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UTILIZATION, BACKGROUND, AND CHEMICAL ANALYSIS OF ACONITUM USING LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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ABSTRACT
The family Ranunculaceae includes the genus Aconitum, which has over 300 species spread throughout Asia, Europe, and North America. Commonly referred to as wolfsbane or monkshood, Aconitum plants are both lethal poisons and powerful cures. Diterpenoid alkaloids such aconitine, mesaconitine, and hypaconitine are the primary source of their pharmacological activity, which has historically been used to treat heart conditions, fever, rheumatism, and neuralgia. Because of its extreme toxicity, quality control and chemical profiling require analytical methods like Liquid Chromatography Combined with Mass Spectrometry (LC–MS). With a focus on contemporary developments in quality assessment and standardization, this review offers a thorough summary of the toxicity, chemical makeup, ethnomedicinal use, and LC-MS-based analysis of Aconitum species.

1. INTRODUCTION

One of the most dangerous yet pharmacologically active groupings of medicinal plants is the genus Aconitum (Zhou et al., 2020). Traditional Chinese and Ayurvedic medicine have traditionally utilized species including Aconitum carmichaelii, A. kusnezoffii, A. napellus, and A. ferox to treat cardiac conditions, inflammation, and discomfort (Tang et al., 2018). However, because aconitine-type alkaloids alter voltage-gated sodium channels and can result in deadly arrhythmias, these advantages come with significant toxicological hazards (Chan, 2016).
The complex alkaloid combinations in Aconitum preparations may now be reliably identified, measured, and tracked using sophisticated chromatographic and spectrometric techniques, especially LC-MS (Liu et al., 2018).

2. BACKGROUND AND APPLICATION OF ETHNOBOTANY

2.1 Conventional Use

To lessen toxicity and improve its warming and analgesic qualities, Aconitum carmichaelii Debeaux (Fuzi) is boiled or steamed in Traditional Chinese Medicine (TCM). According to Tang et al. (2018), it is used to cure ailments like rheumatic pain, heart failure, and yang deficiency.
Aconitum ferox (Vatsanabha) is used in Ayurvedic medicine to treat fever, neuralgia, and paralysis. It is detoxified through Śodhana, which is purification with cow's urine, milk, or ghee (Singh & Sharma, 2016). For these reasons, Aconitum species are also used in traditional systems in Tibet and Japan (Okamoto et al., 2017).

2.2 Detoxification and Toxicity

Diester-diterpenoid alkaloids (DDAs) including aconitine, mesaconitine, and hypaconitine, which are transformed into monoester derivatives during processing, are the primary cause of Aconitum's toxicity. These are changed into less harmful substances such aconine and benzoylaconine during detoxification (Ameri, 1998; Zhao et al., 2021). Traditional procedures are a crucial safety measure since proper processing significantly lowers toxicity (Fujita et al., 2019).

3. CHEMICAL COMPOSITION

3.1 Classification of Alkaloids

Alkaloids from Aconitum can be divided into:
Aconitine, mesaconitine, and hypaconitine are diester-diterpenoid alkaloids (DDAs).
Benzoylaconine, benzoylmesaconine, and other monoester-diterpenoid alkaloids (MDAs)
Aconine and mesaconine are examples of amine-diterpenoid alkaloids (ADAs).
Both therapeutic and harmful effects are caused by these substances (Zhao et al., 2021; Singh et al., 2017).

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Additional Components
Flavonoids, polysaccharides, fatty acids, and saponins are examples of non-alkaloid substances that have anti-inflammatory and beneficial antioxidant qualities (Li et al., 2019; Lin et al., 2021).
4. Methods of Analysis for Alkaloids in Aconitum

4.1 Conventional Methods of Analysis
Because alkaloids have structural similarities, traditional techniques including TLC, UV-Vis spectroscopy, and HPLC were first employed but had low resolution and specificity (Peng et al., 2015**).**

4.2 Mass spectrometry-liquid chromatography (LC–MS)
For both qualitative and quantitative investigation of Aconitum alkaloids, LC-MS has become the gold standard. It enables the simultaneous detection of many alkaloids in complicated mixtures by fusing the molecular identification capability of MS with the separation power of LC (Liu et al., 2018**).**

5. THE LC-MS APPROACH
5.1 Preparing the Sample
Methanol, ethanol, or acetonitrile are used to extract the samples (root powders or herbal formulations), which are then centrifuged and filtered. Impurities are eliminated through cleanup utilizing liquid-liquid extraction (LLE) or solid-phase extraction (SPE) (Wang et al., 2016).

5.2 Conditions for Chromatography
C18 reverse-phase column
Acetonitrile–water (0.1% formic acid) is the mobile phase.
Alkaloids with different polarities can be better separated via gradient elution.
To identify DDAs and MDAs, retention durations and chromatographic resolution are maximized (Zhou et al., 2020).

5.3 Detection via Mass Spectrometry
Positive-ion mode electrospray ionization (ESI) is the favored method. Each alkaloid is distinguished by its distinctive molecular ions ($[M+H]^+$) and fragment ions:

Compound	$[M+H]^+$	Major Fragments	MS Mode
Aconitine	646	586,368,226	ESI^+
Mesaconitine	632	572,368	ESI^+
Hypaconitine	616	556,368	ESI^+

Multiple reaction monitoring (MRM) in tandem mass spectrometry (LC–MS/MS) offers quantitative sensitivity and precision down to the nanogram level (Fujita et al., 2019; Li et al., 2019).

6. LC-MS Applications in Aconitum Studies
Quality Control: Verifies safe alkaloid concentration and identifies adulteration (Zhou et al., 2020).
According to Singh and Sharma (2016), detoxification studies track the conversion of DDAs to MDAs.
Alkaloid absorption, distribution, and elimination in vivo are assessed by pharmacokinetics (Wang et al., 2016).
Aconitine toxicity in bodily fluids is detected by forensic toxicology (Chan, 2016).
Metabolomics: The identification of new bioactive compounds is facilitated by UHPLC-QTOF–MS and LC–Orbitrap–MS (Liu et al., 2018; Lin et al., 2021)**.**

7. SAFETY AND STANDARDIZATION
Aconitine, mesaconitine, and hypaconitine shall not exceed 0.020% in processed Aconitum products, according to the Chinese Pharmacopoeia (2020). Safe medication use is made possible by LC-MS, which guarantees adherence to these requirements (Chinese Pharmacopoeia Commission, 2020).

8. PROSPECTS FOR THE FUTURE
To correlate chemical fingerprints with medicinal efficacy, future studies will probably integrate chemometric modeling, bioassay-guided analysis, and LC-MS-based metabolomics. The safety assessment and authentication of Aconitum species may be further improved by integrating multi-omics methods and AI-based pattern recognition (Li et al., 2019; Lin et al., 2021).

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9. CONCLUSION

Understanding Aconitum's pharmacological potential and chemical complexity has changed as a result of research using LC-MS-based analysis. This ancient yet powerful medicinal genus will continue to be used safely thanks to the combination of cutting-edge analytical techniques and traditional wisdom.

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