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REVIEW ARTICLE

A review on efforts for improvement in medicinally important chemical constituents in*Aconitum* through biotechnological interventions

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Abstract

The genus *Aconitum* belongs to the family Ranunculaceae, is endowed with more than 350 species on the earth. Medicinally important aconitine type of diterpenoid alkaloids are the characteristic compounds in most of the *Aconitum* species. The present review endeavored the major research carried out in the field of genetic resource characterization, pharmacological properties, phytochemistry, major factors influencing quantity, biosynthetic pathways and processing methods for recovery of active ingredients, variety improvement, propagation methods, and important metabolite production through cell/organ culture of various *Aconitum* species. More than 450 derivatives of aconitine-type C19 and C20-diterpenoid alkaloids along with a few other non-alkaloidal compounds, such as phenylpropanoids, flavonoids, terpenoids, and fatty acids, have been identified in the genus. A few *Aconitum* species and their common diterpenoid alkaloid compounds are also well characterized for analgesic, inflammatory and cytotoxic properties. However, the different isolated compound needs to be validated for supporting other traditional therapeutical uses of the plant species. Aconitine alkaloids shared common biosynthesis pathway, but their diversification mechanism remains unexplored in the genus. Furthermore, the process needs to be developed on secondary metabolite recovery, mass-scale propagation methods, and agro-technologies for maintaining the quality of products. Many species are losing their existence in nature due to over-exploitation or anthropogenic factors; thus, temporal monitoring of the population status in its habitat, and suitable management programs for ascertaining conservation needs to be developed.

Keywords Aconite · Monkshood · Diterpene alkaloids · Ethnopharmacology · Omics studies · Tissue culture

Introduction

The genus *Aconitum* (Ranunculaceae family) is one of the most recognized medicinal plants representing over 350 species distributed majorly in temperate regions of the Northern Hemisphere (Been 1992). *Aconitum* is a perennial (sometimes biennial) herb having stout leafy stems, bulbs, or rhizomes (Jabeen et al. 2013). East and South-East Asia and Central European represent a rich diversity of *Aconitum* species; however, a few species have also been recorded from Western and Eastern parts of North America. Among Asian countries, over 200 species have been recorded from China (Li and Kadota 2001) of which more than 76 species are used for medicinal purposes in various parts of the country (Xiao et al. 2006). The Hengduan Mountain region has been recognized as a primary center of diversity and speciation of the genus *Aconitum,* which is represented by a total of 166 recorded species (Yan and Qin-Er 2005). A total of 33 species are found in the Himalayan region spanning India, Pakistan, Nepal, Bhutan, and Tibet (Dar and Nordenstam 2014), of which 26 species are reported from Kashmir Himalaya (Jabeen et al. 2013), and have been recognized in local and traditional system of medicine of the region. However, 94 taxa, including 22 native species and 28 nothospecies and a few intra-specific natural hybrids, have been recorded from

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Europe (Mitka et al. 2021). A total of 6 species of *Aconitum* (e.g., *A. columbianum*, *A. delphiniifolium*, *A. maximum*, *A. noveboracense*, *A. reclinatum* Gray., and *A. uncinatum*) have been reported from North America (Brink et al. 1994; Kuchenreuther 1996). Different *Aconitum* species found in Asian countries have diverse medical uses and therapeutical efficacy. Indian species, *A. ferox* and *A. chasmanthum*, were reported three-to-seven times more effective than European species *A. napellus*. *Aconitum* has been used as a powerful poison since ancient times and was used in arrows and bait poison (Been 1992). *Aconitum* species have an important place in the Asian herbal medicine system which is evident from its larger applications in the cardio and neurological disorders in India, China, Japan, and some other Asian countries; however, wrong species identification or improper processing may cause a high risk of severe toxicity (Chan 2014). Its major medicinal application has been recognized as anti-rheumatic, anesthetics, analgesic, diaphoretic, diuretic, anti-periodic anodyne, anti-diabetic, anti-phlogistic, and anti-pyretic agent in different traditional systems of medicine of Asia (Dar and Naqshi 2001; Shah 2005; Yue et al. 2009; Yan et al. 2010; Nyirimigabo et al. 2015).

Proper processing reduces the toxicity of *Aconitum* and sometimes enhanced its efficacy (Liu et al. 2017). Detoxified tubers and roots are only permitted for oral administration for clinical decoctions after proper processing by the Food and Drug Administration of China. Currently, more than 70 processing techniques are available for detoxification in the Chinese traditional system of medicine (Nyirimigabo et al. 2015). Thus, various species are used for the treatment of rheumatoid arthritis, various types of pain, inflammatory diseases, trauma and fractures, intoxication, plaque, immune-suppression-induced ailments, colds, and coughs (Hao et al. 2015). The characteristic aconite-type diterpenoid alkaloids found in *Aconitum* species have a strong medicinal application in the modern pharmaceutical market. An array of derivatives of diterpenoid alkaloids present in different species has similar or sometimes different biological activity with diverse biological efficiency (Bessonova and Saidkhodzhaeva 2000; Shen et al. 2020). Owing to their pharmacological values, diterpenoid alkaloids in *Aconitum* have attracted the attention of researchers. Besides, various other phytochemicals, e.g., flavonoids, phenylpropanoids, phenolic acids, terpenoids, and steroids, have been isolated from *Aconitum*. In different pharmacological activities, analgesic and anti-inflammatory activities have been well explored in extracts as well as isolated diterpenoid alkaloids.

Due to high demand in the international market, *A. spicatum* is among the top ten highly traded medicinal plants from the Nepal Himalaya (Olsen and Larsen 2003). *A. heterophyllum*, an endangered medicinal plant, has an average price of 50 USD per kg and market demand has been estimated at approximately 2000 tons per annum, while its availability is decreasing in the range of 26–50% annually due to a continuous decrease in its wild stock (Olsen and Larsen 2003; Gupta and Souravi 2020). Similarly, *A. carmichaelii* Debx. and *A. kusnezoffii* Reichb. have a huge market in China, as these species are used in medicinal and well as traditional culinary recipes (Brinckmann 2016). Increasing global trade of different *Aconitum* species has imposed a heavy demand for the supply of raw material leading to unscientific and indiscriminate harvesting of many species from the wild. Due to the rapid reduction of wild populations, a few species of *Aconitum*, (*A. chasmanthum*, *A. heterophyllum*, *A. violaceum*, and *A. corsicum*) have been placed in different threat categories of IUCN (IUCN 2020). Due to the availability of large diversity of genetic resources in nature, beneficial therapeutical effects, and the presence of a large array of compounds, *Aconitum* genus has attracted the attention of researchers in medical, pharmaceutical, agriculture, and environmental sciences. Being a factory of diverse potent diterpenoid alkaloids, the genus can be a model plant for the study of diterpenoid alkaloid biosynthesis.

Although various review papers have already been published on mass propagation methods, pharmacological, and phytochemical aspects in this genus (Srivastava et al. 2010; Nyirimigabo et al. 2015; Kumari et al. 2017; Ali et al. 2021; Wani et al. 2022), the present review highlighted recent research on these aspects along with a few additional research areas, including best growth influencing factors for quantity control, optimum recovery methods for secondary metabolite, and transcriptome studies for elucidation of major biosynthetic pathways of active ingredients. Also, available information on diversity in genetic resources (including genetic diversity studies and phylogenetics) and genomic resources along with full plastid genome sequencing studies has been systematically analyzed for improving the quality and quantity of active ingredients. Research gaps have also been highlighted for crop improvement programs and conservation and sustainable utilization of the genus *Aconitum*.

Medicinal uses and pharmacological properties

Medicinal uses

Aconitum has been traditionally used in different traditional medicine systems, including, Ayurveda, Unani, Chinese medicine system, and Homeopathy. The species has been used in treating different types of pains and inflammations, anxiety, neurological disorders, gastrointestinal disorders, edema, bronchial asthma, rheumatic fever, and some endocrinal disorders (Dar and Naqshi 2001; Nyirimigabo et al. 2015). In the traditional Indian medicine system Ayurveda,

Aconitum species are used as anti-pyretic, anti-inflammatory, anti-hemorrhoid, antidotes, anti-tussive, and anti-diarrheal agents (Adams et al. 2013). Roots of Himalayan species, such as *A. balfourii*, *A. ferox,* and *A. heterophyllum,* are used in the treatment of vomiting, diarrhea, abdominal pain, body ache, and arthritis in India (Bisht et al. 2013). Roots of *A. heterophyllum* are also used as a remedy for the nervous system, digestive system, cough, cold, fever, and rheumatism (Shah 2005). However, the roots of *A. spicatum* have extensively been used to treat fever, headache, muscular pain, cuts, and wounds in Nepal and other parts of the Himalaya (Shyaula 2011). *A. delavayi* Franch. roots are used to treat rheumatism, traumatic injuries, pain, swelling, hematemesis, blood disease, hematochezia, and piles (Jiang et al. 2007). Roots of *A. deinorrhizum*are are effective against headache and rheumatic fever (Pullaiah 2006). However, a fermented preparation of *A. violaceum* flowers is useful in cough, fever, colds, stomach, and liver disorders, and preparations of root powder are effective in painful joints and boils, toothache, and preventing tooth cavities (Fig. 1) (Lone et al. 2014). Similarly, *A. bulleyanum*is traditionally used in influenza, rashes, and snake bite. *A. orochryseum* Stapf is used in bilious fever, dysentery, cough and cold, and as a febrifuge for malarial fever, stomach ulceration, and kidney dysfunction (Wangchuk et al. 2010). *A. carmichaelii* Debx, a species found in East Asia and eastern Russia, has been known for its anti-inflammatory, analgesic, diuretics, and cardio-tonic properties (Yan et al. 2010; Yue et al. 2009). *A. brachypodum*, one of the commonly used Chinese traditional herbs, has anti-rheumatic and analgesic properties. *A. napellus* L. a native and endemic species of Western and Central Europe is used in homeopathic preparations (Watad et al. 1995). *A.kusnezoffii* Rchb. found in Eastern China, Serbia, and Korean peninsula is used as an analgesic and anti-rheumatic agent for heart gout, neuralgia, failure congestion, and rheumatism (Zhao et al. 2009). *A. coreanum* has rheumatic arthralgia, anti-arrhythmia, analgesic, and anti-inflammatory properties to cure migraine headache, cardialgia, vertigo, epilepsy, and infantile convulsion (Park et al. 2017a, b). Similarly, *A. taipeicum* Hand-Mzt, *A. finetianum* Hand-Mazz, *A. sungpanense* hand. Mazz, *A. vulparia* Rchb, *A. naviculare* (Bru¨hl) Stapf, *A. kirinense* Nakai, and many other species have various health-beneficial effects and are used in different parts of the world (Wu et al. 1996; Fico et al. 2003; Wang et al. 2004; Jiang et al. 2007; Shrestha and Jha 2010; Zhang et al. 2013; Zhang et al. 2014).

Pharmacological properties

Anti-inflammatory potential

Different *Aconitum* species have been used as anti-inflammatory and analgesic agents in different systems of medicines since ancient times (Huang et al. 2011). The ethanolic root extract of *A. heterophyllum* has been reported to inhibit inflammation by reducing the weight of cotton pellet in cotton pellet-induced granuloma in rats and the activity was comparable to diclofenac sodium, which is a strong

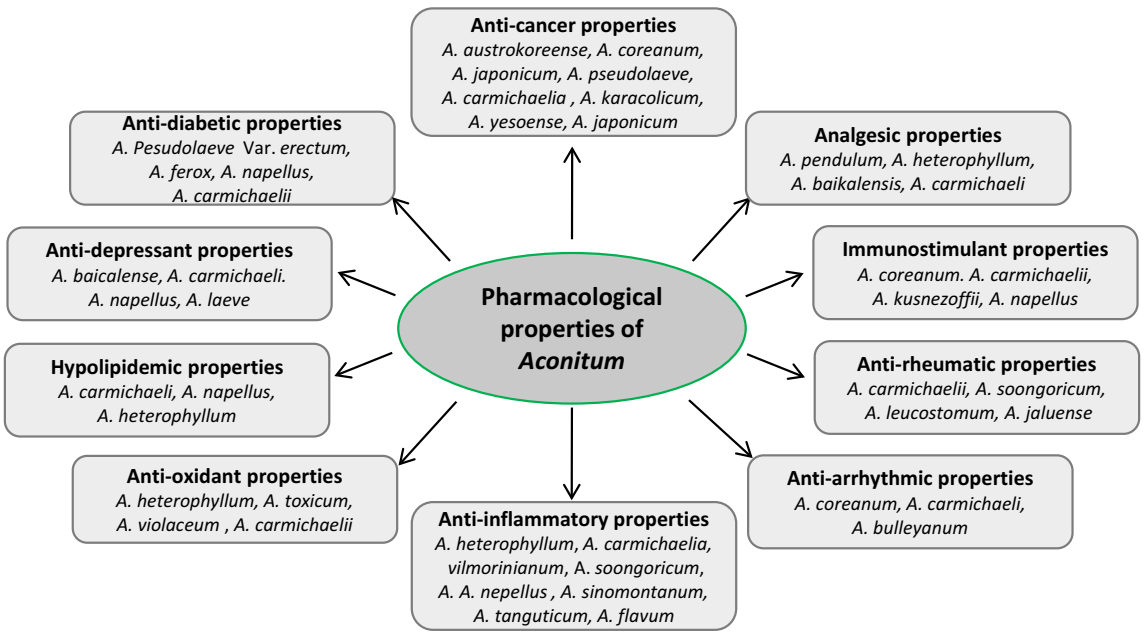

Fig. 1 Pharmacological properties of various species of *Aconitum* validated in various studies

non-steroidal anti-inflammatory drug (Verma et al. 2010). *Aconitum* roots (*Pendulous monkshood*) displayed significant anti-inflammatory properties on lipopolysaccharides-induced mouse peritoneal macrophages at the dosage of 4–200 µg/ml body weight (Huang et al. 2013). Similarly, *A. vilmorinianum* Kom. exhibited improvement in swelling, hyperemia, allodynia, and vascular permeability in arthritic knee joints (Li et al. 2013). Butanol and ethanol extract of *A. flavum* possesses significant anti-inflammatory activity in dimethylbenzene-induced ear vasodilatation and acetic acid-induced capillary permeability enhancement in mice and carrageenan-induced paw edema in rats. The plant extracts showed significant anti-nociceptive activity using acetic acid-induced writhes, hot-plate test, and formalin test in mice, and it did not show any cytotoxicity at the tested dose (Zhang et al. 2015). Different plant parts of *A. carmichaelii* have shown analgesia and anti-inflammatory, and were found useful in the retreatment of rheumatoid arthritis and these activities showed no significant difference among leaves and stem parts used (Ya-Nan et al. 2018). Oral administration of the ethanolic extract of *A. napellus* L. (200 mg/kg) showed potent anti-inflammatory activity by inhibiting the acute as well as chronic inflammation adjuvant carrageenan-induced inflammation in rats (Gupta et al. 2019). Chloroform extract of *A. sinomontanum* showed significant anti-nociceptive and anti-inflammatory activities by improving the alleviated pain induced by acetic acid and significantly reducing the xylene-induced mouse ear edema (Zhang et al. 2020). Similarly, extracts of *A. tanguticum* (Maxim.) Stapf have displayed potential anti-inflammatory effects in rats (Wu et al. 2014).

Guiwuline, a franchetine type C_{19}-diterpenoid alkaloid isolated from *A. carmichaelii, s*howed potential analgesic activity in mouse hot-plate test and showed little toxicity to mice (Wang et al. 2012). Four new C_{19}-diterpenoid alkaloids, taronenines A–D, isolated from the roots of *A. taronense* showed inhibition activities of interleukin-6 in LPS-activated RAW 264.7 cells with IC_{50} values between 18.87 and 29.60 µg/mL (Yin et al. 2018). Diterpene alkaloids isolated from *A. baikalense* (e.g., napelline, songorine, hypaconitine, mesaconitine, and 12-epinapelline N-oxide) improved histamine-induced acute inflammation condition by reducing swelling (more than 20%) of the injured limb. However, the reference drug diclofenac sodium salt exhibited 1.5 times higher activity than these isolated compounds (Nesterova et al. 2014). Two new diterpenoid alkaloids, 7,8-epoxy-franchetine and N-(19)-en-austroconitine A, isolated from *A. iochanicum* exhibited inhibitory activity against nitric oxide (NO) production in LPS-activated RAW264.7 macrophages. The anti-inflammatory activity of these compounds was ~ 25% weaker than diclofenac sodium drug (Guo et al. 2017). Also, paconitine and puberanine alkaloid isolated from the aerial parts of the leaves of *Aconitum* Royle. exhibited significant anti-inflammatory activity by decreasing the neutrophil accumulation in inflammatory regions (Shaheen et al. 2005). Aconitine, Songorine, 16,17-dihydro-12β, 16β-epoxynapelline, and 12-epi-napelline application significantly improved the lipo-polysaccharide-induced cellular inflammatory responses improving the content of IL-6, IL-1β, TNF-α, and PGE-2 (Zhang et al. 2021a). A vakognavine-type alkaloid found in different *Aconitum* species inhibited the activity of cyclooxygenase-2, which was found comparable to aspirin (Liang et al. 2016). Lappaconitine also exhibited significant analgesic properties by inhibiting the voltage-dependent sodium channels, increasing epinephrine release in synaptic cleft, and inhibiting the release of P substance (Li et al. 2019).

Besides, diterpenoid alkaloids, four caffeoyl derivatives, caffeic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid methyl ester isolated with activity guided fractionation process of *A. koreanum* root extract exhibited strong anti-inflammatory effects by inhibiting nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells and inhibited the expressions of iNOS and COX-2 related genes at mRNA levels in a dose-dependent manner (Park et al. 2009). A brief mechanism of the inflammatory response of aconitine alkaloid is summarized in Fig. 2.

Anti-cancer and anti-proliferative properties

Aconitum plant extracts exhibit significant anticancer and cytotoxic properties by inhibiting the growth of different cancer cell lines. Lipopolysaccharide-rich aqueous fraction of dried tuberous roots of *A. austrokoreense* Koidz significantly inhibited nitric oxide production induced in RAW 264.7 cell lines and inhibited cytotoxic activity against human cancer cell lines (Ju et al. 2020). Ethanolic extract of *Aconitum* roots (*Pendulous monkshood*) inhibited the proliferation of HepG 2 cells, sP 2/0 cells, and Hela cells in a dose-dependent manner (Huang et al. 2013). The aqueous solution of aconitine-containing extract administered to mice having ascites or a solid form of Ehrlich's carcinoma showed effectiveness against growth tumor growth and the average life span of experimental animals (Dasyukevich and Solyanik 2007). Similarly, aconitine-containing herbal extract significantly inhibits the growth of tumors and metastasis of Lewis lung carcoma (LLC-R9) cell lines (Solyanik et al. 2004). Oral administration of crude polysaccharides' extraction of *A. coreanum* stems inhibits the growth of H22 tumor cells and prolonged the life span of H22 ascites tumor-bearing mice (Liang et al. 2012).

A new vakognavine-type alkaloid displayed strong inhibitory effects on HT-29, SGC-7901 and HepG2 cell lines with IC_{50} values between 0.948 and 3.645 µM (Liang et al. 2016). Taipeinine alkaloids found in various species of *Aconitum* blocked the cell cycle at G1/S phase and exhibited

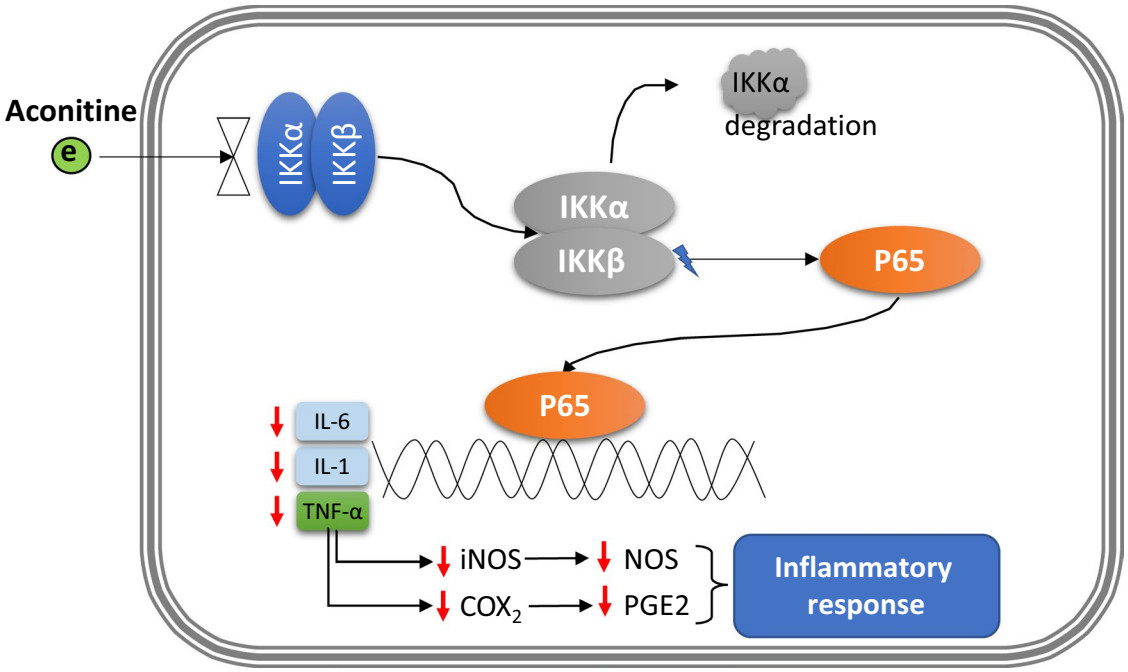

Fig. 2 The molecular mechanisms of anti-inflammatory response of aconitine alkaloid (*IKKα* IκB kinase-α, *IKKβ* IκB kinase-α, *IκBα* nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor-α, *NF-κB* Nuclear factor kappa-light-chain-enhancer of activated B cells, *P65, P65* protein of transcription factor, *TNF-α* tumor necrosis factor-α, *IL-6* interleukin-6, *IL-1* interleukin-1, *COX-2* cyclooxygenase-2, *PGE2* Prostaglandin E2, *iNOS* inducible nitric oxide synthase, *NOS* nitric oxide synthase). Red arrow (↓) indited inhibition (Lan et al. 2021; Li et al. 2022)

its usefulness in the inhibition of HepG2 cell proliferation (Zhang et al. 2014). Also, veatchine-type C20-diterpenoid alkaloids including 12-acetylluciculine and its derivatives isolated from *A. japonicum* inhibited the growth of human malignant A172 cells (Wada et al. 2007). Lappaconitine, a C19-diterpenoid alkaloid found in the roots of *Aconitum pseudolaeve,* inhibits the proliferation of the human lung cancer cells A549. At a higher concentration of lappaconitine, apoptosis rate increased with the down-regulated expression of Cyclin E1A in 549 cells along with an increase in G1 + G0 phase and a decrease in S and G2 + M phases (Sheng et al. 2014). Lycaconitine also has potent inhibitory effects on human fibro-carcinoma KB V20C, which was resistant to 20 nM vincristine, but it was found non-cytotoxic to NK cells (Kim et al. 1998). Five compounds (e.g., oxonitine, deoxyaconitine, hypaconitine, mesaconitine, and crassicauline A) were isolated from the roots of *A. carmichaelii* Debx. showed significant cytotoxic activities against various cancer cell lines belonging to leucocythemia, breast cancer, and liver cancer. Compounds having two ester groups contained in the structure possess a stronger effect than others (Gao et al. 2012). Also, 8-*O*-azeloyl-14-benzoylaconine isolated from the roots of *A. karacolicum* Rapcs. has two ester groups that exhibited significant anti-proliferative activities against cell lines of the colon (HCT-15), lung (A549), and breast (MCF-7) cancer (Chodoeva et al. 2005). Ten new acylated alkaloid derivatives prepared from the natural diterpenoid alkaloids of *A. yesoense* and *A. japonicum* inhibited the growth of A549 cells by arresting the cell cycle at G1 stage. Further, cytotoxic properties showed a significant increase after the addition of an acyl group at both C-11 and C-15 positions (Wada et al. 2011).

Effects on the central nervous system

Aconitum has been traditionally considered an analgesic agent and it significantly reduced neuropathic pain in the rat chronic constriction injury model (Xu et al. 2006). The diterpenoid alkaloids present in *Aconitum* exhibit potent *N*-cholinolytic activity and induced signals to neuronal nicotinic acetylcholine receptors (nAChRs) (Turabekova et al. 2010). Furthermore, Bullatine A, a diterpenoid alkaloid found in many species of *Aconitum,* can attenuate ATP-induced BV-2 microglia apoptosis through P2X receptor pathways (Li et al. 2013). Diterpene alkaloids often displayed anti-epileptic properties by guarding the Na^+ channels as Na^+ channels are involved at the beginning of epilepsy. Lappaconitine normalizes neuronal activity by blocking Na^+ channels (Ameri 1998a). A few structure–relationship studies indicated that aromatic substitutes of different compounds have better anti-epileptic activity. Aromatic substitutes of aconitine-type diterpene alkaloids, such as

6-benzoylheteratisine, 1-benzoylnapelline, lappaconitine, and 14 benzoyl-talatisamine, exhibited better hippocampal excitability in rat model system than many commercially available compounds, such as heteratisine, napelline, lappaconidine, and talatisamine (Ameri 1997, 1998b). However, aconitine alkaloids can also suppress delayed rectifier K^+ current in NG108-15 neuronal cells. However, alterations in action potentials caused by aconitine might be concerned with abnormal neuronal excitability (Lin et al. 2008).

Anti-arrhythmic effect

Guan-Fu base S (a diterpenoid alkaloid) isolated from *A. coreanum* exhibited inhibitory effects on blocking the ventricular-specific sodium current using a whole-cell patch voltage-clamp technique (Xing et al. 2014). Alkaloids isolated from *A. carmichaelii* species exhibited strong anti-arrhythmogenic properties (Liu et al. 2012). Similarly, allapinin isolated from different *Aconitum* species prevented paroxysmal atrial fibrillations and hence exhibited strong application as an anti-arrhythmic drug (Mazur et al. 1986).

Anti-diabetic and hypolipidemic effects

Different solvent extracts of *A. pseudolaeve* var *erectum* root showed over 90% of α-amylase inhibition and over 60% of α-glucosidase inhibition, which is an important mechanism to regulate glucose uptake from the diet (Kim et al. 2013). Ethanolic extract of *A. ferox* showed strong inhibitory activity against α-glucosidase and improved the blood level of Alloxan-induced diabetic rats (Shoaib et al. 2020). Aqueous extract of *A. napellus* exhibited promising hypoglycaemic activity comparable to reference standard glibenclamide to reduce blood glucose levels in alloxan-induced hyperglycaemic adult Wistar albino rats (Chhetree et al. 2010). Also, *A. carmichaelii* extract appeared more effective in insulin immune reactivity than the control and treated group after oral administration on blood sugar in Type II diabetic mice (Jung et al. 2010). Similarly, the methanolic extract of *A. heterophyllum* exhibited hypolipidemic potential (Subash and Augustine 2012). Also, polysaccharides found in *A. carmichaelii* inhibited high-cholesterol levels by up-regulating the mRNA expression of cholesterol 7α-hydroxylase (Zhou et al. 2015).

Other biological properties

Major *Aconitum* diterpene alkaloids are toxic due to their ability to interact with voltage-gated sodium channels, which increases the permeability of the smooth muscle membrane for sodium ions, followed by an increase the calcium ion availability (Fig. 3). Consequently, during muscular contraction, the release of neurotransmitters and changes in receptors and the promotion of lipid peroxidation create severe toxic effects followed by cell apoptosis in the heart, liver, and other tissues (Chan 2009). *Aconitum*

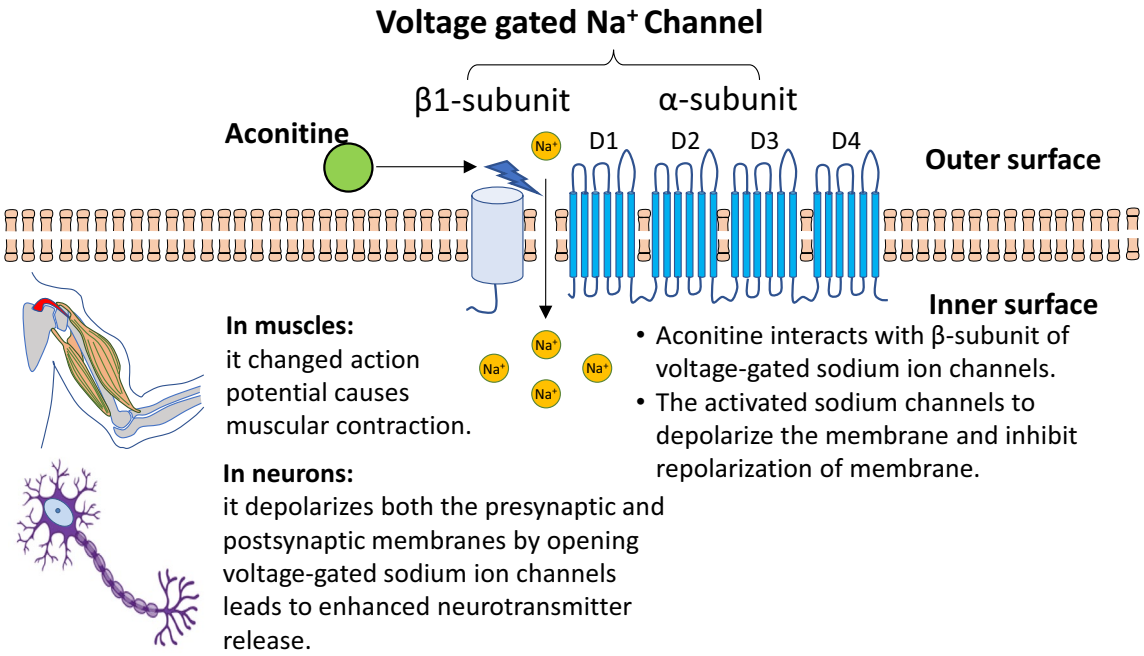

Fig. 3 Mechanism action of aconitum alkaloid through the interaction with voltage-gated sodium ion pump of muscle and cardiac cells

species are also well known as immune-stimulant agents and many reports indicated that polysaccharides in these species have significant immune-stimulant properties (Zhao et al 2006; Gao et al. 2010; Fu et al. 2022). FPS-1, a water-soluble polysaccharide isolated from roots of *A. carmichaelii* Debx., showed potent stimulating effects on splenocyte antibody production and murine lymphocyte proliferation induced by concanavalin A or lipopolysaccharide (Zhao et al 2006). The water-soluble polysaccharides from *A. kusnezoffii* Reichb. (WKCP-A fraction) showed notable splenic lymphocyte proliferation activities and macrophage phagocytosis activities (Gao et al. 2011). Recently, some neutral polysaccharides isolated from *A. carmichaelii* leaves also exhibited promising immune modulatory properties (Fu et al. 2022), The roots of the plant *A. heterophyllum* traditionally used for curing dyspepsia, abdominal pain, and diarrhea showed antisecretory and antimotility effect mediated through the nitric oxide pathway (Prasad et al. 2014). Besides, antimicrobial, antivirus, anti-plasmodial, and antioxidant activities are reported from extracts, fractions, polysaccharides, diterpenoid alkaloids, and non-alkaloidal compounds obtained from different *Aconitum* species (Reviewed by Nyirimigabo et al. 2015; Zhou et al. 2015; Ali et al. 2021).

Major active ingredients of Genus

Aconitum species are well known for the presence of diterpenoid alkaloid components, which have nitrogen-containing cyclic organic compounds with complex chemical structures. Aconitine was the first alkaloid isolated from the *Aconitum* in 1833 by Geiger (Nyirimigabo et al. 2015). Furthermore, other alkaloids, such as hypaconitine, mesaconitine, benzoylmesaconine, atidine, isotisine, hetidine, heteratisine, hetsinone, heterophyllisine, heterophylline, and heterophyllidine, were isolated from roots of different species of *Aconitum* (Fig. 4). Now, more than 1000 diterpene alkaloids, structurally classified into four categories, C18-, C19-, C20-, and bis-subtypes have been isolated from this genus. The diterpene alkaloids in *Aconitum* plants have captured the focus of modern research in recent years to develop therapeutical agents for anti-inflammatory, analgesic, or anti-pyretic drugs. The major active ingredients identified in some important species of *Aconitum* have been summarized in Table 1. Structure–activity relationship of aconitum alkaloid indicated that diterpenoid alkaloid having aroyl or aroyloxy group at R-14 position exhibited 30-times higher analgesic potential than the diterpenoid alkaloid with aroyloxy group at R-4 position (Bello-Ramírez et al. 2003).

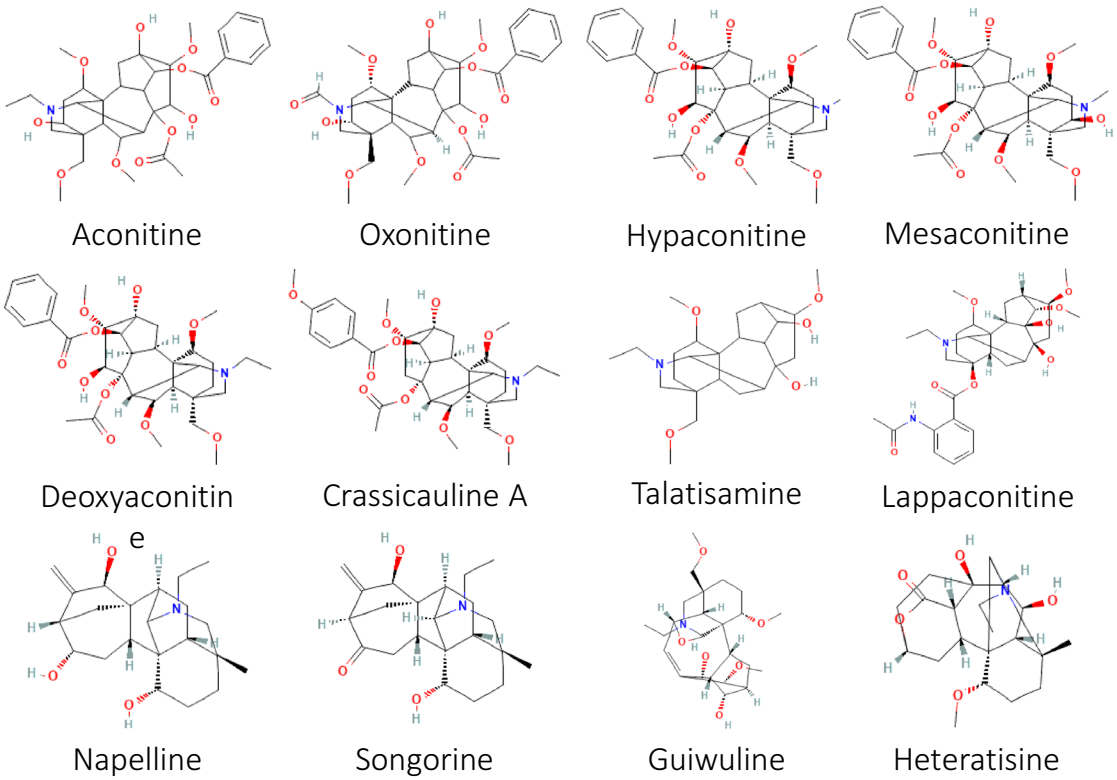

Fig. 4 Structure of some important pharmacologically potent diterpene alkaloids isolated various *Aconitum* species

Table 1 Alkaloids identified in different *Aconitum* species and their content in root part (if quantified)

Species	Alkaloids (quantitative value)	References
A. apetalum	Aconitine, aconorine, cammaconine, taurenine, songorine, songomine, 8-*O*-ethylcammaconine, 3-deoxyaconitine, apetaldines A−G, talassicumine A, acobretine E,	Hu et al. (2019)
A. balfourii	Pseudaconitine, aconitine, benzylaconitine, picroaconitine, andhaemonepellene	Khetwal and Pande (2004), Khetwal (2007)
A. barbatum	Puberunine and puberudine	Mu et al. (2012)
A. baikalense	Napelline, songorine, hypaconitine, mesaconitine, 12-epinapelline N-oxide	Nesterova et al. (2014)
A. chasmanthum	Aconitine, indaconitine, chasmanine, homochasmaninehomochasmaconitine, chasmoaconitine, chasmanthinine 14-*o*-benzoyl-8-ethoxybikhaconine, 14-o-benzoyl-8-methoxybikhaconine	Achmatowicz Jr and Marion (1964), Parvez et al. (1998)
A. carmichaeli	Benzoylmesaconine (0.017%); mesaconitine (0.027%); aconitine (0.003%); hypaconitine (0.179%); deoxyaconitine (0.013%);	Wang et al. (2006)
A. falconeri	Faleoconitine, mithaconitine, 3-methoxyacoforestinine, karakoline, 3-hydroxy-2-methyl-4 h-pyran-4-one; 3,4-dimethoxymethylbenzoate	Atta-ur-Rahman et al. (2000)
A. ferox	Bikhaconitine, pseudaconitine, veratroylbikhaconine, veratroylpseudaconine, diacetyl pseudaconitine	Hanuman and Katz (1993)
A. franchetii	chasmaconitine, chasmanthinine, talatisamine, indaconitine, leueandine	Csupor et al. (2006)
A. heterophyllum	Atisine, isoatisine, aconitic acid, hetisine, heteratisine, atidine, hetidine, hetisone, heterophyllisine, heterophylline, heterophyllidine, heterophyllinine-A, heterophyllinineB, lycoctonine, delphatine and lappaconitine	Wang et al. (2006), Ahmad et al. (2008), Nisar et al. (2009)
A. hemsleyanum	Benzoylmesaconine (0.004%); mesaconitine (0.013%); aconitine (0.004%); hypaconitine (0.014%); deoxyaconitine (0.002%);	Wang et al. (2006), Tang et al. (2009)
A. laciniatum	Pseudaconitine, 14-veratroylpseudaconine, 14-*o*-acetylneoline, neoline, senbusine a	Wangchuk et al. (2010)
A. laeve	Swatinine, delphatine, puberanine, *n*-acetylsepaconintine, swatinine a, swatinine b, foresticine, neoline, delvestine, chasmanine, 8-methylaconitine, 14-dimethylaconitine, *n*-deethyllyaconitine-naldehyde, lappaconitine, lycaconitine, lapaconidine, Lycoctonine	Ulubelen et al. (2002), Shaheen et al. (2005)
A. orientale	Demethylappaconitine; 7, 11, 14-trihydroxy-2,13-dioxohetisane; 6, 13, 15-trihydroxyhetisane; *n*-deethyldelphatine lappaconitine, lycoctonine, browniine	Ulubelen et al. (1996)
A. pendulum	Benzoylmesaconine (0.008%); mesaconitine (0.014%); aconitine (0.484%); hypaconitine (0.020%); deoxyaconitine (0.008%);	Wang et al. (2006)
A. palmuturn	Vakhmatine, vakhmadine, atisine, hetisine	Jiang and Pelletier (1991)
A. rotundifolium	Rotundifosines A–G, heterophyllidine, chellespontine	Frejat et al. (2017); Zhang et al. (2018)
A. spicatum	Indaconitine, chasmaconitine, ludaconitine, spicatine a, and spicatine b	Gao et al, (2006), Shyaula et al. (2016)
A. transsectum	Benzoylmesaconine (0.114%); mesaconitine (0.003%); aconitine (0.002%); hypaconitine (0.004%)	Wang et al. (2006)

Also, the molecular weight of diterpenoid alkaloid inversely correlated with its analgesic properties. The analgesic effect is not only mediated by the central nervous system but by many other possible pathways, such as activation of voltage-dependent Na^+ channels, inhibition of prostaglandin synthesis, and inhibition of noradrenaline uptake (Ameri 1998c). Toxicity of aconitine in neural, cardiac, and muscular tissues has been mediated by permanent activation of the voltage-dependent Na^+ channel by shifting toward the hyperpolarized direction (Faber and Rudy 2000). Also, the presence of three groups [R(I)-C3', R(I)-C5', and R(I)-C2'] mainly the C5' of the aromatic ring also determined the analgesic potency of these alkaloids. Furthermore, benzoyl ester and its position at aconitum alkaloid have been identified as an essential group that determined the binding efficacy of molecules at voltage-dependent Na + channels.

High toxicity occurs if these ester groups are found at C8 and C14 positions and it also determines arrhythmia and analgesic properties (Isono et al. 1994). Besides alkaloidal constituents, various other active constituents, including, phenylpropanoids, flavonoids, terpenoids, steroids, free fatty acids, and polysaccharides, were isolated and identified from different *Aconitum* species. Major flavonoids present in *Aconitum* consist of glycosides of kaempferol and quercetin. To date, 55 known flavonoids have been isolated and identified from different species of the *Aconitum*; among them, 29 are new glycosides and have not been detected in other species (Yin et al. 2019). Similarly, 22 phenylpropionic acids, some steroids, and free fatty acids have been identified and quantified in different species of *Aconitum* (Yue et al. 2010; Weber et al. 2015; Liang et al. 2018; Yin et al. 2019). These non-alkaloidal constituents have significant antioxidant, antiparasitic, anti-phlogistic, antineoplastic, and immunoregulatory effects, and have also potential utility in discoveries of novel compound identification for drug discoveries and chemotaxonomical significance.

Major factors influencing the content of active ingredients

Being a sessile organism, toxic diterpene alkaloids acted as a plant defense system against environmental stresses and pathogenic attacks. Biosynthesis and accumulation of secondary metabolites are generally influenced by genetic architecture, developmental stage, environmental condition, metabolic expenditure of metabolites, and their other importance in plant tissue (Suyal et al. 2019; Wang et al. 2023). Besides the genetic makeup, epigenetic factors (e.g., DNA methylation and miRNA regulation) control the production of secondary metabolite with the influence of environmental stresses, such as drought, salinity, cold, pathogens, and heavy metals in pants by regulating stress-responsive genes (Wang et al. 2023). Thus, the accumulation of aconitine-type diterpene alkaloids exhibited great variation in different growing conditions within a species (Yu et al. 2017; Venkatasubramanian et al. 2018). Aconitine alkaloid is mainly accumulated in the hypogynous organs, while N-di-ethyl-aconitine accumulated in the epigeous organs. A significant variation across the seasons has been reported in total alkaloid content among the different *Aconitum* species. A high quantity of total alkaloids has been recorded in the leaf parts during the growth period. However, with the age, plant alkaloid content decreases (Colombo et al. 1988). Mesaconitine and aconitine in roots of *A. japonicum* have been reported in higher quantities in the samples harvested in May month, while hypaconitine has been reported in samples harvested in December month. Also, total alkaloid content was exhibited higher in the samples collected in May month, and lower in the samples collected in September month (Ito et al. 1996). The biosynthesis of total alkaloids and aconitine in the root of *A. nagarum* and *A. elwesii* was found to be highest in November month. However, in the leaf parts, the alkaloid content was highest in August month (pre-flowering season) in both these species (Sinam and Devi 2011). A considerable quantity of alkaloids was also reported in the stems and leaves of *A. carmichaelii*, and among the different tissues, total alkaloid contents were reported higher in the stem than in other parts. Among seasons, the total alkaloid of the stem part peaked in June, while in leaves, it was highest in July month (Li 2019).

Similarly, in the aerial parts of *A. zeravschanicum,* the highest total alkaloid content was observed at the initiation of the vegetation stage (Nigmatullaev and Salimov 2000). Under the conditions of adequate vegetative growth, the plant initiates sexual reproduction. *A. kusnezoffii* plant, inhibits its vegetative growth and reduces the metabolite accumulation in asexual organs to enhance the success of reproduction. Plant sacrifices their defense system by reducing the turnover of potentially active compounds to provide maximum resources for the important biological process during reproduction. Asexual organs (main storage root) acted as a sink of resources ensuring the storage, utilization, and allocation of resources from the storage root. However, lateral roots (i.e., initial asexual organ) accumulated higher biomass at the end of the growing season and become a mode of asexual reproductive method in the lateral stage (Tang et al 2021). Biomass of the aerial part (weight or the size) can be used as a marker for determining diester alkaloid content in *A. carmichaelii*, as a positive relationship was found between the weight of the aerial part and weight of the new tuberous root and the weight of the aerial part and diester alkaloid content in stem (Kawasaki et al. 2011).

Best recoveries of active ingredients

Diterpenoids alkaloids are the main active constituents in *Aconitum* with some toxicity. Quantification of these alkaloids and keeping the low level of toxic compounds important steps for the quality control of *Aconitum*-based drugs. Thus, various actions have been initiated to reduce its toxicity and increase the availability of non-toxic metabolites. Among the compound separation methods, a high-performance liquid chromatography (HPLC) method has been developed using the C18 column coupled with a photodiode array detector (DAD) for the simultaneous quantitative determination of six *Aconitum* alkaloids (i.e., aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine, and benzoyl-hypaconine) from *Aconitum* roots. The best recovery (90–100%) was achieved in gradient elution using solvents of acetonitrile and ammonium bicarbonate buffer (pH 10.0) (Xie et al. 2005). Also, a capillary zone electrophoresis method has been developed

for the simultaneous determination of six major alkaloids (i.e., aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine, and benzoyl-hypaconine) from *Aconitum* roots. The best recovery was achieved in running buffer having a mixture of 200 mm Tris, 150 mm perchloric acid, and 40% 1,4-dioxane (pH 7.8), and the best operation temperature of the capillary was found 25 °C (Song et al. 2010).

Processing methods also significantly influenced the aconitine alkaloid in roots and herbal formulations. It has been observed that various processing steps cause the removal of the acetyl group at C8 position of diterpenoid alkaloid molecules and formed benzoylaconine with 100–400 times lesser toxicity than natural diterpenoid alkaloid molecules (Fig. 5). Toxicity is further reduced after the loss of benzoyl ester group at C14 to convert it into aconine molecules (Mizugaki and Ito 2005). In China, such processing of *Aconitum* is performed through soaking, heating, and decocting in water or alkaline solution (Liu et al. 2007a). After such processing, the pharmacological activity of plant extract or preparation did not exhibit any significant change (Liu et al. 2007b). However, during the steaming process, the quantity of diester–diterpenoid alkaloids decreased and the contents of monoester–diterpenoid alkaloids increased initially and reached to maximum level after 40 min, but then started to decrease gradually. However, the concentration of aconine alkaloids (e.g., mesaconine, aconine, and hypaconine) increased throughout the processing. On contrary, the concentrations of fuziline, songorine, karacoline, and salsolional remain constant or decreased slightly (Yang et al. 2014). During the baking process, the total and diterpenoids' alkaloids changed significantly along with the total toxicity. However, a slight reduction in the concentration of diester–diterpenoid alkaloids has been obtained during the steaming process, and monoester–diterpenoid alkaloids, aconine alkaloids, and the total alkaloids have been reported to be degraded at different temperatures (Yang et al. 2014). Thus, optimization of processing parameters, such as temperature and time duration, appeared an important step for improving medicinal efficacy.

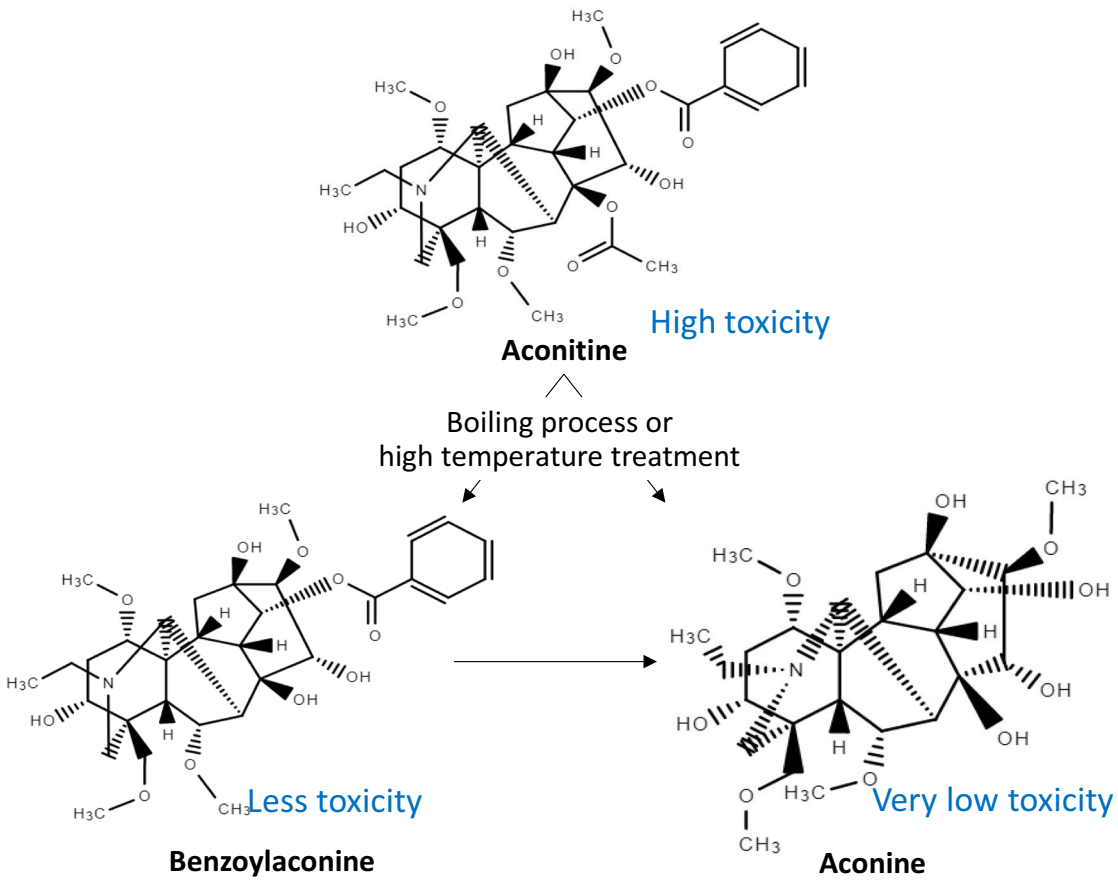

Fig. 5 Change of toxic aconitine into benzaconine and acinine after hating treatment or boiling process (see Liu et al. 2017 for detail)

Biosynthetic pathways of diterpenoid alkaloids

The active ingredient diterpene alkaloids can be broadly classified into three types, viz., diester–diterpene alkaloids, monoester–diterpene alkaloids, and unesterified diterpene alkaloids (Zhou et al. 2015). Ester hydrolysis can transform diester–diterpene alkaloids into monoester–diterpene alkaloids or ester-free amine-diterpene alkaloids, which have relatively lower toxicity and better pharmacological effects. In rats, after hydrolysis, the toxicity of monoester–diterpene alkaloid remained up to less than 150 times that of toxicity of diester–diterpene alkaloid, while their pharmacology remained unchanged (Wen et al. 2013; Zhou et al. 2015). To date, over one thousand diterpene alkaloids have been isolated from different species of *Aconitum*, and after successful clinical testing, only a few compounds (lappaconitine, 3-acetylaconitine and bulleyaconitine) have been used as analgesics (Teng et al. 2021).

Diterpene alkaloids and their derivatives followed a common biosynthetic pathway and have been investigated in many species with modern technological advancements. Aconitine biosynthesis is initiated with MVA (Mevalonate) and MEP (methylerythritol) pathways (Fig. 2). The process is initiated with the condensation of three isopentenyl pyrophosphate (IPP) molecules for the formation of geranyl–geranyl pyrophosphate with the help of the enzyme geranyl–geranyl pyrophosphate synthase (GGPPS). Geranyl–geranyl pyrophosphate formed copalyl diphosphate through proton-induced cyclization in the presence of copalyl–diphosphate synthase (CDPS) (Zi et al. 2014). Furthermore, kaurene synthase (KS) formed kaurene through cyclization and rearrangement of copalyl diphosphate and atisane is formed through its alternate rearrangement reaction. After subsequent oxidation, hydroxylation, and addition of β-ethanolmaine, an atisine skeleton is formed.

Following further oxidation and modification of the atisine skeletal through various cytochrome P450s, different C20-type diterpene alkaloids are formed (Cherney et al. 2014; Devkota and Sewald 2013). The main toxic and pharmacological active constituents of *Aconitum* are aconitine-type C19-diterpene alkaloids (Jiang et al. 2022). The C19-type diterpene alkaloid skeletons were derived from various substitutions on the aconitine skeleton, leading to the formation of different alkaloids (Fig. 6).

Transcriptome studies for major biosynthetic pathways' elucidation

In recent years, technological advancement created an opportunity to generate large information on genomics, proteomics, or transcriptomics in very less time in an inexpensive manner. Among these, transcriptomic studies aim at the genome-wide characterization of mRNA expression in cells, tissue, organs, or organisms as a function of a particular treatment, growth condition, ontological stage, environment, season, or pathological condition, and elucidated and/or characterized gene, transcription factors, and their epigenetic control of various biological processes (Druka et al. 2010; Wai et al. 2022; Yang et al. 2022). The technique has extensively been used to dissect the expression and genetic regulation of biosynthetic pathways of the pharmacologically important secondary metabolite. Rai et al. (2017) detected the candidate genes involved in the mevalonate (MVA) and methylerythritol (MEP) biosynthetic pathways for the synthesis of geranyl–geranyl pyrophosphate as a precursor of diterpene alkaloids. Also, a few unigenes involved in the biosynthesis of diterpene alkaloids were identified after geranyl–geranyl pyrophosphate formation (Rai et al. 2017). Among these, ent-CPP synthases (CPS), ent-kaurene synthases (KS), kaurene oxidases (KOX), cyclases, aminotransferases, monooxygenases, methyltransferase, and BAHD acyltransferases were found to be active during the biosynthesis of C19-diterpene alkaloids in *Aconitum*. Furthermore, regulating transcription factors (TFs) involved in the accumulation of diterpene alkaloids have been identified (Yang et al. 2020).

In a comparative transcriptome study of root and areal parts, genes like 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), mevalonate kinase (MVK), mevalonate diphosphate decarboxylase (MVDD), and 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase (HDS) were found to be involved in root part for the synthesis of aconitine-type diterpene alkaloids (Pal et al. 2015). Atisine biosynthesis is regulated by the simultaneous accumulation of two molecules, e.g., atisenol and steviol, in *A. heterophyllum.* network link between different metabolic pathways identified genes of glycolysis (e.g., G6PI, PFK, ALD, and ENO), serine biosynthesis (e.g., PGDH and PSAT), diterpene biosynthesis (e.g., KO and KH) and phosphorylated pathway as candidate genes for steviol and atisine biosynthesis (Kumar et al. 2016).

In *A. carmichaelii*, key genes encoding enzymes, such as ent-copalyl diphosphate synthases, ent-kaurene synthases, kaurene oxidases, cyclases, and aminotransferases, were found involved in the early steps of diterpenoid alkaloids' biosynthetic pathway. Furthermore, candidate genes responsible for modification in diterpenoid alkaloids, such as monooxygenases, methyltransferase, and acyltransferases skeletons, were identified through transcriptome sequencing (Zhao et al. 2018a). Terpene synthase genes (CDPS4 and KS2) were correlated with 21 unigenes annotated as cytochrome P450 in *A. carmichaelii* (Rai et al. 2017).

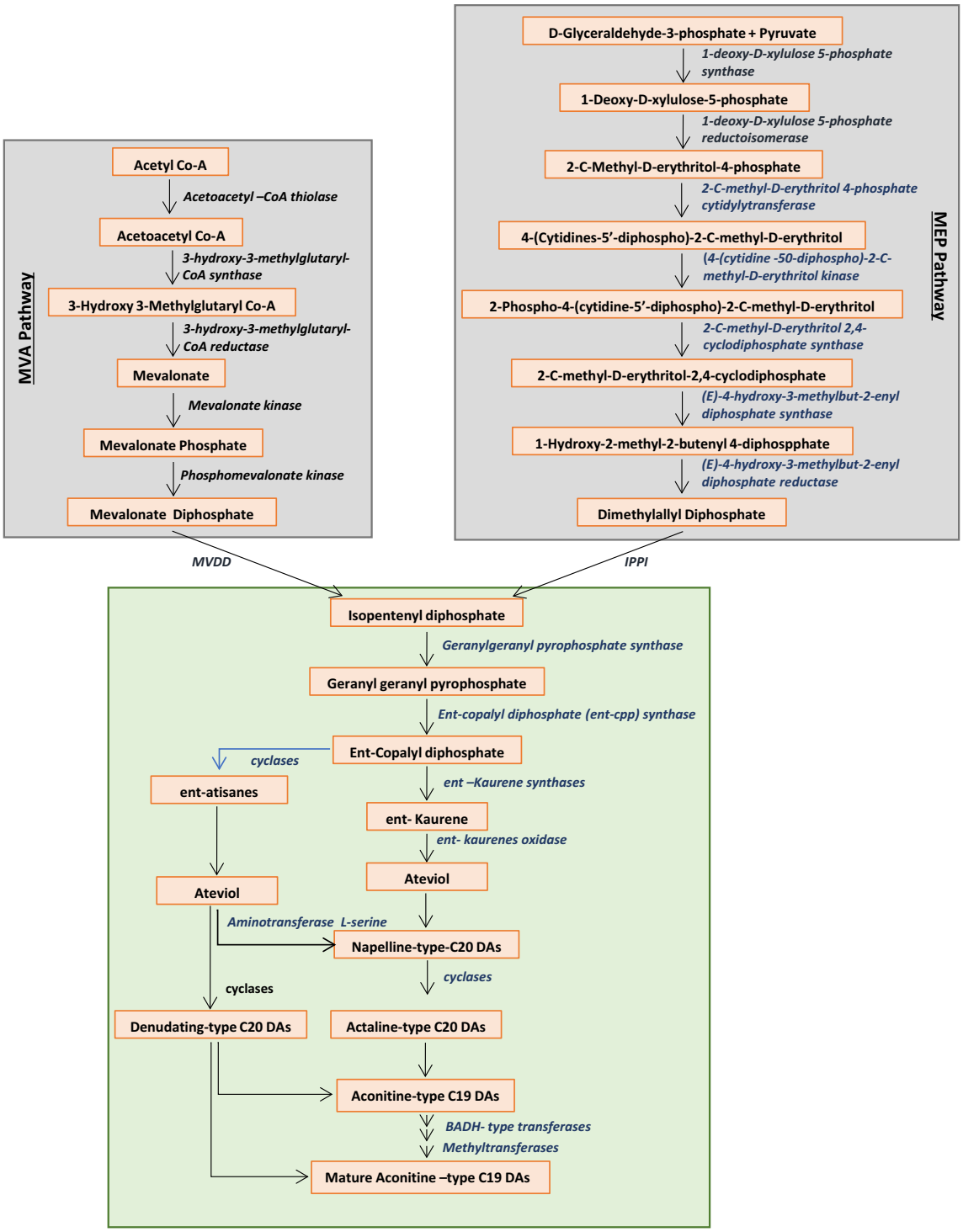

Fig. 6 Biosynthetic pathway for aconitine-type diterpenoid alkaloid (DAs) in *aconitum* genus. The biosynthesis pathway is initiated with the formation of mevalonate diphosphate formation from acetyl co-A in MVA pathway and dimethylallyl diphosphate from D-glyceraldehyde-3-phosphate and pyruvate in MEP pathway. After condensation of the end products of these two respective pathways, isopentenyl diphosphate is formed which is further condensed into geranyl–geranyl pyrophosphate as a precursor of diterpene alkaloids (see Rai et al. 2017; Yang et al. 2020 for detail)

In *A. heterophyllum*, higher expression of eight genes (out of 18 genes) encoding GDP-mannose pyrophosphorylase (GMPase), SHAGGY, Expansin, RING-box protein 1 (RBX1), SRF receptor kinase (SRF), b-amylase, ADP glucose pyrophosphorylase (AGPase), and auxin-responsive factor 2 (ARF2), was observed in roots than shoots which indicated their major contribution in accumulation and storage of primary metabolites in the tuberous root (Malhotra et al. 2016). Digital gene expression analysis in *A. heterophyllum* revealed that four genes (e.g., 3-hydroxy-3-methylglutaryl-CoA reductase, mevalonate kinase, mevalonate diphosphate decarboxylase, and 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase) of aconite biosynthesis pathway and five genes of primary metabolic pathways (e.g., geranyl diphosphate mannose pyrophosphorylase, SHAGGY, RING-box protein 1, SRF receptor kinases, and b-amylase) over-expressed in root parts compared to shoots for the biosynthesis of aconitine and related alkaloids. Also, ABC transporters (399 nos.) involved in the biosynthesis and storage of aconitine-type diterpene alkaloids were identified (Pal et al. 2015).

In *A. kusnezoffii*, among the different plant parts, the mevalonate pathway was relatively more active than methylerythritol 4-phosphate pathway in the flower, while in the leaf and stem, the situation found the opposite. However, aconitine, mesaconitine, and hypaconitine contents in different tissues remain inconsistent with the expression of biosynthesis genes, indicating that there exists a sink–source relation in the plant (Bai et al. 2022). The transcriptome data also indicated the genes involved in alkaloid biosynthesis are differentially expressed in different varieties, consistent with differences in the accumulation of alkaloids (Yang et al. 2020). However, information on different CYP450s, methyl transferases, hydroxylases, and other enzymes involved in structural modification for the diversification of aconitine-type diterpene alkaloids remains largely unexplored.

Genomic resources and diversity studies

Representing over 350 species, the *Aconitum* genus provides a large genetic resource availability across the world (Nyirimigabo et al. 2015). With high species diversity, eastern Asia has been considered a center of diversification (Kadota 1987). The greatest species diversity has been found in the Hengduan Mountains region of southwestern China (Liang-Qian 1988). The subgenus *Aconitum* includes more than 250 species, mainly distributed in the Eastern Himalaya, southwestern China and Japan. Interestingly, a total of 22 native species are found in Europe and are regarded as a secondary center of *Aconitum* diversification (Mitka 2000). While, with more than 60 species, *Aconitum* subgenus *Lycoctonum* has a wide distribution range and well-known background of polyploidy (Kong et al. 2017a, b). Despite great medicinal value in traditional and modern medicines, fewer efforts have been made in *Aconitum* species in genomic resource creation for variety improvement.

Phylogenetic classification and genetic diversity

Within species, a large genetic diversity has been demonstrated in various studies. *Aconitum* species are well known for taxonomic complexity due to extensive inter-specific hybridization (Kita and Ito 2000). Nuclear ITS markers have been applied to resolve this issue in some recent studies. Many species from northern China exhibited close relationships with the species from Europe, North America, and eastern Asia. ITS-based categorization supports the subgrouping of the subgenus *Aconitum,* which is based on the morphology of seeds and petals, suggesting that seed and petal morphology reflects the phylogenetic relationships within the subgenus in a better way. Other important morphological characters used for traditional taxonomical classification, such as the degree of leaf division, the shape of the upper sepal, and the attitude of the stem, might be unreliable in subdividing the subgenus (Luo et al. 2005). Similarly, based on phylogenetic analysis using multiple nuclear (ITS and ESTs) and chloroplast markers in *Aconitum* L. subgenus. *Lycoctonum*, the removal of many unique arrays of characters (the latter even having the aberrant base chromosome number of $x = 6$) was suggested to achieve monophyly in the section. Furthermore, the subgenus *Lycoctonum* was suggested to be redefined to include only two sections, the unspecific sect. *Alatospermum* and the relatively species-rich sect. *Lycoctonum* (Hong et al. 2017).

In different species of *Aconitum*, moderate-to-high level of genetic diversity was reported in various studies. RAPD and ISSR as preliminary marker systems used for genetic diversity characterization of *Aconitum* genus were successfully used in diversity characterization of *A. noveboracense*, *A. columbianum* Nutt, *A. carmichaelii,* and *A. kongboense* L. species and based on genetic diversity parameters, suitable conservation priorities were formulated (Cole and Kuchenreuther 2001; Zhao et al. 2015; Luo et al. 1994; Meng et al. 2015). However, these marker systems lack the issue of repeatability thus, AFPL emerged as another choice of technique for diversity characterization of *A. brachypodum*, *A. kongboense, A. plicatum,* and *A. firmum* (Mitka et al. 2015; Du et al. 2018; Meng et al. 2014).

More recently, sequence-specific markers, such as SSRs' markers, have emerged as a new method for genetic characterization. With the development of sequencing technology, expression-based markers become helpful for the development of functional markers. The ubiquity and usefulness of SSRs or microsatellites have been well recognized in eukaryotes for constructing framework genetic maps, comparative

mapping, marker-assisted selection, genetic diversity, and gene targeting due to high frequency, co-dominance, multiallelic nature, reproducibility, extensive genome coverage, and easiness in detection (Sharma et al. 2020). These markers have already been successfully used in the genetic diversity characterization of *A. coreanum, A. carmichaelii, A. gymnandrum, A. napellus* L, *A. austrokoreense,* and many other species (Won et al. 2012; Yang and Zhou 2017; Lee et al. 2018). Various genetic diversity studies available in *Aconitum* species using different marker systems are presented in Table 2.

Availability of genomic resources

Information on genetic resources provides insight into the expression, regulation, and heredity of traits, which can be harnessed for variety improvement and quality maintenance during molecular breeding efforts. Thus, the use of DNA markers has become useful assays for diversity analysis, phylogenetics, genetic mapping, marker-assisted breeding, genotyping, and genome analysis in *Aconitum*. The level of genetic diversity determined by various types of markers can be utilized for formulating conservation strategies for different species of *Aconitum*. Various efforts for genomic resource creation, such as microsatellite loci or SSR marker development, etc., are summarized in Table 3. To date, no efforts have been made the improvement of variety in *Aconitum* using the molecular marker; however, it might be beneficial in terms of time, labor, and cost-efficiency.

In *A. kusnezoffii*, 19 microsatellite loci were developed and these markers were found useful in quantifying male and female fitness in *A. kusnezoffii* and evaluating the effects of clonal growth on sexual reproduction (Ge et al. 2016). In *A. brachypodum*, 12 microsatellite markers were developed from two microsatellite-enriched libraries (AG, AC) constructed using an FIASCO method (Li et al. 2015). Hou et al. (2020) identified 19 polymorphic microsatellite loci for *A. gymnandrum*. Among these, seven loci showed significant deviation from Hardy–Weinberg equilibrium and showed potential for pollination ecology and population genetic studies in Qinghai–Tibet plateau. In *A. gymnandrum,* 16 out of the 32 loci were identified as polymorphism and were found useful in characterization in genetic diversity analysis (Xu et al. 2011). In *A. coreanum* (*H. Lév.*), ten microsatellites were developed, and only two markers were polymorphic and showed F_{ST} as 0.205 and 0.275, respectively (Won et al. 2012).

Currently, Next-Generation Sequencing (NGS) technology has been found useful in large number marker development in a shorter time. In *A. reclinatum*, sequencing of a genomic library using Illumina HiSeq technology, ten polymorphic primer pairs were developed. These microsatellites isolated were found useful in genetic diversity and conservation genetics studies (Zhou et al. 2018). Also, in *A. austrokoreense*, 9 novel microsatellite markers were developed from sequencing of genomic DNA and these novel markers were found valuable for assessing genetic diversity and for germplasm conservation (Yun et al. 2015). Similarly, in *A. vilmorinianum*, 18 novel microsatellite markers were developed using next-generation sequencing technology of genomic DNA (He et al. 2015). Cadre et al. (2005) identified and characterized 16 polymorphic microsatellite markers in *A. napellus* through sequencing of a partial genomic library and found them an efficient tool for detailed investigations of the population genetic structure of the species. These polymorphic microsatellite loci will be especially useful for genetics studies. In the future, these microsatellite loci can be valuable for further interpretation of the fine population structure and conservation strategies of this rare, threatened species.

Furthermore, NGS-based transcriptome analysis could provide a large dataset on the expression part of genomics and could be useful for the development of functionally relevant markers. This has explored the development and utilization of sequence-based markers for genome mapping and molecular breeding in a faster manner (Unamba et al. 2015). In comparative root and shoot transcriptome analysis of *A. heterophyllum*, a total of 177,438 potential SSRs were identified and these potential functional markers could be linked with quality-related traits (Pal et al. 2015). Similarly, in *A. carmichaelii*, the de novo transcriptome assembly yielded a total of 16,068 SSR from 14,168 unigenes motifs with at least ten repetitions of mono- to hexa-nucleotide (Rai et al. 2017) wherein mono-nucleotide repeats had the greater frequency (63.68%) followed by tri- and di-nucleotide repeats (22.3% and 12%, respectively). Based on the functional classification of SSR containing unigenes, the development of quality-related functional markers related to secondary metabolites, high-altitude adaptation, yield, and productivity could be developed.

Full genome sequencing has not been carried out in any species of *Aconitum*. However, the full sequence of the chloroplast and mitochondrial genome is available in many species of *Aconitum*. A comparative overview of full genome sequencing is summarized in Table 4. The chloroplast genome was found as a typical quadripartite structure with a Large Single-Copy region (LSC) and a Small Single-Copy (SSC) separated by a pair of inverted repeat regions. IR region was found more conserved as compared to the LSC and SSC regions (Chen et al. 2015; Sungyu, et al. 2016; Kong et al. 2017a, b; Park et al. 2017a; Park et al. 2017b; Meng et al. 2018; Kong et al. 2017a, b; Yang et al. 2018; Kim et al. 2019; Liu et al. 2020; Meng et al. 2019; Cheng et al.2020; Li et al. 2020; Lim et al. 2020; Wang and Li 2020; Zhang et al. 2021a, b; Ni et al. 2022;). Among all the species, the smallest size of cp genome (151,214 bp) was

Table 2 Genetic diversity studies in different species of *Aconitum* using diverse markers

Species name	Sample size and location	Marker type used	Diversity and other description	References
A. noveboracense & *A. columbianum*	USA	Isozyme and ISSR	PP = 86% by Isozymes and 89.7% by RAPD, high level of F_{ST} = 0.24 and less gene flow (Nm = 0.68.) was recorded	Cole and Kuchenreuther (2001)
A. kusnezoffii, A. soongaricum A. carmichaelii & *A. leucostomum*	3 population in Xinjiang Province	RAPD and ISSR	PP = 97.25% (RAPD) and 98.92% (ISSR). Hs was higher than Ht. G_{ST} was 0.4358 (RAPD) and 0.5005 (ISSR). Nm was 0.6473 for ISSR and 0.4991 for RAPD)	Zhao et al. (2015)
A. carmichaeli	Jiangyou, China	ISSR	PP was 69.39%, Na = 1.6939 Ne = 1.3715, H = 0.230 8, and I = 0.3530	Luo et al. (1994)
A. kongboense L	Motuo, Tibet Plateau	ISSR	At species level, PP = 58.42%. Ne = 1.564; H = 0.320; and H = 0.469. All the cultivars cluster into 3 groups according to their geographical origin and genetic backgrounds	Meng et al. (2015)
A. plicatum & *A. firmum*	Carpathian Mountains Europe	AFLP	Gst was greater within the Sudetic A. plicatum (F_{ST} = 0.139, P < 0.001) than within the Carpathian A. firmum (F_{ST} = 0.062, P < 0.001) populations due to the long-lasting geographic isolation	Mitka et al. (2015)
A. brachypodum	Guizhou area	AFLP	Relatively high level of genetic diversity (He = 0.322 9 ± 0.179, I = 0.472 0 ± 0.251 7, PP = 80.57% at species level and Genetic differentiation index (Gst) was 0.864 2	Du (2018)
A. kongboense	Eastern China	AFLP	NPL = 77, PPB = 68.75%, Na = 1.688, Ne = 1.412) and lowest in population 2 (NPL = 57, PPB = 50.89%, Na = 1.509, Ne = 1.273)	Meng et al. (2014)
A. coreanum	Korea	Microsatellite markers	Low gene flow (1.126), heterozygosity deficit, low level of among-population differentiation, small size of gene flow, and lack of sequence variation of the organelle suggest that species are reproductively isolated from other species	Won et al. (2012)
A. carmichaeli	Central china	Microsatellite markers	Ne = 3.25, H_o = 0.612, H_e = 0.493, I = 0.851 and h = 0.505. F_{ST} = 0.149) and Nm = 1.432 was limited	Yang and Zhou (2017)
A. austrokoreense	Korea	Microsatellite markers	High pairwise F_{ST} = 0.35 (mean) suggested significant differentiation among populations. Low within population genetic variation was recorded	Lee et al. (2018)

NPL number of polymorphic loci, *PP* Percentage of Pymoorphic loci, *Na* observed number of alleles, *Ne* effective number of alleles, *Ho* observed heterozygosity, *He* expected heterozygosity, *H* Nei's gene diversity, *I* Shannon's index, *Hs* inter-population diversity *Ht* intra-population diversity, *Nm* gene flow, G_{ST} efficient of gene differentiation, *h* Nei's genetic diversity index, F_{ST} genetic differentiation

Table 3 Microsatellite maker development in different species of *Aconitum*

Name of species	Method for marker development	Marker detail	Application and validation	References
A. austrokoreense	Next-generation sequencing technology	9 novel microsatellite markers were developed	These novel markers were found valuable for assessing the genetic diversity and germplasm conservation	Yun et al. (2015)
A. carmichaelii	Transcriptome sequencing	A total of 16,068 SSR across 14,168 unigenes	Potential genetic resource for genetic diversity assessment and comparative genetics across different *Aconitum* species	Rai et al. (2017)
A. brachypodum	Fast isolation by AFLP of sequences containing repeats	12 microsatellite markers were developed	Among the 12 markers, three deviated from HW equilibrium significantly and have shown usefulness in population genetics	Li et al. (2015)
A. coreanum	Next-Generation Sequencing	10 microsatellites were developed	Only two markers were polymorphic and showed *FST* of 0.205 and 0.275, respectively	Won et al. (2012)
A. gymnandrum	Direct genomic DNA sequencing	19 polymorphic microsatellite loci were developed	Seven loci showed a significant deviation from Hardy–Weinberg equilibrium followinga Bonferroni correction, which might be due to non-random mating of individuals	Hou et al. (2020)
A. gymnandrum	Using the combined biotin capture method	16 polymorphic microsatellite loci were identified	50% loci were polymorphic and the number of alleles per locus ranged from 4 to 15 in 66 individuals from 6 population	Xu et al. (2011)
A. heterophyllum	Transcriptome sequencing	A total of 177,438 potential SSRs were identified	Functional markers could be identified for quality-related traits	Pal et al. (2015)
A. kusnezoffii	Genomic enrichment approach	A total of 19 microsatellite loci were developed	Among these, 13 loci polymorphic and 5 loci among the 6 amplified in *A. barbatum*var. *puberulum*were polymorphic,	Ge et al. (2016)
A. napellus	Sequencing of partial genomic library	16 polymorphic microsatellite markers were characterized	These microsatellites offer an efficient tool for detailed investigations on population genetic structure of the species	Cadre et al. (2005)
A. pseudolaeve & A. longecassidatum	Complete sequencing of chloroplast (CP) genome	A total of 61 indels and 62 SNPs detected between both the species	A. pseudolaeve, A. barbatum and A. longecassidatum can be clearly distinguished using the novel indel markers [AcoTT (trnK-trnQ) and AcoYN (ycf1-ndhF)]	Park et al. (2017a)
A. reclinatum	Sequencing of a genomic library using Illumina HiSeq technology	10 polymorphic primer pairs were developed	These microsatellites were found useful in genetic diversity and conservation genetics studies	Zhou et al. (2018)
A. reclinatum	Sequencing of a genomic library using Illumina HiSeq technology	55 pairs of microsatellite primers were obtained	14 pair (~25%) high polymorphism primers could be used for identification of genetic resources, genetic diversity analysis and DNA fingerprint	Yang & Zhou (2017)
A. vilmorinianum	Next-generation sequencing technology	18 novel microsatellite markers were developed	These polymorphic microsatellite locican be useful for genetics studies	He et al. (2015)

Table 4 Available information in the full chloroplast genome of different *Aconitum* species

Species name	Genome size (bp)	Number of genes	GC content	Other important feature	References
A. coreanum	155,880	131 genes—86 protein-coding, 8 rRNA genes, and 37 tRNA genes	38.13%	LSC (86,338 bp) and SSC (16,946 bp) regions separated by a pair of IR regions (26,294 bp)	Park et al. (2017b)
A. carmichaelii	157,040p	131 genes—86 protein-coding, 8 rRNA genes, and 37 tRNA genes	37.99%	LSC (87,628 bp) and SSC (16,924 bp) regions separated by a pair of IR regions (26,244 bp)	Park et al. (2017b)
A. pseudolaeve	155,628	112 genes—78 protein-coding, 4 rRNA genes, and 30 tRNA genes	38.0%	A pair of inverted repeat regions separated by LSC (86,683 bp) and SSC (17,091) regions	Park et al. (2017a)
A. longecassidatum	155,524	112 genes—78 protein-coding, 4 rRNA genes, and 30 tRNA genes	38.10%	A pair of inverted repeat regions separated by LSC (86,466 bp) and SSC (16,950 bp) regions	Park et al. (2017a)
A. vilmorinianum	155,761	132 genes—78 protein-coding, 4 rRNA genes, and 30 tRNA genes	38.10%	A pair of IR regions of 26,209 bp), one LSC (86,394 bp) and one SSC region (16,949 bp) two pseudogenes (*yrps19* and *yycf1*) found	Meng et al. (2018)
A. delavayi	155,769	131 genes—78 protein-coding, 4 rRNA genes, and 30 tRNA genes	38.1%	A pair of IR regions of 26,240 bp), one LSC (86,340 bp) and one SSC region (16,949 bp)	Meng et al. (2018)
A. episcopale	151,214	131 genes—78 protein-coding, 4 rRNA genes, and 29 tRNA genes	38.3%	A pair of IR regions of 26,156 bp and 26,217 bp), one LSC (83,182 bp) and one SSC region (15,598 bp), two pseudogenes (*yrps19* and *yycf1*) found	Meng et al. (2018)
A. hemsleyanum	155,684	132 genes—78 protein-coding, 4 rRNA genes, and 30 tRNA genes	38.1%	A pair of IR regions of 26,235 bp), one LSC (86,929 bp) and one SSC region (16,922 bp), two pseudogenes (*yrps19* and *yycf1*) found	Meng et al. (2018)
A. contortum	155,653	132 genes—78 protein-coding, 4 rRNA genes, and 30 tRNA genes	38.1%	A pair of IR regions of 26,221 bp), one LSC (86,267 bp) and one SSC region (16,944 bp), two pseudogenes (*yrps19* and *yycf1*) found	Meng et al. (2018)
A. scaposum	157,688	145 unique genes, 8 rRNA genes and 38 tRNA genes	38.0%	A pair of IR regions of 26,156 bp and 26,232 bp), one LSC (69,309 bp) and one SSC region (16,917 bp)	Zhang et al. (2021a, b)
A. coreanum	157,024	132 genes (86 protein-coding genes, 8 rRNAs, and 37 tRNAs)	38.0%	87,637 bp of LSC and 16,901 bp of SSC, are separated by two 26,243 bp IRs	Kim et al. (2019)
A. puchonroenicum	155,631	111 genes (4 rRNA genes, 29 tRNA genes, and 78 protein-coding genes	38.05%	86,689 bp LSC and 17,088 bp SSC regions are separated by 25,927 bp of a pair of IR regions	Lim et al. (2020)
A. piepunense	155,836	130 genes (8 rRNA genes, 37 tRNA genes, and 85 protein-coding genes	37.8%	A large LSC, (86,433 bp), a small SSC, (16,945 bp), and a pair of IR regions (26,229 bp)	Ni et al. (2022)
A. reclinatum	157,354	135 genes, including 87 protein-coding genes, 40 tRNA genes and 8 rRNA genes	38.0%	A pair of 26,061 bp IRs regions separated by LSC of 88,269 bp and SSC of 16,963 bp	Kong et al. (2017a, b)
A. flavum	155,654	129 genes, including 83 protein-coding genes, 8 rRNA, and 37 tRNA genes	38.1%	A one LSC of 86,390 bp, one SSC region of 16,968 bp, and two IR regions of 26,148 bp	Liu et al. (2020)
A. austroyunnanense	155,818	131 genes, including 85 protein-coding genes, 37 tRNA genes, 8 rRNA genes and a pseudogene	38.1%	Two IRs, (26,128 bp) regions, one large LSC (86,555 bp) and one SSC (17,007 bp)	Cheng et al. (2020)
A. pendulum	155,597	131 genes, consisting of 86 protein-encoding genes, 8 rRNA, and 37 tRNA	38.1%	A copy of LSC and SSC regions of 86,336 and 16,961 bp, isolated by two IRs regions of 26,150 bp	Wang end Li (2020)

Table 4 (continued)

Species name	Genome size (bp)	Number of genes	GC content	Other important feature	References
A. brachypodum	155,651	132 genes including 85 protein-coding genes, 37 tRNA genes, 8 rRNA genes and 2 pseudogenes	38.0%	A pair of 26,213 bp IRs) separated by LSC region of 86,292 bp and SSC region of 16,933 bp	Meng et al. (2019)
A. carmichaelii	155,737	112 gene species (incl. 78 protein-coding, 30 tRNAs & 4 rRNAs	38.1%	A pair of IR regions of 26,193 bp, separated by LSC region of 86,330 bp and SSC region of 17,021 bp	Yang et al. (2018)
A. tanguticum	157,114	112 gene, including 78 protein-coding, 30 tRNA and rRNA gene	38.0%	A pair of IR regions (26,255 bp), separated by LSC region (87,559 bp) and SSC region (17,045 bp)	Li et al. (2020)
A. volubile var. pubescens	155,872	131 genes including 86 protein-encoding genes, 8 rRNA genes and 37 tRNA) genes	38.12%	LSC (86,348 bp), SSC (16,944 bp) separated by pair of IRs (26,290 bp)	Sungyu, et al. (2016)
A. barbatumvarpuberulum	1 56,749	130 genes, including 84 protein-coding genes, 34 tRNA genes and 8 rRNA genes	38.7%	Include LSC region of 87,630 bp and SCR region of 16,941 bp separated by two IRs of 26,089 bp	Chen et al. (2015)
A. angustius	156,109	126 genes with 84 protein-coding genes, 38 tRNA genes and 4 rRNA genes	38.0%	A pair of IR regions of 26,225 bp, separated by LSC region of 86,719 bp and SSC region of 16,914 bp	Kong et al. (2017a, b)
A. finetianum	155,625	126 genes with 84 protein-coding genes, 38 tRNA genes and 4 rRNA genes	38.0%	A pair of IR regions of 25,927 bp, separated by LSC region of 86,664 bp and SSC region of 17,107 bp	Kong et al. (2017a, b)
A. sinomontanum	157,215	126 genes with 84 protein-coding genes, 38 tRNA genes and 4 rRNA genes	38.0%	A pair of IR regions of 26,090 bp separated by LSC region of 86,074 bp and SSC region of 16,926 bp	Kong et al. (2017a, b)

found in *A. episcopale* (Meng et al. 2018). These genomes were found good source of simple sequence repeats, and interestingly, among the different subgenus, a higher number of SSRs were obtained (64–62, average value 59) in subgus *Lycoctonum* as compared to subgenus *Aconitum* (40–60, average value 54). The maximum SSRs concentration was found in the LSC region (Kong et al. 2017a, b). However, the number of SSRs was found between 259 and 287 in a few other studies (Park et al. 2017b; Meng et al. 2018). Furthermore, cluster analysis based on the chloroplast genome revealed that two separate monophyletically groups formed according to their subgenus of *Aconitum* (Park et al. 2017a; Kong et al. 2017a, b; Meng et al. 2018). These full chloroplast genome sequences have shown potential applicability in phylogenic classification, evolutionary history, and divergence studies.

Tissue culture studies

Mass propagation techniques

Standardization of culture medium is one of the important components of plant tissue culture. Giri et al. (1993) reported standardization of in vitro propagation of *A. heterophyllum* Wall. They induced callus on MS medium supplemented with either 1 mg/l (2,4-D), 0.5 mg/l Kinetin and 10% coconut water or with 5 mg/l NAA and 1 mg/l BAP. Somatic embryos were able to form when calli induced on 2,4-D and NAA were transferred onto MS medium supplemented with 1 mg/ l BAP and 0.1 mg/l NAA following two sub-culturing. Complete plantlets were regenerated from these somatic embryos following 4 weeks on medium with ¼ MS medium. Rooting of plantlets was achieved with 1.0 mg/l IBA. Jabeen et al. (2006) were able to induce callus on MS medium supplemented with a low concentration of NAA (0.5 mg/l) and BAP (0.25 mg/l). The shooting was achieved in MS medium supplemented with 0.25 mg/l NAA with 0.5 mg/l BAP. Likewise, the regeneration of plantlets from callus cultures of leaf and lateral buds was achieved (Singh et al. 1998).

Young leaf explants and lateral buds were cultured on MS medium supplemented with 0.8 mg/l of BAP and NAA each leading to callus induction and finally somatic embryos and shoots. Pandey et al. (2004) could induce callus from the leaf segment of in vitro axillary bud on MS medium supplemented with 4.5 µMBAP and 26.9 µMNAA. They were able to induce shoots with the same concentration of BAP and a lower concentration of NAA. In vitro shooting and rooting of plantlets was achieved with 1 µMBAP and 12.3 µM of IBA. Since in vitro propagated plants are more likely to have variation in morphology, genetic makeup, phytochemical content, or stages of development than seed-grown or

vegetative propagated plants, Pandey et al. (2004) evaluated the difference in the chromosome, protein profile, and alkaloid content of in vitro tubers raised and seed raised plants of comparable age. Rawat et al. (2013a) studied secondary metabolite production in *A. violaceum* Jacq. Among all the tested hormone combinations, 2.5 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.25 µM kinetin (Kn) could promote callus formation. 6-Benzyl amino purine (6-BAP) was found to be effective for shoot regeneration and secondary metabolite production compared to thidiazuron (TDZ). The frequency of plantlet regeneration was highest when calli were transferred to an MS medium supplemented with 1 µM BAP and 0.5 µM α-naphthalene acetic acid (NAA). It was observed that when low concentrations of NAA were added to the culture medium, secondary metabolite production was reduced as compared to cytokinin only. Further, different cytokinin concentrations affected secondary metabolite production in some cases.

Callus-mediated organogenesis from both leaf and petiole segments was reported by Gondval et al. (2016). MS medium supplemented with 0.5 mg/l TDZ and 1.0 mg/l NAA yielded optimum callusing. The shoots and roots were developed on MS medium supplemented with a combination of 0.25 mg/l IAA and 0.25 mg/l NAA. The plantlets were acclimatized and successfully transferred to field conditions. In *A. ferox,* callus was initiated and maintained on MS medium supplemented with 2.26 µM 2,4-D. For the development of adventitious shoots, BAP was found to be responding among all the tested plant growth regulators. Synergistic interaction between IAA and BAP inducted plantlets with well-developed roots and shoots. However, the best rooting response was recorded when shoots were placed on a paper bridge in the liquid MS medium. Phytochemical analysis revealed similar antioxidant activity closer to wild plants. The study leads to the development of the method for sustainable utilization of *A. ferox* (Singh et al. 2020). Rafiq et al. (2021) developed an efficient micropropagation protocol for *A. chasmanthum* Stapf ex Holmes using nodal explants. Nodal explants showed direct multiple shoot regeneration with BAP (0.5 mg/l) and rhizome formation was achieved following 8 weeks in MS medium and finally development of plantlets. These protocols could be used for large-scale propagation and conservation of various *Aconitum* species. A summary of species, explant, and media used in tissue culture studies of *Aconitum* has been summarized in Table 5.

Secondary metabolites' production from callus suspension culture

Micropropagation through plant tissue culture has also been used as a route for the production of secondary metabolites through induction of callus or in suspension cultures in presence of higher concentrations of auxin or in a combination of auxin and cytokinin. Production of important secondary metabolites has been successfully achieved in many *Aconitum* species. Tissue culture-raised plants showed a higher accumulation of aconitine content. In *A. violaceum*, higher aconitine content was recorded in tissue culture-raised

Table 5 Response of different species of *Aconitum* in tissue culture conditions

Species	Explant	Media & growth regulators	References
A. atrox (Bruhl). Muk	Leaf explants and lateral buds	MS + 0.8 mg/L BAP and NAA	Singh et al. (1998)
A. balfourii Stapf	Leaf segment of in vitro axillary bud	MS + 4.5 µM BAP + 26.9 µM NAA	Pandey et al (2004)
A. balfourii Stapf	Leaf and lateral buds, axillary buds, leaf	MS + 4.5 µM BAP + 26.9 µM NAA	Singh et al. (1998)
A. balfourii Stapf	Leaf and petiole segments	MS + 0.5 mg/L TDZ + 1.0 mg/L NAA	Gondval et al (2016)
A. bucovinense Zapał	Leaf	MS + 1.5 mg/L IBA + 1.0 mg/L BAP	Kocot et al. (2022)
A. carmichaeli	Shoot tips and axillary buds	MS + 1.0 mg/L BAP	Hatano et al (1988)
A. chasmanthum Stapf ex Holmes	Nodes, leaves and stems	MS + cytokinins (BAP, Kn), auxins (2,4-D, NAA)	Rafiq et al. (2021)
A. ferox	Seed raised plantlets	MS + 2.26 µM 2,4-D)	Singh et al. (2020)
A. heterophyllum Wall	Leaf and petiole	MS + 1 mg/L 2,4-D + 0.5 mg/L kinetin + 10% coconut water (v/v) or with 5 mg/L NAA + 1 mg/L NAA	Giri et al (1993)
A. heterophyllum Wall	Nodal segments	0.5 mg/L NAA and 0.25 mg/L BAP	Jabeen et al. (2006)
A. heterophyllum Wall	Leaf explants	MS + 1 mg/L BAP + 0.5 mg/L Kin + 0.1 mg/l GA_3	Mahajan et al. (2015)
A. napellus	Shoot tip, stem nodes	Shoots (4 mg/l BA); explants on rafts (0.25 mg/l BA)	Watad et al. (1995)
A. uncinatum	Shoot apex – multiple shoots, plantlets	44.4 µM BA + 46.5PM kinetin	Lim and Kitto (1995)
A. violaceum Jacq	Nodal segment	MS + 0.5 µM 2,4-D + 0.25 µM kinetin	Rawat et al (2013a)
A. violaceum Jacq	Shoot tip, callus	MS + 0.1 µM BAP + 0.5 µM NAA	Chandra (2003)

hardened plants as compared to control plants (Rawat et al. 2013a). In *A. carmichaelii* Debx., MS medium supplemented with 0.5 mg/l IAA exhibited better rooting at 20 °C, and in these roots, aconitine-type alkaloids (mesaconitine and hypaconitine) were found more than 50% higher than plants cultured at 15 °C and 10 °C (Shiping et al. 1998). Also, in *A. ferox*, a higher level of antioxidant activity was found in roots regenerated with the supplementation of 5MS + 6 µM BAP + 3 µM IAA, but total phenolic and flavonoid contents were obtained as lower than in control plants (Singh et al. 2020).

Cell suspension cultures can be efficient for aconitine alkaloids or related secondary metabolite production. In *A. napellus,* various culture conditions exhibited a positive impact on cell biomass and aconitine accumulation. Callus induced in leaf explants was transferred into liquid MS medium with 5% sucrose and showed significant improvement in cell growth and aconitine accumulation after 8 weeks. Salicylic acid and yeast extract supplementation in the MS medium induced a higher accumulation of aconitine and reached up to 0.043% (dry weight basis) (Hwang et al. 2004). In *A. heterophyllum*, total alkaloid (aconites) content was recorded as 3.75 times higher in *Agrobacterium rhizogenes*-mediated transformed roots (2.96%) as compared to non-transformed (control) roots (Giri et al. 1997). In other species, such studies are largely lacking; however, such biotechnological interventions could be promising tools for important metabolite production.

Future prospects and conclusion

More than 350 *Aconitum* have been found worldwide, and the phytochemical and pharmacological studies are still restricted to only a few species. Such a vast diversity in genetic resources created an opportunity for the discovery of novel molecules with higher efficient diterpene alkaloids or with different biological activities. Limited molecules present in *Aconitum* have been explored for pharmacological activities. From pharmacological activities, studies have been carried out in limited areas, such as anti-inflammatory, analgesic, anti-rheumatic, anti-nociceptive, hypoglycemic, multidrug resistance inhibitor, cytotoxic, anti-tumorous, immune stimulant, and antioxidant activities (Ameri 1998a; Ameri 1998b; Xu et al. 2006; Dasyukevich and Solyanik 2007; Verma et al. 2010; Huang et al. 2011; Wang et al 2012; Xing et al. 2014; Nesterova et al 2014; Zhang et al. 2015; Guo et al 2017; Zhang et al 2020; Zhang et al 2021a, b; Liang et al 2016). However, lead from these preliminary finding needs more in-depth investigations for novel drug discoveries. For example, most of the diterpene alkaloids have been validated for anticancer or anti-inflammatory activities; however, after the screening of all the existing diterpene alkaloids, the identification of the most potent molecule and activity–structure relationship might provide a breakthrough in drug discovery. Also, most of the ethnopharmacological uses of the genus need to be validated using suitable model systems followed by clinical trials. The processed *A. carmichaelii* hydrogel patch prepared by matrix prescription optimized through the central composite design-response surface method was found better in quality in terms of appearance, adhesion and in vitro release (Wu et al 2018) and such drug delivery systems can be more useful for determining the new dosage of aconite-type diterpene alkaloids.

High genetic diversity within the genus, hybridization compatibility in nature (existence of natural hybrids), large geographical distribution, and presence of a large array of aconitine-type diterpene alkaloid and other important secondary metabolites indicated tremendous possibilities of variety development in *Aconitum*. Only, *A. carmichaelii* Debeaux has been cultivated on farms in western China without any information on quality attributes. In India, *A. heterophyllum* is cultivated in high-altitude Himalayan regions to some extent. Diversity in quantitative traits at the genetic level provides the basis for successful breeding efforts during variety improvement. Thus, genetic characterization is necessary; however, such studies are largely unavailable in most of the *Aconitum* species. Genetic diversity studies at the global level in the species are only available in a few species (Cadre et al 2005; Xu et al. 2011; Won et al 2012; Li et al 2015; Pal et al 2015; He et al 2015; Yun et al 2015; Ge et al 2016; Rai et al 2017; Park et al 2017a; Zhou et al 2018; Yang and Zhou 2017; Hou et al 2020). Also, due to the scarcity of genomic information in most of the *Aconitum* species, trait-specific functional markers are not available in *Aconitum* species, which can be important for elite parental group identification, gene targeting, mapping studies, and marker-assisted breeding. Modern sequencing platforms, simulation modeling, and computational advancement can reduce time and expense in future Aconitum research. Thus, opportunities for genetic improvement in species through molecular breeding or core genotype selection for cultivation are insignificant in *Aconitum.*

Most of the *Aconitum* species preferred patchy distribution, and thus, low population density exists in nature. Extensive extraction of most of the species in Asia causes pressure on the wild population leading to its extinction. Therefore, regular monitoring of the population status in the wild to ascertain the existence of the species on the earth is required. Many species of *Aconitum*, e.g., *A. chasmanthum* (critically endangered), *A. heterophyllum* (endangered), *A. violaceum* (vulnerable), *A. corsicum* (vulnerable), *A. austrokoreense* (near threatened), *A. firmum* subsp. *moravicum* (near threatened), and *A. lasiocarpum* (near threatened), are categorized under the threat list of IUCN (IUCN 2020).

However, many species restricted to very small geographic regions have yet not been evaluated and have a larger threat of extinction. Propagation protocols (tissue culture or conventional) are only available for a few species (Hatano et al 1987, 1988; Giri et al 1993; Watad et al 1995; Lim and Kitto 1995; Singh et al. 1998; Chandra 2003; Pandey et al 2004; Jabeen et al. 2006; Rawat et al. 2013b; Gondval et al. 2016; Singh et al. 2020; Rafiq et al. 2021) and robust method of propagation for commercial cultivation need to be developed. Although, seed germination methods have been standardized for many important species (Beigh et al 2006; Vandelook et al 2009; Shang et al 2011; Priyanka and Priyanka 2012; Rana and Sreenivasulu 2013); however, agro-techniques are only available in few species such as in *A. carmichaelii* (Gao et al 2021).

The availability of huge diversity in diterpene alkaloids and variable bioactivity induces synergistic effect on many disease conditions and improve overall therapeutic effects. However, a quantitative assessment of diterpene alkaloids in many *Aconitum* species has not yet been carried out. Instead, chemical derivatization of existing molecules became the area of modern research; however, these more effective modern aconitine-derived semi-synthetic compounds required screening of full clinical parameters as it may induce other side effects. Low content of alkaloid causes problems of individual compound isolation and its pharmacological characterization. Thus, the increased production of important secondary metabolites through cell culture or *Agrobacterium rhizogenes*-mediated transformed roots can reduce production costs. Small-scale protocols for suspension culture or *Agrobacterium rhizogenes*-mediated transformed roots for metabolite production have been successfully developed in a few *Aconitum* species with promising results (Giri et al. 1997; Shiping et al. 1998; Hwang et al. 2004; Rawat et al. 2013b; Singh et al. 2020; Nguyen et al. 2021). However, commercial production of cell or hairy root cultures in bioreactors required precise process development methods. Cell cultures are susceptible to shear stress that causes disorganization of cells leading to callus formation and lower biomass productivity. Although, it has been found useful for alkaloid production from *Catharanthus trichophyllus*, *C. roseus,* and many other transformed plants (Davioud et al 1989; Toivonen et al. 1989; Eibl and Eibl 2002; Sevón and Oksman-Caldentey 2002). Currently, newly emerged mathematical modeling for large-scale bioreactors can be helpful for designing the scale-up conditions and projecting the impact of a large-scale environment on biomass and metabolites' production (Straathof et al 2019). Furthermore, pathway engineering, culturing of genetically transformed cells, and use of elicitors are other research areas that need to be explored in *Aconitum* species.

More than 70 traditional or modern processing methods are available for the detoxification of aconitum poison, and it is claimed that this processing increased the efficacy of extract of preparations after the structural modification in molecules. The stability and bioactivity of diterpene alkaloids under different temperature conditions and processing methods have not been carried out. Thus, more emphasis is needed on the analysis of derivatives of diterpene alkaloids generated through processing. Without proper analytical mapping and quantification, the safety and efficacy of aconitine-containing drugs for human use cannot be determined (Borcsa et al. 2011). A quantitative assessment of potent molecules after different processing techniques and assurance of quality will reduce the risk associated with the toxicity of the plant. Furthermore, optimization of suitable potent molecule recovery methods from different *Aconitum* species in extraction using Response Surface Methodology (RSM), Taguchi method, or other techniques might be useful for improving yield and economic value (Wu et al. 2018).

Thus, the review analysis revealed that the anti-inflammatory, analgesic activity, anticancer, and cytotoxicity activities of extract and some diterpene alkaloids are well explored. Further, research needs to be carried out on the pharmacological activity of many isolated compounds. Identification of suitable drug delivery systems for reducing the toxic effects on the body and bioprocessing methods for reducing the toxicity needs to be explored and developed. Production of important metabolite through calls, callus, or *Agrobacterium rhizogenes*-mediated genetic transformation or other advanced techniques, and their optimum recovery process needs to be developed for production of higher content of active metabolites. Among the available options, exploration of existing genetic resources, and information on genomic resources might be very important. Identification of candidate genes and transcription factors determining and regulating biosynthetic pathways related to quality might be important. Lack of genomic information is a hurdle in the current scenario, and modern genomics, transcriptomics or metabolomics techniques can help understand the inheritance of quality-related traits, trait-specific functional marker development for genetic characterization, and molecular breeding. Development of varieties for cultivation, agrotechnologies for maintaining the quality of products, robust propagation methods for propagation for farmers, and temporal monitoring of the population status in wild for ascertaining conservation have been identified for future research. Thus, this review would be helpful in new drug discoveries, disease management, conservation of genetic resources, and economic upliftment of farmers.

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