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# Distribution of toxic alkaloids in tissues from three herbal medicine Aconitum species using laser micro-dissection, UHPLC–QTOF MS and LC–MS/MS techniques

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ABSTRACT

Aconite poisoning continues to be a major type of poisoning caused by herbal drugs in many countries. Nevertheless, despite its toxic characteristics, aconite is used because of its valuable therapeutic benefits.

The aim of the present study was to determine the distribution of toxic alkaloids in tissues of aconite roots through chemical profiling. Three species were studied, all being used in traditional Chinese Medicine (TCM) and traditional Indian medicine (Ayurveda), namely: *Aconitum carmichaelii*, *Aconitum kusnezoffii* and *Aconitum heterophyllum*.

Laser micro-dissection was used for isolation of target microscopic tissues, such as the metaderm, cortex, xylem, pith, and phloem, with ultra-high performance liquid chromatography equipped with quadrupole time-of-flight mass spectrometry (UHPLC–QTOF MS) employed for detection of metabolites. Using a multi-targeted approach through auto and targeted LC–MS/MS, 48 known compounds were identified and the presence of aconitine, mesaconitine and hypaconitine that are the biomarkers of this plant was confirmed in the tissues. These results suggest that the three selected toxic alkaloids were exclusively found in *A. carmichaelii* and *A. kusnezoffii*. The most toxic components were found in large *A. carmichaelii* roots with more lateral root projections, and specifically in the metaderm, cork and vascular bundle tissues. The results from metabolite profiling were correlated with morphological features to predict the tissue specific distribution of toxic components and toxicity differences among the selected species. By careful exclusion of tissues having toxic diester diterpenoid alkaloids, the beneficial effects of aconite can still be retained and the frequency of toxicity occurrences can be greatly reduced.

Knowledge of tissue-specific metabolite distribution can guide users and herbal drug manufacturers in prudent selection of relatively safer and therapeutically more effective parts of the root. The information provided from this study can contribute towards improved and effective management of therapeutically important, nonetheless, toxic drug such as Aconite.



## 1. Introduction

Traditional Chinese Medicine (TCM) and Ayurveda from India are the two most ancient traditional medical systems, which were developed over millennia and are currently being increasingly used worldwide. Three species of Aconitum, namely *Aconitum heterophyllum* Wall, *Aconitum carmichaelii* Debx., and *Aconitum kusnezoffii* Reichb. (Fam. Ranunculaceae), are commonly used as herbal medicines in India and China, in spite of their toxicity. The monograph of dried roots of *A. heterophyllum* is listed in the Ayurvedic Pharmacopoeia of India. The roots of *A. heterophyllum* are considered relatively less toxic and they are used for treatment of emesis, inflammation, fever, cough, diarrhea and helminthiasis. The roots are also included in formulations like Rodhrãsava (infusion), Mahãvisagarbha Taila (medicated oils), and Śivã Guikã (pills), as listed in the Ayurvedic formulary of India (Anonymous, 1977, 1987). The roots of *A. carmichaelii* and *A. kusnezoffii* are recorded in the Chinese Pharmacopoeia for the treatment of rheumatalgia, rheumatoid arthritis, cold and pain, as well as a part of formulations like Sini Tang, Guifu Dihuang Wan, and Fuzi Lizhong Wan (Chinese Pharmacopoeia Commission, 2010). However, these formulations can be prescribed only by registered medical practitioners, and the procedures for prescription are strictly regulated by the government (Chinese Pharmacopoeia Commission, 2010). Official formulations of Aconitum are listed in this manuscript to

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emphasize the importance of it in both Ayurveda and TCM, in spite of its toxic characteristics. *A. heterophyllum* is also listed among the critically endangered species of plants by the International Union for Conservation of Nature (IUCN) and is banned for export through the circular of Govt. of India Ministry of Commerce Public Notice No. 47 (PN)/92-97 dated 30th March 1994 (Shah, 2005). *A. carmichaelii* and *A. kusnezoffii* are extensively cultivated in the southwestern and northern parts of China.

'Aconite' is a common name used for different plant species belonging to genus *Aconitum* L. Of all the toxic herbal drugs, aconite is one of the most important because poisonings due to its inappropriate use have occurred in Asia and some Western countries and can be fatal (But et al., 1994; Chan, 2011, 2012; Fatovich, 1992; Poon et al., 2006; Tomlinson et al., 1993). Aconitine (**1**), which is also referred to as the Queen of Poisons, is a constituent of this herb, and is primarily responsible for its fatal effects (Chen et al., 1956; Li et al., 1997; Singh et al., 2012). However, apart from the inherent toxicity of the drug, other factors plausibly contribute to the occurrence of poisoning due to plants belonging to the genus Aconitum: these include improper dose selection, inappropriate method of processing, consumption of the unprocessed raw drug, and lack of awareness regarding the toxicity of the drug among patients (Chan, 2011). If these ancillary contributing factors are controlled, this poisonous drug can be safely used (Bisset, 1981; Judith et al., 2009; Mishra, 2000; Pelletier, 1984; Sarkar et al., 2012a).

The toxic alkaloidal components of plants belonging to the genus Aconitum, can be classified into three classes (Wang, 2008), according to the different substitution types at the $C_8$ and $C_{14}$ positions. The first class comprises Diester Diterpene Alkaloids (DDA's) that have "high acute toxic" effects and include aconitine (**1**), mesaconitine (**2**) and hypaconitine (**3**). The second class of compounds consist of Monoester Diterpene Alkaloids (MDA's) that have a "low acute toxic" nature due to lack of an acetyl group at the $C_8$ position, and include compounds like delphatine (**4**), atisine (**5**), benzoylaconine (**6**) and deltaline (**7**). The third class of compounds consists of De-esterified Diterpenoid Alkaloid (DEDA) compounds that lack both acetyl and benzoyl groups at the $C_8$ and $C_{14}$ positions. These compounds lack acute toxic effects and are "non-toxic", and include karakoline (**8**) and lycoctonine (**9**) (Gutser et al., 1998; Li et al., 1997; Shim et al., 2006; Zhang et al., 2012). Structures of some DDA compounds and other alkaloids found in Aconitum species are shown in Fig. 1.

Apart from differences in their therapeutic effects and uses, the roots of selected aconitum species also differ in their morphologies. In ancient China, there have been two different viewpoints about the quality evaluation of the roots of *A. carmichaelii* and *A. kusnezoffii* based upon morphological characteristics. According to the *Compendium of Materia Medica* (Ben Cao Gang Mu, published in 1593), roots with a broad base and fewer lateral root projections are of good quality (Li, 1985). In contrast, the *Origins of the Materia Medica* (Ben Cao Yuan Shi, published in 1612) records that roots

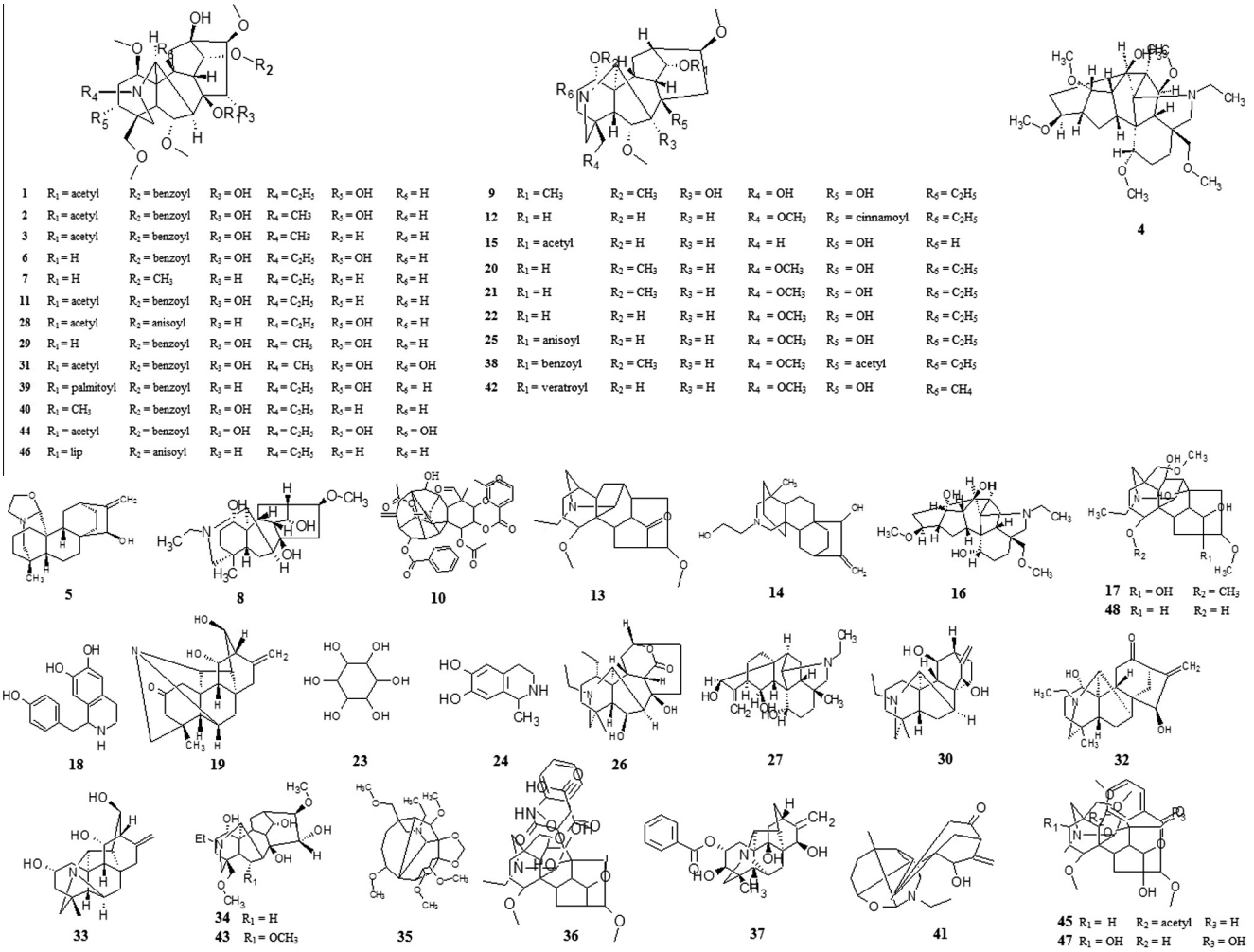


**Fig. 1.** Structures of some alkaloid compounds detected in Aconitum species selected for this study.

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