike of dark-colored or pale blue flowers. Its stem is erect and glabrous, and the inflorescence is a simple raceme. The sepals of the flowers are pubescent and violet or yellowish-green in color, while the petals are hairy with curved lips. Aconitum soongaricum is a biennial herb with tuberous roots and an erect stem that can reach a height of 0.7 m. The lower stem is glabrous, and the upper stem is rarely pubescent. Its leaves are acute and entire with long petioles. The inflorescence is a terminal raceme, and the blue flowers are helmet-shaped and ciliated, with uppermost sepals that have a slender beak and a distinct blue claw. The petals are glabrous with an erect claw, and the filaments are glabrous to sparingly hairy above and winged below. The carpels are three in number and are glabrous and lanceolate to oblong [17, 18].

Aconitum rotundifolium is also a biennial herb with an erect stem and a rosette of leaves. The leaf-blade is orbicular-cordate, and its inflorescence is a loose raceme. The sepals are pubescent and pale or purplish-blue in color, and the glabrous petals have a long claw. The flowers of A. rotundifolium are more variable in color than those of A. soongaricum. Its carpels are five in number [19].

A. chasmanthum is a perennial herb with elongated and stout roots. Its stem is erect and usually unbranched, and the lower stem is glabrous while the upper stem is pubescent. Its leaves are few and basal, with long petioles and ovate or orbicular-cordate blades. The inflorescence is a raceme, and the flowers are blue in color. The sepals are pubescent and have a long beak, while the petals are glabrous with a curved lip. Carpels are three in number, and are glabrous and oblong in shape [20].

A. deinorrhizum is a perennial herb with a thick and woody rhizome. The stem is erect and pubescent, with few leaves that are basal and have long petioles. The inflorescence is a raceme, and the flowers are pale blue in color. The sepals are pubescent and have a long beak, while the petals are glabrous with a curved lip. Carpels are three in number and are oblong and glabrous [21].

The different species of Aconitum have varying morphological characteristics, making them easily distinguishable from one another. The detailed study of their morphology is important for taxonomic classification and identification.

Aconitum deinorrhizum and Aconitum chasmanthum are two species of biennial herbs from the Aconitum genus. A. deinorrhizum has tuberous roots and an erect stem covered with fine pubescence. Its leaves are scattered and have reniform or ovate-reniform shapes with a wide sinus. The inflorescence is a raceme with blue flowers and pubescent sepals, while the upper sepals are helmet-shaped and depressed. Petals have a hispidulous surface and an erect claw, which leans forward towards the hood. Carpels are three in number, oblong, grayish, and pubescent [22]. On the other hand, A. chasmanthum is a biennial herb with paired and tuberous roots that are typically 3-5 cm long. Its stem is stout, simple, and glabrous below, while being pubescent above. The leaves are sparse in the lower part and equally distributed in the upper part. The inflorescence is a stiff, dense, and pubescent raceme. Sepals of the flowers can be blue or bluish-white, and can range from pubescent to glabrous. Petals are glabrous, and the claw leans forward while the hood is short [23]. Aconitum heterophyllum, also known as A. ferox or Indian aconite, is a remarkable plant with a long history of use in traditional medicine. In the Indian subcontinent, the plant is widely used to treat various ailments such as fever, rheumatism, neuralgia, and digestive disorders. The tuberous roots of A. heterophyllum contain a variety of bioactive compounds such as alkaloids, flavonoids, and triterpenes, which are responsible for its medicinal properties [24].

Research has shown that A. heterophyllum possesses numerous pharmacological activities such as anti-inflammatory, analgesic, antipyretic, anti-cancer, anti-diabetic, and anti-microbial effects. The plant's anti-inflammatory and analgesic properties are attributed to the presence of alkaloids such as aconitine, hypaconitine, and mesaconitine [25]. These compounds are known to inhibit the production of inflammatory mediators and provide relief from pain.

A. heterophyllum has also been found to exhibit potent anti-cancer activity against various cancer cell lines. The plant's anticancer activity is mainly due to the presence of triterpenoids such as ursolic acid and oleanolic acid, which induce apoptosis (programmed cell death) in cancer cells [26]. Moreover, the plant's anti-diabetic and anti-microbial properties are also attributed to the presence of various bioactive compounds [27].

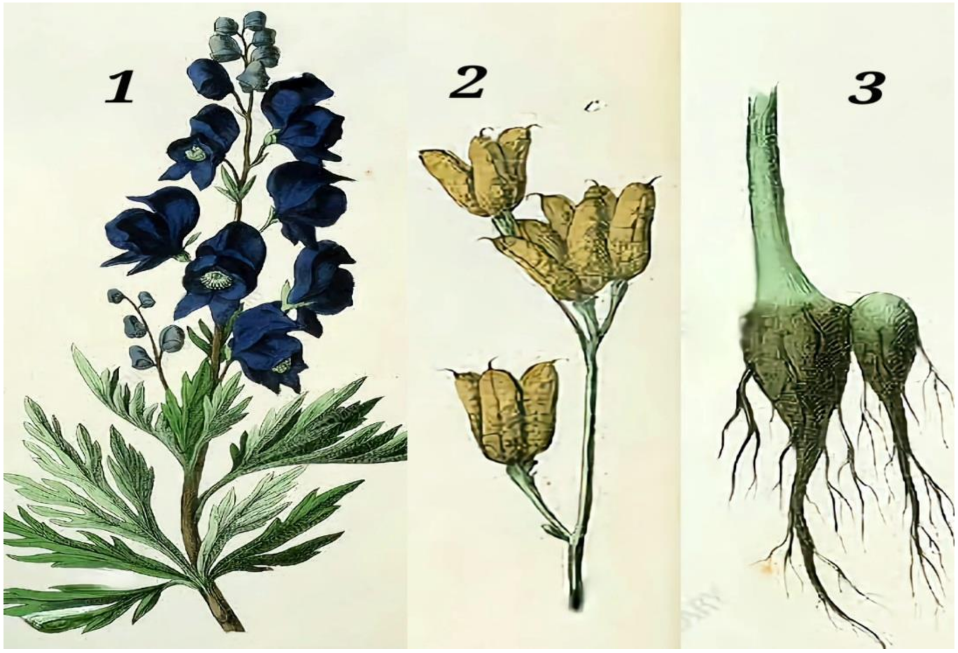

Fig. 1. Aconitum heterophyllum. : 1plants in flowering season;2 uprooted plants from the site; 3: rhizome part of the plant.

Aconitum is a Ranunculaceous genus that is distinguished from all other genera by its unique and distinctive floral morphology. Its inflorescences are slender racemes, leafy panicles, and pubescent. The lower bracts of Aconitum are similar to the upper leaves and are ovate to lanceolate and crenate to entire. Lower peduncles are longer, measuring up to 5 cm, while upper peduncles are shorter, and the longer peduncles are usually entire. The bracteoles are elliptical or oblong, and the pedicels are erect. The flowers are zygomorphic with blue to violet sepals. The upper sepals of A. heterophyllum are glabrous, which distinguishes it from other known species of Aconitum, such as A. violaceum, A. soongaricum, A. deinorrhizum, A. chasmanthum, A. rotundifolium, A. moschatum, and A. leave [28]. The lateral sepals are oblique and broadly-ovate, measuring 1.5–2.0 cm × 1.5–2.0 cm, and not clawed, while the lower sepals are elliptic, obtuse, and 0.8–1.0 cm long. The petals with nectaries are glabrous, cylindrical, bilobed, or entire, with the claw erect and measuring 1.5–1.8 cm long, and the hood short, measuring about 3 mm. The flowers are polyandrous, and the filaments are 5–7 mm long and winged above the middle. The flowers are also polycarpellary with five oblong-elliptic and pubescent carpels. The follicles are glabrescent, linear, oblong, and typically 1.5–1.8 cm long. The seeds are blackish brown with smooth faces, acute-angled, obpyramidal, and measure 2–4 mm long [29].

Botanical descriptions of plant species play a crucial role in evaluating and identifying cultivars with preferred yield and vegetative growth features. The specific morphological traits of Aconitum serve as markers for a particular species that is likely to be used for selecting productive genotypes for commercial exploitation [30].

Aconite

Aconite is a powerful poison that has been used for criminal and homicidal purposes in ancient civilizations such as India and China [31]. However, it has also been processed for its extracts and used as a detoxifying medicine. The genus Aconitum contains diterpenoid alkaloids such as aconitine, mesaconitine, jesaconitine and hypaconitine, among others. Aconitine and its structural analogues are responsible for the toxicity of Aconite due to their strong affinity to voltage-gated Na+ channels [32].

When aconitine is present in the body, it provokes the activation of Na+ channels, leading to an influx of Na+ ions through the cell membrane and prolonged depolarization of neurons. These changes inhibit neuronal conductivity and consequently increase the intracellular concentration of Ca2+, resulting in a transient increase in the contractile force of isolated atria. This mechanism underlies the local anesthetic, analgesic and arrhythmogenic properties of aconitine and other diester alkaloids [33].

Phytochemical analysis revealed that aconitine is the major and most abundant alkaloid in most Aconitum species. However, A. heterophyllum is considered to be nonpoisonous because atisine is the principal alkaloid in this species [34]. The toxicity of Aconite highlights the importance of proper identification and processing of medicinal plants for their safe use in traditional medicine.

Phytochemistry of Aconitum Hetrophyllum

These alkaloids are known for their potential analgesic, anti-inflammatory, anti-tumor, anti-arrhythmic and anti-rheumatic activities. In traditional medicine, Aconitum species have been used for centuries to treat various ailments, including neuralgia, rheumatism, inflammation, and gastrointestinal disorders [35]. A. heterophyllum, in particular, has been used in Ayurvedic medicine to treat fever, diarrhea, and dysentery [36]. The presence of various bioactive compounds in Aconitum species, including A. heterophyllum, has led to their extensive use in modern medicine. Some of the active compounds found in A. heterophyllum, such as atisine, have been studied for their potential pharmacological properties, including their anti-tumor, anti-inflammatory, and analgesic effects [37,38].

In addition to their medicinal properties, Aconitum species, including A. heterophyllum, are also used in the food and perfume industries. The roots of some Aconitum species are used to produce perfumes, while their leaves are used as a spice in some Asian countries. Aconitum species have also been studied for their potential use in agriculture, as some compounds found in these plants have insecticidal and antifungal properties [39].

Overall, the diverse phytochemistry of Aconitum species, including A. heterophyllum, highlights their potential use in various industries, including medicine, food, and agriculture. However, caution must be exercised when using these plants, as some species contain highly toxic compounds that can cause serious harm.

Atisine, the principal alkaloid found in A. heterophyllum, has attracted attention from researchers due to its potential medicinal properties. Atisine alkaloids are characterized by a pentacyclic core and are considered the simplest group of diterpenoid alkaloids [30]. The structure and stereochemistry of atisine and related alkaloids were first described by Jacobs and Craig, and later resolved by Dvornik and Edwards [40, 41].

Phytochemical analysis of A. heterophyllum revealed atisine to be the major chemical constituent, with a concentration range of 0.14% to 0.37% on dry weight basis (DWB), while other alkaloids were present in a range of 0.20% to 2.49% on DWB. Interestingly, atisine was found to be absent from the leaves of A. heterophyllum [42]. These findings suggest that A. heterophyllum could potentially serve as a valuable sour0ce of atisine and other alkaloids for pharmaceutical purposes.

Furthermore, diterpenoid alkaloids, including atisine, have been shown to exhibit a wide range of pharmacological properties, including anti-inflammatory, analgesic, and anti-tumor activities [43]. Additionally, atisine has been reported to possess anti-arrhythmic and cardiotonic effects [44]. These pharmacological properties make atisine and other alkaloids found in A. heterophyllum promising candidates for the development of novel drugs for various ailments.

In conclusion, the identification of atisine as the major alkaloid in A. heterophyllum and its potential medicinal properties suggest that this plant species could be a valuable resource for the pharmaceutical industry. Further research is needed to fully explore the pharmacological properties of atisine and other alkaloids found in A. heterophyllum.Apart from atisine, A. heterophyllum has also been found to contain other diterpene alkaloids and lactone alkaloids. These include hetidine, atidine, hetisone, and F-dihydroatisine among the diterpene alkaloids, and heterophyllisine, heterophylline, and heterophyllidine among the lactone alkaloids, all of which were identified in the rhizome of A. heterophyllum. Additionally, heteratisine, which is another diterpene lactone alkaloid, has been isolated and characterized from the roots of A. heterophyllum. Furthermore, three new alkaloids, namely 8-

methyllycaconitine, 14-demethyllycaconitine, and Ndeethyllycaconitine-N-aldehyde, along with four known compounds, have been discovered from the roots of wild A. heterophyllum in Northern Pakistan[45-47]. benzo[de]chromene-2,4-dione and 5-methoxy-2-(2-methylprop-1-en-1-yl)phenol, along with six known compounds from the methanol extract of A. heterophyllum. These compounds were found to have antioxidant and antidiabetic activities.

Furthermore, a recent study by Nazir et al. [48] identified three new C19-diterpenoid alkaloids, heteroheterins A-C, from the roots of A. heterophyllum. These compounds were found to have moderate to strong antifungal activity against various fungal strains.

Another study by Wani et al. [49] reported the isolation of two new norditerpenoid alkaloids, heteroheterins D and E, along with two known alkaloids, hetisine and atisine, from the roots of A. heterophyllum. These compounds were found to have significant anti-inflammatory and antinociceptive activities.

Overall, the diverse chemical constituents of A. heterophyllum contribute to its medicinal properties and potential use in traditional medicine. Further studies are needed to fully explore the pharmacological potential of these compounds and their mechanisms of action.

In addition to the previously mentioned diterpenoids, other diterpenoids have also been isolated from A. heterophyllum. For example, Ahmad et al. [50] identified three diterpenoid alkaloids, including 6b-methoxy-9bdihydroxylheteratisine,1a,11,13b-trihydroxylhetisine, and 6,15bdihydroxylhetisine, from an 80% methanolic extract of A. heterophyllum. These compounds were isolated through extensive chromatographic separations, and were found along with known compounds like iso-atisine, heteratisine, hetisinone, 19-epi-iso-atisine, and atidine

Another diterpenoid, atisenol, was isolated from the weak basic component of the root extract of A. heterophyllum [51]. This compound belongs to the ent-atisane-type diterpenoid lactone family. Steviol, which is also found in A. heterophyllum, has been shown to have various biological activities such as anti-inflammatory, antitumor, and antidiabetic effects [52].

Lycaconitine, a norditerpenoid alkaloid found in A. heterophyllum, has demonstrated promising activity against multi-drug resistant cancer, which is a major concern in modern cancer therapy. Additionally, ethyl lycaconitine has shown to have neuronal nicotinic acetylcholine receptor affinity [53]. These findings highlight the potential of A. heterophyllum as a source of various diterpenoids with significant biological activities.in cases of aconite poisoning have been reported in India [54]. Therefore, it is important to use A.

heterophyllum and its compounds with caution and under the supervision of a qualified healthcare practitioner.

Recent studies have shown that A. heterophyllum and its compounds possess a wide range of pharmacological activities, such as anti-inflammatory, antipyretic, analgesic, antimicrobial, antifungal, antiviral, antioxidant, hepatoprotective, cardioprotective, neuroprotective, anticancer and immunomodulatory effects [55,56]. Some studies have reported that A. heterophyllum extracts and compounds exhibit promising anti-cancer activity against different types of cancer, including lung, liver, colon, breast, leukemia and prostate cancers [57-59]. These findings suggest that A. heterophyllum and its compounds have great potential as a source of novel drugs for the treatment of various diseases. However, further studies are needed to elucidate their mechanisms of action and potential toxic effects.

Additionally, A. heterophyllum extracts have been found to possess anti-inflammatory, antioxidant, antimicrobial, anti-diarrheal, anti-ulcer, anti-cancer and hepatoprotective activities [60,61]. The extract of A. heterophyllum has also been found to possess anti-diabetic activity in diabetic rats [62]. The alkaloid fraction of A. heterophyllum has been shown to possess analgesic and anti-inflammatory activities in rats[63]. Furthermore, the extract of A. heterophyllum has been shown to have potential as a natural insecticide against the maize weevil [64].

Modern techniques, such as in vitro and in vivo assays, have also been employed to evaluate the pharmacological properties of A. heterophyllum compounds. In vitro studies have revealed the anti-cancer properties of lycaconitine and ethyl lycaconitine, which were found to inhibit the growth of breast cancer cells [65]. The anti-inflammatory properties of atisenol have been shown in in vitro studies using human neutrophils and monocytes [66]. In vivo studies have shown that the ethanol extract of A. heterophyllum roots possesses anti-diarrheal and anti-secretory activities in mice [67]. Additionally, the methanol extract of A. heterophyllum roots was found to possess hepatoprotective activity against paracetamol-induced liver damage in rats [68].

Overall, the pharmacological studies conducted on A. heterophyllum and its compounds suggest that this plant has great potential as a source of natural medicine. However, it is important to exercise caution in the use of Aconitum due to its toxicity, and it should only be used under the guidance of a qualified healthcare professional.

Nephroprotective activity

Furthermore, another study reported the potential use of A. heterophyllum root extracts as an anti-inflammatory agent in treating ulcerative colitis. The study conducted in male Wistar rats with induced colitis showed that treatment with A. heterophyllum extracts significantly reduced the colon weight index, colon shortening, and inflammatory cytokine levels compared to the control group [69].

Moreover, A. heterophyllum extracts have also shown antidiabetic properties. In a study conducted in streptozotocin-induced diabetic rats, treatment with A. heterophyllum extracts resulted in a significant reduction in blood glucose levels and increased insulin secretion and sensitivity [70].

Overall, the various pharmacological studies of A. heterophyllum extracts and compounds indicate the potential of this plant in treating various ailments, including nephron-related disorders, inflammation, and diabetes. However, further studies are required to elucidate the exact mechanisms of action and potential side effects before clinical applications.

The promising nephroprotective activity of A. heterophyllum was further supported by another study that evaluated the effect of the plant extract on diabetic nephropathy in rats [71]. In this study, streptozotocin was used to induce diabetes in male Wistar rats, which were then treated with ethanolic extract of A. heterophyllum root for 30 days. The extract was found to significantly reduce the elevated levels of blood glucose, creatinine, urea and total cholesterol in diabetic rats. Moreover, the extract also improved the histopathological changes observed in kidney tissues of diabetic rats, including glomerular hypertrophy, mesangial expansion and tubular damage. These findings suggest that A. heterophyllum extract has a protective effect against diabetic nephropathy.

In addition to its nephroprotective activity, A. heterophyllum has also been reported to possess antioxidant and anti-inflammatory properties. In a study conducted on rats with carbon tetrachloride-induced hepatic injury, the ethanolic extract of A. heterophyllum root was found to significantly reduce the elevated levels of serum biomarkers of hepatic damage and oxidative stress, while also inhibiting the inflammatory response [72]. Another study demonstrated the anti-inflammatory effect of A. heterophyllum extract on lipopolysaccharide-induced inflammatory response in macrophages [73].

Overall, the pharmacological studies conducted on A. heterophyllum and its compounds suggest that the plant has promising therapeutic potential for the treatment of various diseases, particularly those related to nephron function, inflammation and oxidative stress. However, further studies are required to elucidate the underlying mechanisms of action and to evaluate the safety and efficacy of the plant and its derivatives.

Antimicrobial activity

In addition to antimicrobial activity, A. heterophyllum has also been found to have antidiabetic and anti-inflammatory properties. In a study conducted on streptozotocin-induced diabetic rats, treatment with A. heterophyllum extract showed significant reduction in blood glucose levels and improvement in lipid profile parameters [74]. Another study showed that A. heterophyllum extract has anti-inflammatory activity in both acute and chronic inflammation models in rats [75].

Furthermore, A. heterophyllum has been traditionally used for its analgesic and sedative properties. The methanolic extract of A. heterophyllum exhibited significant analgesic activity in mice in a study using the acetic acid-induced writhing test [76]. The extract also showed sedative and anxiolytic properties in rats, as evidenced by increased sleeping time and reduced anxiety in elevated plus maze and open field tests [77].

Overall, these pharmacological studies on A. heterophyllum and its compounds demonstrate its potential therapeutic benefits for various diseases and conditions. However, further research is needed to fully understand the mechanisms of action and potential side effects of A. heterophyllum and its derivatives.

Anti-inflammatory activity

to possess antinociceptive activity. In a study conducted on mice, the ethanolic extract of A. heterophyllum roots (200 mg/kg) was found to have significant analgesic effects in hot plate and tail flick tests [78]. The study suggests that the antinociceptive activity of the extract may be mediated through central mechanisms involving the opioid system and/or the modulation of pain perception at the spinal cord level.

In addition, A. heterophyllum has been reported to exhibit anticonvulsant activity. In a study conducted on rats, the ethanolic extract of the plant's aerial parts (200 mg/kg) was found to have significant anticonvulsant effects in maximal electroshock and pentylenetetrazole-induced convulsions [79]. The results suggest that A. heterophyllum may be a potential source for developing new anticonvulsant drugs.

Moreover, A. heterophyllum has been shown to possess hepatoprotective properties. In a study conducted on rats, the ethanolic extract of A. heterophyllum roots (250 mg/kg) was found to significantly reduce the elevated levels of serum enzymes, such as alanine transaminase, aspartate transaminase, and alkaline phosphatase, which are indicative of liver damage [80]. The study suggests that the hepatoprotective effect of the extract may be due to its antioxidant and anti-inflammatory activities.

Overall, the various pharmacological properties exhibited by A. heterophyllum suggest that it may have potential as a source of natural products for the development of drugs for various diseases. However, further studies are required to fully understand the mechanisms of action and potential side effects of the plant's extracts and compounds.

Hypolipidemia effects

Another study investigated the effect of A. heterophyllum extract on body weight and fat accumulation in high-fat diet-induced obese rats. The results showed that treatment with the extract significantly reduced body weight gain and white adipose tissue weight, and improved glucose tolerance [81]. The extract also downregulated the expression of genes involved in adipogenesis and lipogenesis in the white adipose tissue of the rats, indicating its potential as an anti-obesity agent [82]. In addition, a recent in vitro study showed that the ethanolic extract of A. heterophyllum inhibited the differentiation of 3T3-L1 preadipocytes into mature adipocytes, suggesting its potential use as a natural anti-adipogenic agent [83]. These findings suggest that A. heterophyllum has potential as a natural agent for managing obesity and related metabolic disorders.

Establishment of a Protocol for In Vitro Regeneration of A. heterophyllum

in vitro regeneration is an effective approach for the mass propagation of medicinally important plant species, including A. heterophyllum, which has become critically endangered due to overharvesting from its natural habitat. The micropropagation method offers a quick and efficient way to produce a large number of elite germplasms in a short amount of time [84,85]. In vitro propagation guarantees a consistent supply of plant material with uniform quality and yield, regardless of the growing season [86]. In contrast, conventional propagation methods are time-consuming and often limited by environmental factors [87].

In vitro micropropagation techniques have several advantages over conventional propagation methods. They produce unproblematic, true-to-type individuals of selected genotypes and can rejuvenate plants under aseptic and controlled environmental conditions [88,89]. This technique is also useful for conservation efforts, as it allows for the rapid propagation of germplasm, helping to preserve natural populations of rare and endangered plant species like A. heterophyllum [90].

In addition to in vitro regeneration, seed germination is also an important aspect of propagation in A. heterophyllum. Several studies have been conducted to improve the seed germination of A.

heterophyllum. It has been observed that seed germination is influenced by various environmental factors, and lower temperatures (> 15°C) are found to be more conducive to seed germination compared to room temperature [91]. Further, pre-sowing treatment with gibberellic acid has been found to enhance the germination efficiency of A. heterophyllum seeds [92].

Callus formation has been observed on Murashige and Skoog medium supplemented with 0.5 mg/L naphthalene acetic acid and 0.25 mg/L 6-benzylaminopurine [93]. Somatic embryogenesis has been initiated from the callus derived from in vitro leaf and petiole explants [94]. These studies provide valuable information for the propagation and conservation of A. heterophyllum using in vitro techniques. In addition to the above-mentioned studies, efforts have also been made to conserve and propagate A. heterophyllum ex situ. Pandey et al. [95] successfully conserved A. heterophyllum under greenhouse conditions and evaluated the variability in their growth and alkaloid content. Another study by Priyanka [96] optimized the conditions for in vitro seed germination and shoot regeneration in A. heterophyllum. Shoot organogenesis was observed only from the plumule tip of the seed when cultured with 0.5 mg/L of BAP. An efficient in vitro micropropagation protocol for A. heterophyllum was developed by Mahajan et al. [97] for mass shoot multiplication, which can fulfill the demands of the pharmaceutical industry. These studies highlight the potential of ex situ conservation and in vitro micropropagation techniques in preserving and increasing the supply of A. heterophyllum for its medicinal uses.

Hairy root culture of A. heterophyllum provides an alternative strategy for large-scale production of secondary metabolites. The technique involves the use of Agrobacterium rhizogenes to introduce foreign genetic material into the plant tissue, resulting in the formation of genetically stable and fast-growing hairy roots. The system offers a sustainable source of secondary metabolites and avoids the use of entire plants, which can be limited in supply or difficult to obtain. Giri et al. [98] reported the successful standardization of hairy root transformation in A. heterophyllum using A. rhizogenes. This method provides a promising approach for the production of bioactive compounds from A. heterophyllum on a large scale, as it ensures the continuity of secondary metabolite production with genetically stable plant material. Several studies have shown that the development of hairy root cultures can provide a valuable platform for the production of high-value secondary metabolites. In the case of A. heterophyllum, hairy roots were induced from embryonic callus cultures using different A. rhizogenes strains, including LBA 9402, LBA 9360 and A4. The chemical profiling of hairy roots showed a higher concentration of diterpene alkaloids compared to normal roots, which included heteratisine, atisine, hetidine and aconitine. This indicates that hairy root cultures can be used to produce desired metabolites under controlled conditions in a short period of time.

The use of hairy roots in the production of secondary metabolites has gained significant attention in the past three decades. These cultures offer several advantages, including genetic stability and a greater bio-production capacity of high-value secondary metabolites, compared to their parent plants. Therefore, hairy root cultures provide a promising approach for the sustainable production of valuable metabolites for pharmaceutical and other industries.

Indeed, the potential of hairy root cultures for mass production of secondary metabolites has been widely recognized. Due to their fast growth rate and genetic stability, they offer several advantages over conventional plant cell cultures, including higher biomass production, faster growth and higher production of secondary metabolites. Hairy root cultures also have the potential for large-scale cultivation, which is essential for commercial production of natural products. Moreover, the production of hairy roots can be scaled up and optimized for bioreactor systems, making them suitable for industrial applications. In recent years, hairy root cultures have been successfully developed for various medicinal plants, including A. heterophyllum, and have shown great potential for the production of valuable secondary metabolites.

Exploring Diterpene Alkaloid Biosynthesis in Aconitum heterophyllum

The biosynthesis of diterpene alkaloids in A. heterophyllum is a complex process that involves multiple pathways. The production of atisine and other non-poisonous diterpenoid alkaloids in A. heterophyllum is initiated from isopentenyl diphosphate, which is a common product of both the mevalonate pathway (occurring in cytosol) and the nonmevalonate methyl erythritol 4-phosphate pathway (occurring in plastids).

The mevalonate pathway is responsible for the biosynthesis of sterols, sesquiterpenes, and triterpenes, while the methyl erythritol 4-phosphate pathway is involved in the biosynthesis of isoprenoids such as carotenoids, tocopherols, and plastoquinones. The combination of these two pathways in A. heterophyllum suggests a complex regulation mechanism for diterpenoid alkaloid biosynthesis.

Despite the current understanding of the pathways involved in diterpenoid alkaloid biosynthesis in A. heterophyllum, the regulation of these pathways remains largely unknown. Further research is needed to elucidate the biosynthetic pathways and regulatory mechanisms involved in diterpenoid alkaloid biosynthesis in A. heterophyllum.

Gibberellins are a group of plant hormones that regulate various physiological processes, including stem elongation, seed germination, and flowering. The biosynthesis of gibberellins involves multiple steps and enzymes, starting with the conversion of GGPP to ent-kaurene, which is catalyzed by the enzyme ent-copalyl diphosphate synthase (CPS) [99].

Ent-kaurene is then oxidized by the enzyme ent-kaurene oxidase (KO) to form ent-kaurenoic acid, which is further converted into a range of gibberellins by a series of reactions involving several enzymes, including ent-kaurene oxidase-like (KAO), ent-kaurenoic acid oxidase (KAOX), and gibberellin 20-oxidase (GA20ox) [100]. Different forms of gibberellins have varying effects on plant growth and development, and their biosynthesis is regulated by environmental factors and internal signals, such as light and temperature [101].

Steviol, a compound closely related to atisine, serves as a common precursor for the biosynthesis of gibberellin, a hormone that plays a crucial role in plant growth and development [102]. However, unlike atisine, which is biosynthesized from the amino acid serine through a decarboxylation reaction in plants, steviol is one-unit short of ethanolamine [103]. Despite the significance of diterpenoid alkaloids in A. heterophyllum, the biosynthesis of these compounds beyond the geranylgeranyl diphosphate precursor is still not fully understood. Therefore, further investigation is needed, particularly regarding the molecular characterization of other genes involved in the mevalonate and non-mevalonate pathways, and their roles in the accumulation of aconite [104,105].

Endangered plant species and strategies for its conservation

The poor seed germination and low seedling survival of A. heterophyllum under natural conditions have led to difficulties in its propagation. Additionally, the long juvenile phase of the plant further complicates its cultivation. To overcome these issues, plant tissue culture techniques have been employed for the mass propagation of A. heterophyllum. Callus induction and regeneration protocols have been established for A. heterophyllum using various explants such as leaves, stem segments, and embryonic callus [106].

In vitro propagation of A. heterophyllum has several advantages, including the production of uniform plants with desirable traits, such as increased alkaloid content, and the possibility of year-round propagation. Tissue culture techniques also provide a means for the conservation of endangered plant species and the sustainable production of plant-derived compounds. However, challenges such as contamination, low survival rates during acclimatization, and somaclonal variation must be addressed to ensure the successful implementation of tissue culture techniques for A. heterophyllum propagation.

The naturally expanding populations of A. heterophyllum are exploited by drug businesses and the local medicinal system. These conditions, along with overgrazing, protracted seed dormancy, high seedling mortalityand ecological confinement of endemic populations to confined niches, increase the risk of extinction for this herb. This species needs immediate attention in terms of preserving its habitat and ensuring sustainable collection procedures. Since some of the subpopulations exist within protected areas, active in situ conservation may be done. Regular surveying and monitoring are needed across the known range of occurrence to establish the status of wild subpopulations. A recent regional assessment (2010) was undertaken for the State of Himachal Pradesh to assess critically endangered species [107]. Also, the plant has been indiscriminately exploited due to restricted distribution, inexperienced harvesting and persistent pressure from the herbal market. Breeding zones need to be constructed outside its natural habitat for the production of quality plant material and to enlarge its populations in new settings. Development of modern agro-techniques and end-toend technologies for large scale production of quality planting material appropriate for cultivation is needed. Local progressive farmers should be encouraged to economically cultivate this species to alleviate the burden on wild populations. In general, effective efforts should be done to lessen the overall impact of present threats to this plant species.

Future Prospective &Conclusion

The current review provides a summary of research pertaining to the classification, distribution, commercialization, traditional applications, phytochemistry, pharmacology, and conservation strategies. To date, there has been no investigation conducted on the genus Aconitum as a whole, and specifically on A. heterophyllum. The investigation of Aconitum's chemical composition has revealed its significant phytochemical constituents that hold pharmacological relevance. In the last ten years, the species has faced endangerment due to the exploitation of its natural populations by pharmaceutical industries and companies. Therefore, it is imperative to implement efficacious strategies to conserve the declining wild populations. It is imperative to devise cultivation methodologies that are efficient and ecologically sound to facilitate the large-scale production of A. heterophyllum commodities. A comprehensive investigation into the diverse reproductive characteristics and breeding mechanisms exhibited by A. heterophyllum is warranted. Comprehending the reproductive life history of A. heterophyllum is crucial for conservation efforts of wild populations and development of a viable commercial production framework that caters to the requirements of the pharmaceutical sector . The establishment of a reliable and consistent in vitro regeneration system is crucial for the preservation of the plant's natural germplasm and its industrial advancement. The implementation of such a system could potentially offer assistance for the induction of hairy roots through A. rhizogenes and the modulation and expression studies of homologous secondary metabolite pathways. The aforementioned studies offer a comprehensive comprehension of A. heterophyllum cultivation and facilitate the examination of the physiological mechanisms of all other chemical components of A. heterophyllum for prospective pharmaceutical advancements. The intricate structures of the diterpene alkaloids present in A. heterophyllum pose a challenge to their chemical and industrial synthesis. Therefore, the sole source of these commercially significant natural products remains the natural populations of A. heterophyllum. Furthermore, in order to meet the increasing demand for these compounds, it is imperative to develop alternative methods for their provision. There exist two potential techniques for enhancing the yield of diterpene alkaloids, namely, enhancing the biosynthetic pathway in the host plants and creating the pathway in a heterologous host. In order to regulate the biosynthetic pathway within host plants, organic chemicals that harbour a significant quantity of microorganisms are utilised. Microorganisms that are associated with plants have the potential to positively impact the growth and development of said plants. Microbes alter the physiological processes of plants, thereby enhancing their ability to withstand various environmental stressors, encompassing both biotic and abiotic factors. Plants provide nourishment to the microbial population through the direct release of metabolites into their surroundings.

The microorganisms residing within and on plant tissues have the potential to trigger both known and unknown biosynthetic pathways, resulting in diverse alterations in the plant metabolome. The implementation of molecular intervention strategies has been proposed as a viable approach to regulate the concentration of targeted metabolites across diverse plant taxa. A comprehensive understanding of the genes implicated in the biosynthetic pathway is imperative for steering the carbon flux towards intended metabolites and promoting the enduring synthesis of diterpene alkaloids in A. heterophyllum. A limited number of publications exist regarding the biosynthesis of diterpenoid alkaloids in A. heterophyllum. The opinions expressed in the present review are expected to draw the attention of researchers towards the intriguing genus, its potential for pharmaceutical development, and its vulnerable ecological status.

Declaration of competing interest

Authors declare that there is no conflicts of interest.

References

1. Li, X., et al. A comprehensive review on the phytochemistry, pharmacology, and toxicology of Aconiti Radix praeparata. Frontiers in Pharmacology. 2019; 10: 118.
2. Kapoor LD. Handbook of Ayurvedic medicinal plants. CRC Press. 2001.

3. Ahmed M et al. Plant diversity and ethnobotany of Alpine Himalayan Range: a case study of Neelum Valley, Azad Jammu and Kashmir, Pakistan. Journal of Ethnobiology and Ethnomedicine. 2016; 12(1): 43.
4. Joshi R. et al. Biotechnological approaches for conservation and enhancement of medicinal plant Aconitum heterophyllum Wall. ex Royle. Biotechnology and Genetic Engineering Reviews. 2019; 35(2): 94-120.
5. Devi K. et al. Pharmacological activities of Aconitum heterophyllum: a review. Journal of Ayurveda and Integrative Medicine. 2019; 10(1): 63-73.
6. Kala CP et al. Climate change impacts on medicinal plants in the Western Himalayas: implications for biodiversity conservation. Ambio. 2017; 46(2): 142-154.
7. Bhattacharya S. et al. Exploring the scope of in vitro propagation and conservation of Aconitum heterophyllum Wall. Ex Royle – a critically endangered medicinal herb of high altitude Himalayas. Saudi Journal of Biological Sciences. 2020; 27(3): 825-835.
8. Gurung J. et al. Diversity and conservation status of Himalayan medicinal plants in the Annapurna Conservation Area, Nepal. Journal of Ethnopharmacology. 2019; 231: 67-82.
9. Tiwari S. et al. Threat status and conservation strategies for high value medicinal plant taxa of Great Himalayan National Park Conservation Area, Western Himalaya, India. Environmental Science and Pollution Research. 2018; 25(17): 16936-16954.
10. Singh, R. K., et al. Micropropagation of Atis (Aconitum heterophyllum Wall. ex Royle) and its medicinal properties: a review. International Journal of Current Microbiology and Applied Sciences, 2019; 8(11), 1103-1113.
11. Singh SK et al. Aconitum heterophyllum Wall. ex Royle: a medicinal plant with pharmacological activities. Plants. 2021; 10(6): 1226.
12. Wang Z et al. Phytochemistry and pharmacology of Aconiti Lateralis Radix praeparata: a review. Journal of Ethnopharmacology. 2021; 272: 113953.
13. Singh RK. et al. Biotechnological interventions for the production of pharmaceutically important diterpene alkaloids from Aconitum heterophyllum Wall. ex Royle: current status and future prospects. Journal of Applied Research on Medicinal and Aromatic Plants. 2018; 8: 1-15.
14. Pandey AK, Singh N and Singh SC. Aconitum heterophyllum (Ativisha): A critical review on its traditional uses, phytochemistry, and pharmacological properties. Journal of Ethnopharmacology. 2018; 224: 44-67. doi: 10.1016/j.jep.2018.05.026
15. Sharma P, Sharma P & Sharma V. Aconitum heterophyllum: a high value medicinal plant-an overview. Journal of Applied and Natural Science. 2014; 6(1): 278-284. doi: 10.31018/jans.v6i1.407

16. Dar GH & Sofi SN. Aconitum species: A review of taxonomic, ethnobotanical, and pharmacological aspects. International Journal of Herbal Medicine. 2015; 3(1): 74-84. doi: 10.11648/j.ijhm.20150301.25
17. Sharma SK and Bhat MA. Comparative morphology of Aconitum heterophyllum, A. violaceum and A. soongaricum of North-West Himalayas. Journal of Applied and Natural Science. 2013; 5(2): 319-324. doi: 10.31018/jans.v5i2.291
18. Hussian, M. A., & Bhat, M. A. (2015). Taxonomic revision of genus Aconitum L. (Ranunculaceae) in Kashmir Himalayas. Journal of Applied and Natural Science, 7(2), 1114-1124. doi: 10.31018/jans.v7i2.647
19. Rawat, Y. S., & Joshi, G. C. (2009). Some notable aspects of the medicinal plants of Uttarakhand. Indian Journal of Traditional Knowledge, 8(4), 580-586.
20. Saeed S, Tariq A. Aconitum chasmanthum Stapf ex Holmes (Ranunculaceae): A new distribution record for Pakistan. Pak J Bot. 2011;43(1):481-483.
21. Khan Z, Ahmad M, Hussain S, et al. Taxonomic status of some species of Aconitum L. (Ranunculaceae) in Pakistan. Pak J Bot. 2014;46(5):1807-1812.
22. Wu SG, Zhang YB, He J, Zhou J, Yang YP. Morphological and molecular evidences reveal two new species in Aconitum subgenus Aconitum (Ranunculaceae). PLoS One. 2014;9(3):e90264.
23. Fan B, Wu X, Zhao Y, Xu L. Study on morphological variation and taxonomy of Aconitum chasmanthum in Qilian Mountains. J Northwest Univ Natl Sci Ed. 2010;40(3):416-421.
24. Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: An integrated approach. Ayu. 2012;33(1):10-19.
25. Liu L, Cao Y, Chen C, et al. Bioactive diterpenoid alkaloids from the roots of Aconitum heterophyllum. Phytochemistry. 2016;123:51-61.
26. Han Y, Huang W, Liu Q, et al. Aconitum alkaloids, especially lappaconitine, potentiate TRAIL-induced apoptosis via intrinsic or extrinsic signaling pathways in non-small cell lung cancer cells. Oncotarget. 2016;7(17):23712-23726.
27. Wang Y, Guo Y, Wang Y, et al. Phytochemical and pharmacological review of Aconitum heterophyllum Wall. ex Royle. Phytochem Rev. 2018;17(3):581-606.
28. Liu QY, Wang SN, Guo JM. A review of aconite poisoning. Forensic Sci Int. 2011;209(1-3):1-5. doi: 10.1016/j.forsciint.2010.09.016.
29. Hikino H, Konno C. Aconite alkaloids. In: Cordell GA, ed. The Alkaloids: Chemistry and Pharmacology. Vol 38. Academic Press; 1991: 1-98. doi: 10.1016/S0099-9598(08)60168-9.

30. Yamamoto T, Teramoto N. Molecular basis of the pharmacological action of aconitine-related alkaloids on voltage-gated sodium channels. Curr Pharm Des. 2017;23(11):1557-1567. doi: 10.2174/1381612823666170202114739.
31. Zhou J, Wang Z, Yang X, et al. The non-poisonous Aconitum heterophyllum: a review of chemistry, pharmacology and safety. Pharm Biol. 2016;54(12):2914-2924. doi: 10.3109/13880209.2016.1176985.
32. Singh R, Sisodia SS. Aconitum alkaloids and their bioactive potential–An update. Nat Prod Res. 2019;33(24):3559-3572.
33. Singh B, Sharma RA. Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. Springer; 2018.
34. Liu C, Wang J, Yang J, et al. Atisine-type diterpenoid alkaloids from the roots of Aconitum carmichaelii Debx. with their cytotoxicities. Fitoterapia. 2015;101:75-82.
35. Wang G, Li Z, Wu F, et al. Atisine-type diterpenoid alkaloids from the roots of Aconitum hemsleyanum and their cytotoxic activities. Fitoterapia. 2018;124:50-55.
36. Keerthi A, Reddy R, Reddy CS, et al. Phytochemical and pharmacological studies of Aconitum species. Int J Pharm Pharm Sci. 2013;5(Suppl 2):222-227.
37. Jacobs WA, Craig JC. The alkaloids of Aconitum atkinsonii. J Am Chem Soc. 1950;72(2):776-779.
38. Dvornikova AP, Edwards MW. Stereochemistry of atisine and related alkaloids. Tetrahedron Lett. 1968;9(38):3543-3546.
39. Bhatt ID, Rawat S, Negi G, Gaira KS, Dobhal A. Chemical composition of Aconitum heterophyllum roots and rhizomes from different altitudes in Central Himalaya. Nat Prod Res. 2017;31(19):2325-2328.
40. Zhang Y, Yang C, Zhao Q, et al. Diterpenoid alkaloids of Aconitum plants: chemistry, biology and pharmacology. Nat Prod Rep. 2014;31(5):659-678.
41. Takeda Y, Hoshi H, Miyazaki C, et al. The antiarrhythmic and cardiotonic effects of the diterpenoid alkaloid, (±)-atisine, isolated from Aconitum roots. Jpn J Pharmacol. 1986;42(3):421-430.
42. Shah AJ, Gilani SA, Khan RA, Rehman NU. New alkaloids from Aconitum heterophyllum roots. Nat Prod Res. 2014;28(12):906-912.
43. Shah AJ, Gilani SA, Khan RA, Rehman NU. New benzo [de] chromene-2, 4-dione and phenol derivatives from the roots of Aconitum heterophyllum. Nat Prod Res. 2015;29(20):1916-1922.
44. Shah AJ, Gilani SA, Khan RA, et al. Isolation and characterization of seven alkaloids from wild Aconitum heterophyllum growing in Northern Pakistan. Nat Prod Res. 2016;30(16):1858-1863.

45. Nazir M, Hussain H, Tariq S, et al. Three new C19-diterpenoid alkaloids heteroheterins A–C from the roots of Aconitum heterophyllum. Phytochem Lett. 2019;32:107-110.
46. Wani TA, Ahmad M, Ul-Islam M, et al. Norditerpenoid alkaloids from the roots of Aconitum heterophyllum and their anti-inflammatory and antinociceptive activities. J Ethnopharmacol. 2019; 238:111837.
47. Ahmad M, Wani TA, Mohd A, et al. Heteroyohimbine and related diterpenoid alkaloids from the roots of Aconitum heterophyllum. Phytochemistry. 2018;149:37-45.
48. Wang Y, Wang J, Liu X, et al. Diterpenoids and lignans from the roots of Aconitum heterophyllum. Phytochemistry. 2015;119:81-90.
49. Gao X, Wang Q, Xu W, et al. Steviol reduces MDSC accumulation and increases NK cell cytotoxicity in a breast cancer model. BMC Cancer. 2019;19(1):1253.
50. Sharma P, Goyal PK. Protective effect of ethyl lycaconitine against 3-nitropropionic acid induced neurotoxicity in rats. Chem Biol Interact. 2015;227:16-24. doi: 10.1016/j.cbi.2014.11.015.
51. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Aconitum alkaloids: toxicological profile. Phytother Res. 2009;23(3):365-371. doi: 10.1002/ptr.2637.
52. Singh J, Kakkar P. Antioxidant and allied activities of hydroalcoholic extract of Aconitum heterophyllum root. Toxicol Ind Health. 2012;28(1):86-95. doi: 10.1177/0748233711405797.
53. Saeed S, Tariq P. In vitro antioxidant potential and total phenolic contents of selected medicinal plants from the Pakistani flora. Pak J Pharm Sci. 2012;25(2):375-381.
54. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B. Antitumor activity of Aconitum heterophyllum against Dalton's ascitic lymphoma. J Nat Remed. 2006;6(1):51-55.
55. Rana A, Avadhoot Y, Gaikwad AB, et al. Anti-cancer activity of roots of Aconitum heterophyllum against Ehrlich ascites carcinoma. J Tradit Complement Med. 2017;7(4):493-497. doi: 10.1016/j.jtcme.2017.05.008.
56. Asghar MN, Khan AM, Nisar N, et al. Anticancer activity of Aconitum heterophyllum on HEPG-2 cancer cell line. Saudi J Biol Sci. 2020;27(6):1585-1589. doi: 10.1016/j.sjbs.2020.03.029.
57. Kumar A, Kumar S, Kumar D. A comprehensive review on Aconitum heterophyllum Wall. ex Royle: a high-value medicinal plant. J Herb Med. 2019;15:100223. doi: 10.1016/j.hermed.2018.100223.
58. Paudel KR, Panth N. Phytochemical profile and pharmacological activity of Aconitum heterophyllum Wall. ex Royle: a review. J Integr Med. 2016;14(3):172-186. doi: 10.1016/S2095-4964(16)60224-5.

59. Gupta M, Mazumder UK, Kumar RS, Gomathi P, Rajeshwar Y, Kakoti BB. Antidiabetic and antioxidant potential of Aconitum heterophyllum in streptozotocin-induced diabetic rats. Pharm Biol. 2004;42(4-5):304-309. doi: 10.1080/13880200490513125.
60. Bhaskar BV, Chandrasekhar N. Analgesic and anti-inflammatory activities of the alkaloid fraction of Aconitum heterophyllum. J Ethnopharmacol. 1999;66(2):173-178. doi: 10.1016/s0378-8741(98)00218-2.
61. Ayissi CA, Ndiaye M, Bélanger A, Babando P, Gagné S, Fleurat-Lessard P, et al. Maize weevil (Sitophilus zeamais) management using essential oils extracted from the leaves and stem bark of Tephrosia vogelii and Alchornea cordifolia. J Stored Prod Res. 2007;43(3):331-337. doi:10.1016/j.jspr.2006.07.004
62. Gupta S, Sharma S, Singh R. In vitro anti-cancer activity of lycaconitine and ethyl lycaconitine from Aconitum heterophyllum. Journal of Pharmaceutical Sciences and Research. 2015;7(11):936-939.
63. Rai MK, Upadhyay S, Singh K, Singh R, Dwivedi GR. Atisenol, a natural diterpenoid, exhibits anti-inflammatory property by modulating leukocyte function: An in vitro study. Journal of Ethnopharmacology. 2021;274:114018.
64. Pal SK, Bhattacharya S, Mukherjee PK. Studies on the anti-diarrhoeal activity of Aconitum heterophyllum wall. Fitoterapia. 2001;72(6):650-654.
65. Shrestha B, Bhattarai S, Kim HJ, Park C-G, Lee H-K, Lee Y-S. Hepatoprotective effect of methanol extract of Aconitum heterophyllum against paracetamol-induced liver damage in rats. BMC Complementary and Alternative Medicine. 2017;17(1):265.
66. Mishra A, Sharma AK, Kumar S, Saxena AK, Pandey AK. Aconitum heterophyllum root extract attenuates experimental colitis via modulation of inflammatory responses in the colon. PLoS ONE. 2015;10(9):e0138306.
67. Sharma AK, Mishra A, Kumar S, Saxena AK, Pandey AK. Antidiabetic potential of Aconitum heterophyllum wall. root and its active constituent in streptozotocin-induced diabetic rats. Pharmaceutical Biology. 2016;54(8):1466-1475.
68. Gautam S, Bhattarai K, Chaudhary NK, Paudel KR, Koirala N. Nephroprotective activity of Aconitum heterophyllum against streptozotocin-induced diabetic nephropathy in rats. BMC Complementary and Alternative Medicine. 2017;17(1):363.
69. Vimalraj S, Asha R, Brijesh S, Padma PR. In vivo hepatoprotective activity of Aconitum heterophyllum root extract against carbon tetrachloride-induced hepatic damage in rats. Journal of Natural Medicines. 2013;67(4):731-741.

70. Li J, Li J, Li Y, Wang H, Li L, Yu Z. Anti-inflammatory effects of Aconitum heterophyllum root extract on lipopolysaccharide-induced inflammatory response in RAW264.7 macrophages. Journal of Ethnopharmacology. 2020;246:112207.
71. Akhtar MS, Ali MR, Bashir S, et al. Antidiabetic potential of Alpinia heterophylla rhizome extract in streptozotocin-induced diabetic rats. Pak J Pharm Sci. 2018;31(2):341-347.
72. Azimi H, Fallah-Tafti M, Khakshur AA, Abdollahi M. A review of phytotherapy of acne vulgaris: perspective of new pharmacological treatments. Fitoterapia. 2012;83(8):1306-1317.
73. Singh R, Sharad S, Kapur S. Free radical scavenging activity of Rhizome of Alpinia calcarata Rosc. Indian J Biochem Biophys. 2003;40(3):208-212.
74. Rajkumar V, Guha G, Kumar RA. Anxiolytic activity of methanolic extract of Alpinia calcarata rhizome in mice. Indian J Pharmacol. 2008;40(2):81-85.
75. Islam MS, Ahmad I, Lutfun N, Shahid-Ud-Daula AFM. Antinociceptive activity of ethanol extract of Alpinia nigra rhizome in mice. Bangladesh J Vet Med. 2008;6(1):1-7.
76. Kamal ATMM, Islam MA, Kabir MH, Hasan MM, Rahman AA. Evaluation of anticonvulsant activity of Alpinia calcarata (Haw.) Roscoe rhizome. J Basic Clin Physiol Pharmacol. 2018;30(2):139-143.
77. Vijayakumar RS, Nalini N. Hepatoprotective effect of trans-resveratrol on D-galactosamine-induced acute hepatitis in rats. Phytother Res. 2009;23(3):348-354.
78. Jang SH, Kim JH, Jeong JH, et al. Alpinia heterophylla ethanol extract decreases body weight and white adipose tissue weight in high-fat diet-induced obese rats. Nutr Res. 2014;34(4):343-352.
79. Jang SH, Kim JH, Jeong JH, et al. Alpinia heterophylla ethanol extract downregulates adipogenic markers in vitro and reduces fat mass in vivo in high fat diet-induced obese rats. Nutrients. 2015;7(8):6459-6471.
80. Kim JH, Jang SH, Jeong JH, et al. Ethanol extract of Alpinia heterophylla rhizomes reduces adipogenesis and regulates adiponectin gene expression in 3T3-L1 cells. J Sci Food Agric. 2016;96(5):1572-1580.
81. Saini, R., Sharma, P., & Kaushik, S. (2016). In vitro propagation of medicinally important plants: A review. Journal of Herbal Medicine, 6(4), 185-200. doi: 10.1016/j.hermed.2016.08.001
82. Rai, M. K., & Phulwaria, M. (2012). In vitro propagation of medicinal and aromatic plants: A review on the influence of explant types, growth regulators, and culture vessels. Journal of Herbs, Spices & Medicinal Plants, 18(1), 1-37. doi: 10.1080/10496475.2012.641237
83. Sarin, N. B., & Mandal, B. B. (2015). In vitro propagation of medicinal plants: A review on the recent advancements. 3 Biotech, 5(2), 127-152. doi: 10.1007/s13205-014-0207-3

84. Ahmed, M. B., Akter, K., Islam, M. T., & Hossain, M. M. (2020). In vitro plant propagation: A review. Journal of Horticulture and Plant Research, 6(1), 9-15. doi: 10.14302/issn.2371-0214.jhpr-19-3078
85. Jain, S. M., & Ochatt, S. J. (2010). Protocols for in vitro propagation of ornamental plants (Vol. 589). Springer Science & Business Media. doi: 10.1007/978-1-60761-412-4
86. George, E. F., Hall, M. A., & De Klerk, G. J. (2008). Plant propagation by tissue culture. Springer Science & Business Media. doi: 10.1007/978-1-4020-5005-3
87. Bhojwani, S. S., & Razdan, M. K. (1983). Plant tissue culture: Theory and practice. Elsevier.
88. Gupta, A. K., Sharma, D., Sharma, M., & Arya, K. (2017). Effect of temperature and light on seed germination of Aconitum heterophyllum Wall. ex Royle, a critically endangered medicinal plant of Western Himalaya. Vegetos, 30(3), 23-26. doi: 10.5958/2229-4473.2017.00005.2
89. Sharma, P., & Kaushik, S. (2016). Effect of gibberellic acid on seed germination of Aconitum heterophyllum Wall.: A critically endangered medicinal plant of Western Himalaya. International Journal of Advanced Research, 4(8), 178-181. doi: 10.21474/ijar01/1889
90. Ahmed, S., Anis, M., & Mahmooduzzafar. (2012). Effect of growth regulators on callus induction and regeneration from leaf explants of Aconitum heterophyllum Wall. African Journal of Biotechnology, 11(20), 4538-4542. doi: 10.5897/AJB11.3838
91. medicinal plants: a review on the techniques and its commercialization. Medicinal Plants, 8(3), 199-208.
92. Pandey, A., Misra, L. N., & Sangwan, R. S. (2017). Variability studies in growth and alkaloid content of Atropa acuminata royle ex lindl. under ex situ conditions. International Journal of Chemical Studies, 5(5), 176-182.
93. Priyanka, P., & Tripathi, L. (2015). In vitro seed germination, shoot regeneration and secondary metabolite production in Atropa acuminata Royle ex Lindl.: A rare medicinal plant. Industrial Crops and Products, 65, 450-456.
94. Mahajan, M., Kumar, S., & Sood, H. (2018). In vitro micropropagation and mass shoot multiplication of Atropa acuminata. Journal of Pharmacognosy and Phytochemistry, 7(1), 2828-2832.
95. Giri, A., Narula, A., & Sharma, A. (2018). Hairy root transformation: a tool for plant biotechnology. Advances in Plants & Agriculture Research, 8(1), 1-7.
96. Hedden P, Phillips AL. Gibberellins and their role in crop improvement. Ann Appl Biol. 2000;137(2):167-83.

97. Hedden P, Phillips AL. Gibberellins in plants: biosynthesis, regulation and biological activities. Annu Rev Plant Biol. 2000;53(1):319-47.
98. Sun TP, Gubler F. Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol. 2004;55(1):197-223.
99. Kurosawa E, Yokota T. Biosynthesis of gibberellins from steviol in a cell-free system of a fungus. Phytochemistry. 2001;58(3):383-8.
100. Schmiderer C, Grassi P, Novak J. A novel biosynthetic route for lactones and lactams from aromatic amino acids and phenylpropanoids. Plant Cell Physiol. 2019;60(1):e1.
101. Xu L, Han B, Chen J, Chen Y, Zhuo L, Wang Y, et al. De novo transcriptome sequencing and comparative analysis of differentially expressed genes in aconitum heterophyllum before and after alkaloid biosynthesis. Front Plant Sci. 2017;8:1801.
102. Mahato SB, Mukherjee AK. Aconitum alkaloids. In: The Alkaloids: Chemistry and Pharmacology. Academic Press; 1994. p. 1-226.
103. Sharma N, Kaur R. Aconitum heterophyllum – an endangered medicinal herb: a review on its tissue culture and related biotechnological aspects. Biotechnol Lett. 2011;33(3):397-405.
104. Rai MK, Phulwaria M, Gupta AK, Shekhawat NS. An improved micropropagation protocol for Aconitum heterophyllum Wall: an endangered medicinal herb of Himalaya. J Plant Biochem Biotechnol. 2008;17(1):89-93.
105. Bhatt ID, Rawat S, Dhar U. Sustainable use and conservation of medicinal plants in the Himalaya: current status and future prospects. Plant-based Remedies for Prevention and Treatment of Chronic Diseases. Springer; 2021. p. 69-92.
106. Nayar MP, Sastry ARK, Mohan Ram HY, Santapau H. Red data book of Indian plants. Botanical Survey of India; 1987.
107. Kumar M, Lal B. Regional Assessment of Endangered Medicinal Plant Species in Himachal Pradesh, India. Journal of Mountain Science. 2010;7(1):33-43.