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Phytochemicals, Traditional Uses and Processing of *Aconitum* Species in Nepal

Sajan L Shyaula
Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur
e-mail: shyaulasajan@gmail.com

Abstract

The tuberous roots of genus *Aconitum* are commonly applied for various diseases, such as rheumatic fever, painful joints and some endocrinal disorders. It stimulates the tip of sensory nerve fibers. These tubers of *Aconitum* are used in the herbal medicines only after processing. At the mean time, there remain high toxicological risks of the improper usages of *Aconitum*. The cardio- and neurotoxicity of this herb are potentially lethal. Some of the species like *A. ferox* and *A. spicatum* are deadly poisonous while others like *A. oreochryseum* and *A. bisma* are used as the antidote for *Aconitum* poisoning. It is therefore, a valuable drug as well as an unpredictable toxic material. In the current review, assessments of *Aconitum* species are carried out, to increase knowledge for the safety uses, in context of Nepal. The traditional uses, phytochemical studies, its processing techniques and toxicological principles are reviewed.

Key words: *Aconitum*, antidote, aconite poisoning and phytochemicals

Introduction

The genus *Aconitum*, belonging to the family Rannunculaceae, is widely distributed in the alpine and subalpine regions. The plants are usually perennial or biennial herbs, often with stout leafy stems, bulbs or creeping rhizomes. Leaves are mostly cauline, lobed, rarely divided and dentate. Flowers are simple or branched recemes. It comprises of over 300 species, including some ornamental and medicinal plants (Utelli et al. 2000). In annonated check list of flowering plants of Nepal, 38 *Aconitum* species are reported. However 16 *Aconitum* species are listed in Medicinal and Aromatic Plants Database of Nepal (MAPDON). It is distributed from the west to east Nepal and from the temperate to alpine zones (1,800–4,200 m elevation). It is commonly known as bikh in Nepal and Monk's hood or aconite in English. Taxonomical studies on Nepalese species of genus *Aconitum* was the first time reported in 1982 (Shrestha et al. 1982). In Greek, aconite means arrow. *A. ferox* and *A. spicatum* are very poisonous. From the same genus, *A. oreochryseum* and *A. bisma* are used as the antidote for *Aconitum* poisoning. *A. spicatum* is one of the most poisonous plant among 15 plant species reported from Annapurna and Langtang Himalaya area (Bhandary and Shrestha 1982 and 1986). These are the herbaceous perennial plants growing in moisture retentive but well draining soils of mountain meadows. Aconite is well known to the ancients as a powerful poison, but was the first employed as a medicine by Baron Storck, of Vienna, whose experiments were published in the year 1762 (Harvey et al. 1898).

Traditional Uses

The tubers of *Aconitum* are used as antipyretic and analgesic in the far western Nepal. The tubers are also used for tonsillitis, sore throat, gastritis and debility. In comparing the ethnomedical use with modern pharmacological study, it shows consistency with latest pharmacological finding. Caffeic acid of *A. koreanum* is antioxidative and anti-inflammatory (Kunwar et al. 2010). *A. spicatum* is used for fever and head ache, cuts and wounds and musculo-skeletal problems in Rasuwa district. *A. ferox* is used for the joint pains. The anti-inflamatory properties are due to the alkaloidal extract (Uprety et al. 2010). In Manang district, *A. naviculare* is used for high blood pressure, cold, fever and jaundice. The half spoonful of powder *Aconitum* is mixed with 2 spoonfuls of chauri ghee and taken two times a day for fever and jaundice until recovery. This local tradition converts better *A. naviculare* to the sweeten form. In Manang, about one gram of dried plant material is boiled with two glasses of water for about half an hour and the black, bitter decoction is drunk twice a day. According to the respondents, the bitter decoction makes the patient weak. The decoction is often taken along with ghee to restore energy (Shrestha et al. 2007 and Bhattarai et al. 2006). The root powder of *A. bisma* is mixed with water and the diction is taken as an antidote in food poisoning and snake bite in Makalu-Barun and Kangchenjunga. The root powder is also used to treat, fever, headache and stomach ache. Red variety is considered to be the best among available white yellow, black and red (Chaudhary et al. 2002 and Sherpa 2001). In Dolpa, it has also been used for cough, cold and intestinal problem (Lama et al. 2001). The plant is used in disorders of the gall bladder in Mustang (Pandey et al. 2006). As *Aconitum* is

highly poisonous, it is mixed with other medicinal plants by experienced 'amchis' to inactivate toxicity and having same potency. A root paste is applied for allergy, boils, cuts, wounds and edema after mixing with other plants in Mustang (Bhattarai et al. 2010). But scientific informations are lacking about the herb-herb interactions. The ethnomedical use of *Aconitum* in Humla and Helambu is mentioned by Rokaya and Bhattarai. (Rokaya et al. 2010 and Bhattarai et al. 1989). An extract of this plant is used as an antipyretic in Ayurvedic medicine after detoxification (Mahajani et al. 1990).

Aconitum species are usually used by mixing with other plants rather than a single component. *A. ferox*, *A. palmatum* and *A. heterophyllum* are commonly used species in Ayurvedic formulations. According to Ayurvedic pharmacopeia of India, *ativisa* consists of dried roots of *A. heterophyllum* Wall ex. Royle. The important formulations of the *Aconitum* are Mahavisagarbha taila, Rodhrasava Siva Guika, Lakasminarayana rasa, Rasnairandadi Kvatha Curna, Sudarsan Curna, Pancatikta guggulu ghrta, Bala chaurbhadrika curna for therapeutic use of krmiroga, jvara, kasa, chardi, amatisara (Drabya Gun Bigyan 1975 and Bhaisajya Ratnawali 2006). *Vatsanabha* consists of dried roots of *A. chasmanthum* Staf ex Holmes. The important formulations are Tribhuvanakirti rasa, Anandabhairava rasa, Sutasekhara rasa, Vatavidhwansana rasa, Mahavisgarbha taila for therapeutic use of vataroga, sannipata, vatakaphajvara, jvarastisra, kanharoga. Externally, it is applied to reduce pain and inflammation. It is applied by rubbing with oil and it stimulates tip of sensory nerve fibers. In therapeutic dose, it acts as an appetizer.

Phytochemicals from *Aconitum* species found in Nepal

About 54 species of *Aconitum* have been chemically investigated (Rana 2006). *Aconitum* species are the rich sources of diterpene alkaloids and flavonoids. Chinese group have reviewed the structure of diterpenoid alkaloids from *Aconitum* species (Pan and Chen 1993). It also consists of free fatty acids, polysaccharides and different classes of alkaloids. A Hand book of natural products data, diterpenoid and steroidal alkaloids, covers spectral data of 400 diterpenoid alkaloids reported before the end of 1988 (Atta-ur-Rahman 1993). Hanuman and Katz has published a review compiling the ^{1}H NMR spectra of 52 norditerpenoid alkaloids. The structural relationships of diterpenoid alkaloids and their chemical reactions, synthesis and

biological activities from 1998 to 2008 is reviewed by Wang (Wang et al. 2010). Diterpenoid alkaloids are widely distributed throughout the plant kingdom and have been of great interest since the early 1800 because of their pharmacological properties and interesting chemistry. Biogenetically, these diterpenoid alkaloids are derived in nature from the tetracyclic or pentacyclic diterpenes in which carbon atoms 19 and 20 are linked withnitrogen atom of a molecule of β-aminoethanol, ethylamine or methyl amine to form a heterocyclic ring.

The phytochemical investigation of *Aconitum* species from Nepal, was the first time carried out in 1972 (Faugeras et al. 1973). The acute toxicity of alcoholic extracts of six *Aconitum* species tubers appeared to be directly related to the alkaloid content of the tubers. The most toxic test in mice was the extract from *A. spicatum*. The alkaloid content of *A. spicatum* was 1.21% (Faugerus et al. 1973). Chemical analysis by thin layer chromatography of Nepalese species of *Aconitum* had been carried to study the alkaloidal content (Shrestha 1982). The isolation and characterization of two new diterpenoid alkaloids (Figure 1.), navirine B and navirine C, and five known compounds, (+) chellespontine, kaempferol-7-*O*-β-*D*-glucopyranosyl(1'!3)-α-*L*-rhamnopyranoside, kaempferol-7-*O*-α-*L*-rhamnopyranoside, 3-*O*-β-*D*-glucopyranoside, *p*-coumaric-4-*O*-β-*D*-glucopyranoside acid and ferulic-4-*O*-β-*D*-glucopyranoside acid had been reported from *A. naviculare*. It was used as a folk medicine in Manang against cold, fever and headache, as well as for the sedative and analgesic remedies. The antiproliferative activity of the isolated alkaloids, were evaluated against human tumor cell lines, ovarian and colon adenocarcinoma (Dall'Acqua et al. 2008 and Gao et al. 2004).

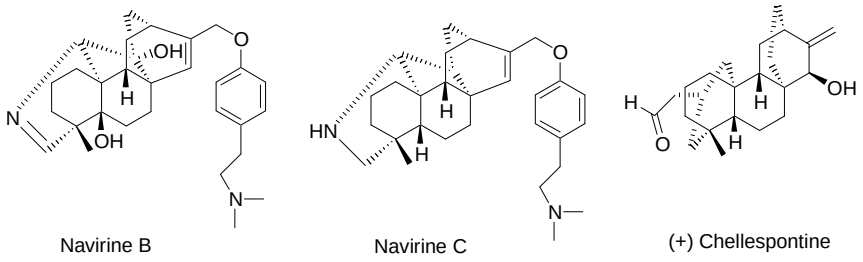


Fig. 1. Representative structures of alkaloids.

Three new flavonoid glycosides were isolated and characterized from aerial parts of *A. navicularie* (Figure 2.). Flavonoid glycosides were identified from the aqueous extract obtained by partition with organic solvents of crude methanolic extract. 3-*O*-[β-*D*-glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranosyl]-7-*O*-[β-*D*-glucopyranosyl-(1→3)-α-*L*-rhamnopyranosyl]kaempferol, 3-*O*-[β-*D*-glucopyranosyl-(1→3)-(4-*O*-*trans*-*p*-coumaroyl)-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranosyl]-7-*O*-[β-*D*-glucopyranosyl-(1→3)-α-*L*-rhamnopyranosyl]quercetin and 7-*O*-[β-*D*-glucopyranosyl-(1→3)-α-*L*-rhamnopyranosyl]quercetin (Shrestha et al. 2006).

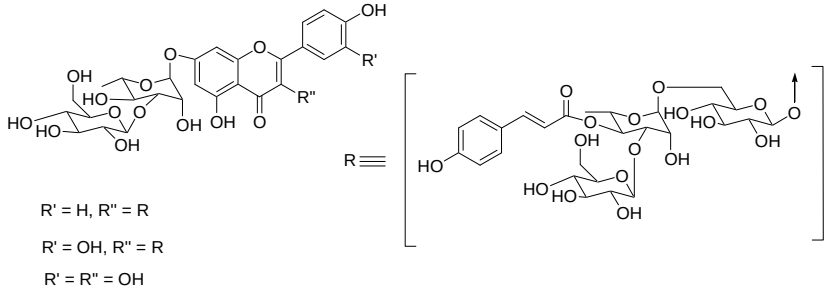


Fig. 2. Structure of Flavonoid glycosides.

he list of chemical compounds isolated from various species of *Aconitum* found in Nepal but collected from different areas are shown in table **1** with their references.

Plant name	Compounds isolated	References
A. balfourii	9-Hydroxysenbusine A	Khewal et al. 2006
	Balfourine, 8-*O*-Methylveratroylpseudaconine	Khewal et al. 1992
A. bisma	Palmatisine, Vakatidine, Vakatisinine, Vakognavine	Singh et al. 1965
	6-Acetylheteratisine, 15-Deacetylvakgnavine, Palmasine, Palmadine	Jiang et al. 1988
	6,8-Diacetylheteratisine	Cheri et al. 1997
	Vakatisine	Singh et al. 1972
	Vakhmadine, Vakhmatine	Jiang et al. 1991
A. heterophylloides	Heterophylloidine	Pelletier et al. 1981
A. ferox	14-*O*-acetylsenbusin A, Bikhaconite, Pseudaconine	Hanuman et al. 1994
	14-*O*-Benzoyl-Lipopseudaconitine, Lipobikhaconitine, Lipopseudaconitine	Hanuman et al. 1994
	Chasmaconitine	Achmatowiez et al. 1964
	Diacetylpseudaconitine, Veratroylpseudaconine	Purushothman et al. 1974
	3,4-Dihydro-6-hydroxy-2(1H)-quinoline	Hanuman et al. 1993
	Pseudaconitine	Klsek et al. 1972
	Veratroylbikhaconine	Hanuman et al. 1993
A. heterophyllum	Anthorine	Lawson et al. 1937
	Atidine	Pelletier et al. 1968
	Atisenol	Pelletier et al. 1982
	6-Benzoylheteratisine	Aneja et al. 1973
	11,13:11,16-Diepoxy-16,17-dihydro-11,12-secohetisan-2-ol	Gonzlez-Coloma et al. 2004
	Dihydroatisine	Pelletier et al. 1978
	Heterophyllidine, Heterophylline, Hetidine, Hetisinone, *O*-Methyl-heterophylline	Pelletier et al. 1968
	Isoatisine	Pelletier et al. 1965
	Hetisine	Jacobs et al. 1942
A. heterophyllum	*N*-Deethyl-*N*-formyllyaconitine	Ulubelen et al. 2002
	O-Methylaconitine	Ross et al. 1992
	Methyl-*N*-succinoylanthranilate	Saheen et al. 2005
A. naviculare	Navirine	Gao et al. 2004
A. orochryseum	Atisiniumchloride	Wangchuk et al. 2007
A.gammiei	16-Acetoxycardiopetaline	Liu et al. 1994
	15-Acetyl-13-dehydrocardiopetamine	Feunte et al. 1989
	15-Acetylcardiopetamine	Gonzlez et al. 1983
	Brachyaconitine	Liu et al. 1996
	Cardiopetamine	Feunte et al. 1989
	N-Deethylaconitine	Arlandini et al. 1987
	1,14-Diacetylneoline, 12-Epiacetyldehydronapelline	Feunte et al. 1988
	Ephedrine	Kopp et al. 1973
	Hokbusine A	Hikino et al. 1983
	Senbusine A	Hikino et al. 1984
	Lpaconitine	Franca et al. 1987
	Merckonine	Desui et al. 1998
	Myriophyllosides F	Lu et al. 2004
	Napelline	Wiesner et al. 1958
	Neoline	Chu et al. 1964
	Norepinephrine	Yoon et al. 1976
	Taurenine	Telnov et al. 1992
A. soongaricum	Acetylsongorine	Zhamerashvili et al. 1981
	Songoramine	Yusunov et al. 1970
	Songorine	Sultankhozhaev et al. 1982
	Songorinine	Samatov et al. 1965
	12-Acetyl-12-epinapelline	Salimov et al. 2004
	Aconine	Pelletier et al. 1979
A. spicatum	Bikhaconitine	Dunstan et al. 1905
	Spicatine A, Spicatine B	Gao et al. 2005
A. violaceum	Indaconitine	Miana et al. 1971

Aconitum poisoning

Aconite tubers were among the most toxic plants known but it had been used in the eastern and western therapeutics for centuries. Aconite was a fast-acting toxin. The root was used to poison the hunting spikes (Manandhar 2002). The root was dried in the mild sun about 4-hours and pounded into paste. The pointed iron of arrow was smeared with the paste, dried then used for hunting. The animal hunted in this manner were not fit for eating but instead were used for their skin, hair, bones and other parts. The poisonous properties of aconites had been mentioned in Rg Veda about BC 1200 indicating that it was used on the weapon (Bisset and Mazars 1984). According to Guoyu, during the Chunqui period of the Zhou dynasty, BC 722-480, a form of aconite, was used to poison the successor to the throne of kingdom of China (Bisset 1979).

The aconite poisoning usually occurred due to ingestion of the wild *Aconitum* plant because of misidentification, contamination, adulteration and miss-processing. The onset of symptoms occurred rapidly within 10 to 20 minutes. A tingling or burning sensation in the fingers and toes were usually seen first, followed by sweats and chills, a generalized paresthesia, a feeling of roughness and dryness in the mouth, numbness and a feeling of intense cold. Later there were violent vomiting, colicky diarrhea, skeletal muscle paralysis, cardiac rhythm disturbances and intense pain. The main causes of death in aconite poisoning were cardiovascular collapse and ventricular arrhythmias. The eight stages (Asta-vegas) of aconite poisoning had been mentioned in Rasa Vagbhata, (Drabya Gun Bigyan 1975 and Bhaisajya Ratnawali 2006). First skin changes followed by tremers, burining allover the body, *vikratavastha*, bubbles from mouth, drooping of shoulders, comatose and death.

Four cases of aconite poisoning had been managed in Manipal teaching hospital (Paudel et al. 2008). Similarly, three cases of aconite poisoning in Nepalese family were managed in Hong Kong after analysis of residual herbs and urine samples (Chan et al. 2010). In the urine analysis, the alkaloids commonly detected in local aconite poisoning cases included bikhaaconitine, pseudoaconitine, indaconitine and yunaconitine. The tribesmen from Nepal usually spent their winter in cities and sell their herbs for earning. In above case, the herbs were brought from Nepal six month before the incident. It occured mainly due to mistakely taken of *Aconitum* instead of another plant 'Nirmasi' (*Delphinium denudatum*) which resembled to it.

Management of aconite poisoning needs immediate attention to the vital functions and close monitoring of blood pressure and cardiac rhythm. Clinical judgments are far more important than the laboratory findings as there is a time-lag before laboratory confirmation. Activated charcoal is advisable if the patient presents within one hour. Patient must be managed in the ICU or cardiac care units. Inotropic therapy is required if hypotension persists. Atropine shall be used to treat bradycardia. Amiodarone and Flecainide are reasonable for the first line treatment. Magnesium is sometimes effective for polymorphic ventricular tachycardia. Cardiopulmonary bypass is recommended if ventricular arrhythmias (Poon et al. 2006). In Ayurveda, the aconite toxicity is managed by giving Tankan (Borax) and ghee or mixture of turmeric juice, borax and ghee to the patient. Excessive cow milk is also drunk to patient for vomiting purpose. A strong coffee or a strong tea or tannic acid can be given to precipitate the alkaloid. A. *oreochryseum* is also used as the antidode of *Aconitum* poisoning (Ghimire et al. 2008). Neermashi or its mixture with root of *Asparagus* are recommended as a preventive measure of aconite poisoning in Annapurna region (Bhandary and Shrestha 1982 and 1986).

What Constituents of Aconitum makes it poisoning as well as medicine

The interest on *Aconitum* mainly based on diterpene alkaloids. Its flavonoid constituents are studied as the chemotaxonomic markers (Lim et al. 1999). The diterpenoid alkaloids can be divided into the following two broad categories on the basis of various substituents, which apparently affect both the chemical and pharmacological properties of these alkaloids. Alkaloids that possess a hexacyclic C19-skeleton and alkaloids bearing a C20-skeleton. C19-diterpenoid alkaloid groups comprise the toxic ester bases and can be further divided into four groups on the basis of the skeleton variations as shown in figure 3.

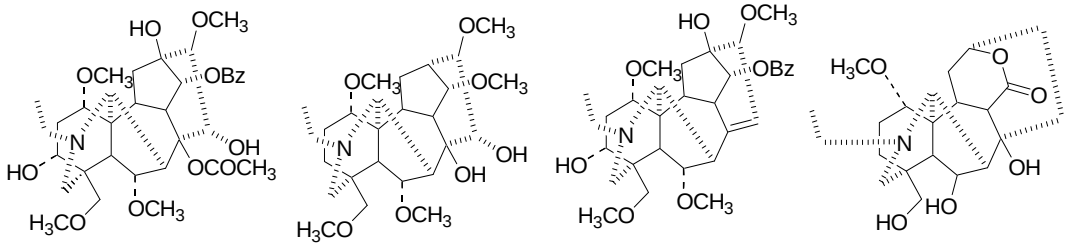


Fig. 3. Class of C-19 diterpenoid alkaloids.

C20-Diterpenoid alkaloids occur as esters but are relatively nontoxic. These are not extensively oxygenated and usually have one methoxy group. These alkaloids have been further classified into three basic types as shown in figure 4.

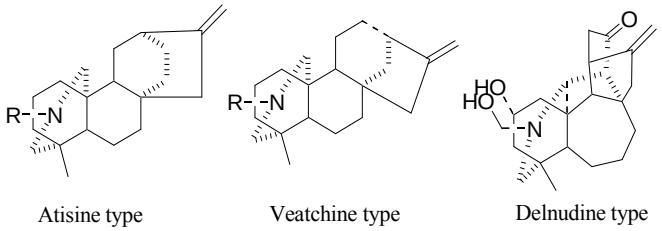
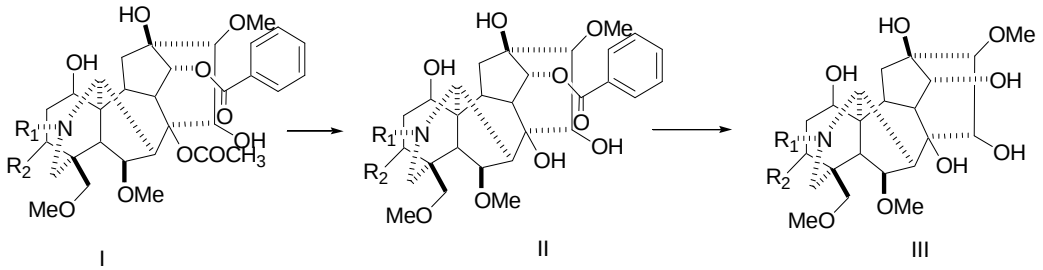


Fig. 4. Class of C-20 diterpenoid alkaloids.

The toxicity of *Aconitum*, mainly derives from the diester diterpene alkaloids including aconitine, mesaconitine and hypaconitine. They can be decomposed into less or non toxic derivatives through different processing method (Figure 5.).



I	II	III	R1	R2
Mesaconitine	Benzoylmesaconine	Mesaconine	CH3	OH
Aconitine	Benzoylaconine	Aconine	CH3CH2	OH
Hypaconitine	Benzoylhypaconine	Hypaconine	CH3	H

Fig. 5. Detoxification of Poisonous alkaloids of *aconitum* sps.

Besides diterpenoid alkaloids, other various alkaloids like higenamine, coryneine, lipohypaconite, lipodeoxyaconitine,lipoaconitine and benzyl mesaconine are also reported from *Aconitum* (Kimura et al. 1988 and Atta-ur-Rahman 1995).

Aconitum processing

The tubers of *Aconitum* have been used as a herbal drug only after processing. There is needed the standardized method for assessing the levels of toxic alkaloids in aconite roots in order to ensure the safe use of these plant materials as medicinal herbs. Processed tubers show lower level of toxic alkaloidal content as compared to unprocessed tubers (Csupor et al. 2009 and Kitagawa et al. 1984). A crude aconite is processed by *Samskaras* in Ayurvedic herbal medication system before being therapeutically used (Thorat and Dahanukar 1991). The root of the plant is boiled with two parts of cow's urine for 7 hours per day for two consecutive days. Then it is boiled with two part's of cow milk for same duration. The root processed in such a fashion is then wash with the lukewarm water, cut into pieces, dried and ground. The study shows that aconite becomes safe after *Samskaras*. It is seen that crude aconite is significantly toxic to mice (100% mortality at a dose of 2.6 mg/ mouse) whereas the fully processed aconite is absolutely non-toxic (no mortality at a dose even 8 times as high as that of a crude aconite). Further, all the steps in the processing are essential for complete detoxification (Sharma 1985).

Mahamrutyunjaya rasa is a kind of herbo-mineral formulation, often uses to treat cardiac disorders. The medicine comprises four kinds of herbs, *A. ferox*, *Solanum indicum, Piper nigrum* and *P. longum*. They are mixed with 1 part each of purified sulphur and sodium metaborate. To this mixture, 2 parts of purified cinnabar (HgS) are added and mixed uniformly. *Shodhana*, a traditional Ayurvedic treatment is used to decrease the toxicity and increase the bioactivity of the active ingredients in the preparation of *Mahamrutyunjaya* rasa. Aconite tubers are cut into small pieces and bundled in a thin cloth and kept in a clay pot. The Go-mutra (cow urine) is added into the pot and kept in hot sunny days for 3 days, replacing the go-mutra everyday. The tuber bark is peeled out and washed and dried to make *vatsanabha* pure and harmless (Pallavi et al. 2010). On comparative toxical evaluation of poly herbal Ayurvedic cardiotonic, the formulation with higher concentration of aconitine shows cytotoxic effects and further not useful for cardiotonic effect (Panda and Debnath 2010).

The herbal decoctions of aconite are generally prepared by soaking the roots in water or saturated lime water and then boiling. This causes hydrolysis of aconite alkaloids to less toxic benzyl aconine and aconine derivatives (Judith et al. 2009). In traditional chinese medicine, *Fuzi* is a collective term for various preparations derived from root of *A. carmichaeli*. The untreated form is called *Shengfuzi*, where as the boiled form is termed as *Nifuzi*. According to the ways of further processing of *Nifuzhi*, it is converted into *Vanfuzi*, *Heishunpian* and *Baifupian*. The following three forms are recorded as the most commonly used *Aconitum* preparations in chinese pharmacopeia, 2005. *Nifuzi* is soaked in a solution of mineral salt. *Heishunpian* is black slices, which are processed by cutting into longitudinal slices and soaked in salt solution and then pieces are backed to half dryness and then completely dried in sun. *Baifupian* is white slices which are processed by boiling in mineral salt solution and the bark is removed and cut into slices and dried in sun. Further if it is roasted along with ginger, it is *Paofupian* and if decocted with *radix glycyrrhizae*, it is called *Danfupian*. In modern methods, the pressure steaming technique is carried out to reduce toxicity of drugs. Optimal processing results are achieved by having the material macerated with water, cut into pieces and then steamed under high pressure (Liu et al. 1994). In Dolpa, the poison is detoxified by boiling the tubers in extract of aru, *Termenalia chebula*. From our current field research in Manaslu Conservation Area, 2010, 'amchis' processed the tubers by frying *A. spicatum* with sand. In some Ayurvedic formulations, the aconite tubers are kept in buffalo excreta and boiled for 3 hours. Aconite root may also be boiled with triphala (in decoction prepared by three myrobalans).

Aconitum is the valuable drug as well as the toxic material. The toxicity of *Aconitum*, mainly derives from the diester diterpene alkaloids. It can be used safely after processing. The aconite poisoning has also been reported in Nepal. Assessment of *Aconitum* species needs to be carried out for safety use in context of Nepal.

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