Biotechnology, 2010
ISSN 1682-296X

Advancement in Research on *Aconitum* sp. (Ranunculaceae) under Different Area: A Review

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Abstract: The present review is the pragmatic approach to ensue the findings on almost every aspect of very important herbal genus *Aconitum,* as a number of species belonging to this genus are renowned for their medicinal benefits in Ayurvedic, Unani, Chinese and Tibetan system of medicine. A lot of important attribute have been assigned to the species of this genus. Numerous works has been done on its various aspects from cultivation, conservation, phytochemical and pharmacological analysis to molecular characterization. The important species of this genus which have been explored so far include *A. heterophyllum, A. carmichaelii, A. balfourii, A. napellus, A. anthora, A. laeve* and *A. kusnezoffii.*

Key words: *Aconitum*, conservation, chemical constituents, pharmacological, diversity

INTRODUCTION

Aconitum is the botanical name of the genus commonly known as aconite, monkshood etc. The genus *Aconitum* belonging to the family Ranunculaceae is widely distributed in the alpine and sub-alpine regions of tropical parts of Northern hemisphere. There are over 250 species that have been reported in this genus (Lane, 2004). These are herbaceous perennial plants growing in moisture retentive but well draining soils of mountain meadows. They are mainly cultivated for their tubers. Aconite produced from the roots of number of different species of *Aconitum* is used in curing wide range if diseases. Different species of *Aconitum* with their medicinal properties and distribution pattern in Himalayas (Shah, 2005) have been listed in Table 1. The genus *Aconitum* finds the key position in the field of research. Many species of this genus has been listed in Red Data Book, due to which many conservation programmes came into existence, these includes *in situ, ex situ/in vitro* mode of conservation. Phytochemical analysis as well as Molecular facet of medicinal plant species of this genus have been and are being explored in many research institutes globally.

The present review discusses the progression in studies on species of *Aconitum* under different research area and importance of these research works has been focused. This sum up of the research efforts contains broader areas of research on *Aconitum spp.* which have been listed as follows:

- Conservation: (a) Seed germination/ Cultivation; (b) Micro-propagation
- Chemical Constituents: (a) Alkaloids, (b) Flavonoids
- Biological and pharmacological properties
- Diversity profile as quality control tool (a) Biochemical markers (b) DNA based markers

CONSERVATION

Inspite of various policy measures, excessive illegal collection of medicinal plant continues to take place on a large scale for gaining more and more financial gains. This includes the collection of species considered endangered also and whose collection is prohibited by law. Red Data Book has a long list of many endangered medicinal plants in which genus *Aconitum*, known as monkshood, wolfsbane, Devil's helmet or blue rocket, belonging to the family Ranunculaceae finds a key position. The degree of threat to natural population of these medicinal plants has increased due to many reasons, viz., overgrazing, prolonged seed dormancy, high seedling mortality and ecological constraints, but the main and important reason is unsustainable exploitation of the medicinal plants for

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the drug industry and local medicinal use (Srivastava *et al*., 2010a). Also, raw material supply can not cope up with the demand of the different herbal drug industries. Therefore, initiatives for their conservation and mass multiplication through various modes have been taken and more advanced technologies are being developed. These modes include seed based regeneration, clonal propagation and micro-propagation.

Seed germination/cultivation: Extraction of many plant species of genus *Aconitum* (Ranunculaceae) from their wild habitat have been occurring on large scale due to their immense medicinal properties and many other uses. This has moved many species towards rarity and now identified at different levels of threat ranging from vulnerable to critically endangered. *In situ* and *ex situ* conservation of the concerned species is thought to be the most easy and cost-effective mode of conservation. These modes need to be encouraged and practiced widely to reduce the pressure on the wild habitats of these species, eventually leading to their conservation.

Seed based multiplication is the most effective, realistic and convenient means for most of the species (Sharma *et al*., 2006). But cultivation through seed in most of the species of *Aconitum* e.g., *Aconitum heterophyllum* is difficult (http://mpcn.frlht.org.in/newsletter.htm) due to poor seed availability and lack of superior germplasm (Nautiyal *et al*., 2009). Also, the seeds of many *Aconitum* sp. are dormant. Limited data and literature is available on the study of seed dormancy of the important representatives of this genus. Germination is said to be complete when the structure called radicle penetrates the area surrounding the embryo, rest of the events including mobilization of major storage reserves are associated with the growth of seedling (Bewley, 1997). But some seeds have underdeveloped embryo, which has to grow to a critical length before germination can occur. Failing to grow may lead to dormant seed. Dormancy can be defined either as- - the absence of an intact, viable seed under favouring conditions within a specific time lapse (Hillhorst, 1995) or an innate seed property that defines the environmental condition in which the seed is able to germinate (Finch-Savage and Leubner-Metzger, 2006). There are different classes of seed dormancy: Morphological Dormancy (MD), Physiological Dormancy (PD), Physical Dormancy (PY), Morphophysiological Dormancy (MPD) and Combinational Dormancy (PD+PY) these classes are further subdivided into different levels and types depending upon the behaviour they show towards the treatment given to break the dormancy (Baskin and Baskin, 2004; Nikolaeva, 1977). These are controlled both genetically as well as environmentally. Additionally, genetic control may be associated with embryo genotypes as the mother plant. Environmental factors affecting the dormancy include temperature, water availability, light quality, photoperiod, altitude and mineral nutrition.

Cold stratification is often the most effective treatment for breaking seed dormancy in Ranunculaceae growing in temperate and alpine climates (Baskin and Baskin, 1994; Forbis *et al*., 2002; Frost, 1974; Walck *et al*., 1999). Chemical stimulants such as Giberrallic Acid (GA), nitrate and thiourea have been found effective in overcoming seed dormancy in Ranunculacea seeds (Bungard *et al*., 1997; Hepher and Roberts, 1985; Probert *et al*., 1987). Various studies have been conceded for the improvement of seed germination and breaking seed dormancy of *Aconitum* sp., some of which have been presented in the current review.

The ability of seed to be in dormancy prevents premature seedling emergence on one hand and on the other it promotes formation of a seed reserve in the soil, which provides conservation of the plant's genetic variability (Baskin and Baskin, 2004; Nikolaeva, 1977). However, not in every circumstances dormancy can be beneficial since dormancy in seeds of economical importance e.g., medicinal plants causes huge problem. A thorough research therefore is necessary for improvement in the seed germination and dormancy breaking systems.

Numerous work are devoted in the improvement of the seed germination and dormancy breaking systems for *Aconitum* sp. Studies of Dalteskaya (1985) have shown that both presence and kind of GA are important in breaking seed dormancy state of the seeds. Different species of the same genus may show dissimilar behaviour towards the treatment. Investigation by Pandey *et al*. (2000) on *A. balfourii* Stapf. and *A. heterophyllum* Wall showed that PGRs like GA_3 (250 µM) significantly enhanced the seed germination in *A. balfourii* (42.5%) in 15 weeks when compared to control (27.5%), while higher concentration of BAP (250 µM) augmented the germination up to 42.5% in *A. heterophyllum* only as compared to control (25%). Nitrogen containing compounds including nitrate, nitrite and cyanide too play vital role in breaking seed dormancy. Thiourea and Potassium nitrate in the same study were used to evaluate their effect on seed germination which showed the promotive effect on *A. balfourii* Stapf. but failed to enhance germination in *A. heterophyllum* Wall.

There have been many studies which shore up with each other but some findings were also there which contradict them. Pandey *et al*. (2005) concluded that *A. heterophyllum* Wall showed significant enhanced germination when the seeds were given hot water

treatment at 40-50°C for 90 sec in the condition other than its natural habitat, while in contrast Beigh *et al.* (2005) examined the same species for its *ex situ* conservation and conclude that plants of *A. heterophyllum* Wall could be cultivated at lower altitude, but the growth was restricted. Chilling but no other treatment could improve seed germination at lower altitude. Similar results were also reported by Dosmann (2002) on *A. sinomontanum*. He observed that chilling improved the seed germination and was found to be an important and pre-requisite condition for its germination.

Temperature plays imperative role in seed germination of *Aconitum* sp. Another research work was performed to resolve the temperature requirement for embryo growth, dormancy break and seed germination of *A. lycoctonum* (Vandelook *et al*., 2009). They showed that embryo growth and germination in this specie occur only at low temperature (<10°C). High temperature pre-treatment was not required for germination and GA_3 does not overcome the chilling requirement. On the basis of these results, *A. lycoctonum* was assigned to have deep complex morpho-physiological dormancy.

These studies show that low temperature is the main aspect for the germination in *Aconitum* sp. However, there are several other reasons behind the poor seed set and inferior germplasm and their dormancy state. One of the most important reasons for the poor seed set may be the low pollen germinatibility in alpine condition (Cabin *et al*., 1991). Pollen fertility is an important feature that can help in establishing the successful adaptation of a plant species (Char *et al*., 1973; Qureshi *et al*., 2002). For conserving, managing and recovery of threatened species, knowledge of Reproductive Biology is essential (Kuniyal *et al*., 2003; Murugan *et al*., 2006). This will also help in improving desired traits or in developing new varieties. A study on floral biology, pollination behaviour and seed production of *A. heterophyllum* Wall at High Altitude Plant Physiology Research Centre (HAPPRC), Tungnath (3550 masl), Uttarakhand, India was carried out (Nautiyal *et al*., 2009). It revealed that flowering occurs from the second week of September to late October, with 20 days of peak flowering. An average of 80,000 pollen grains per flower was estimated with dehiscence timing between 7.30-11.00 am at high temperature. It was also reported that this species is self incompatible, although few fruits were developed but were smaller than the fruits produced by the open pollination and hand crossed flowers. This study may benefit in effective breeding programmes that could be undertaken for better *in situ* and *ex situ* conservation and cultivation for commercial purpose for this critically endangered medicinal plant.

Seed germination and seedling establishment in *A. atrox* is rare even under natural condition (Bhadula *et al*., 2000). Seed germination in *A. atrox* was found to be poor in different testing conditions (Nautiyal *et al*., 1985). Also cultivation cycle of *A. atrox* is very lengthy (5-7 years) to obtain a harvestable raw material (Rawat *et al*., 1992) and a single tuber is produced from a parental stock.

Rawat *et al*. (1992), carried out the vegetative propagation of *A. atrox* (Bruhl.) Muk, a threatened medicinal species via tuber segments in alpine condition (3600 masl) successfully so that the species conservation as well as continuous supply of raw material is ensured. But as no habitation exist in the alpine condition, this will not be acceptable to the farmers. Therefore, similar attempts have been conceded at the lower altitude (1900 m asl). In this study by Kuniyal *et al*. (2006) tuber segments were treated with GA_3, IBA and Kinetin in combination of three, any two or alone for 48 h at room temperature. Vegetative propagation in *A. atrox* at lower altitude was found to be successful as no considerable difference was observes when compared to the results of propagation at higher altitude.

Conservation via cultivation may prove to be beneficial as it is the most trouble-free and lucrative mode. Some modifications and standardization are still needed to be experimented by the researchers and scientists so that an effective cultivation could be done which would benefit the farmers financially and eventually the final consumers.

Micropropagation: Although, many research works had been carried to conserve the *Aconitum* sp. through cultivation, but then also no complete effective method has been primed to bring it in conservation programmes. Tissue culture opens up new area for conserving threatened *Aconitum* sp. as small amount of plant material can generate large no. of disease free propagules which can be re-introduced in their native habitat. In addition, it also overcomes the problems of *ex situ* conservation where seed availability is nearly mandatory. Furthermore, *in vitro* propagation also contributes in broadening of species genetic database; augmenting the yield and production of active constituents and secondary metabolites. *In vitro* propagation of plants holds tremendous potential for the production of high quality plant based medicine (Murch *et al*., 2000). Micro-propagation has many advantages over conventional methods of vegetative propagation which suffer from several limitations (Nehra and Kartha, 1994). Numerous factors are reported to influence the success of *in vitro*

propagation of different medicinal plants (Hu and Wang, 1983; Hussey, 1980; Bhagyalakshmi and Singh, 1988; Short and Roberts, 1991). Also the production of secondary metabolite *in vitro* is possible through plant cell culture (Tripathi and Tripathi, 2003). Zenk (1878) has reported cell lines capable of producing high yields of secondary compounds in cell suspension cultures. Approach for improving secondary products in suspension cultures, using different media for different species; have been reported by Robins (1994).

Several studies have been done and are being extended to conserve the species of *Aconitum* and other genuses. For conservation, tissue culture finds the priority as it requires a small amount of propagules for mass multiplication endowing with large quantity of raw material to herbal drug industries. *A. carmechaeli* an important medicinal sp. indigenous to China prescribed together with other herbal drugs, as an analgesic in the treatment of rheumatism and neuralgia (Shoyama *et al*., 1993) and functions as an important ornamental flower in Japan (Shiping *et al*., 1998). To deal with its large demand, mass multiplication is necessary. Successful clonal multiplication through tip-tissue culture method was done in which multiple shoot formation was achieved on MS medium (Murashige and Skoog, 1962) with 5 mg L^{-1} BAP and optimum rate of root formation with 0.5 mg L^{-1} IAA (Hatano *et al*., 1988). In another study, microtubering was achieved at same concentration of IAA but when incubated under dark for 6 weeks and it was also observed that temperature condition affects the microtubering significantly as 15°C was found to be the best temperature rather than 20°C for microtubering *in vitro* for *A. carmechaeli* Debx., but levels of mesaconitine and hypaconitine (aconitine type alkaloid) were higher at 20°C than at 15 and 10°C, also in clonally propagated level of aconitine type alkaloid were lower as compared to that in cultivated plants (Shiping *et al*., 1998).

Agar, which act as a common support for plant tissue culture may prove to be disadvantageous for the plant growth, as a result of selective adsorption of growth components it (Debergh, 1983). Some types of agar contain inhibitory substances which may prevent morphogenesis in certain cultures (Powell and Uhrig, 1987). Therefore, to enhance the *in vitro* multiplication rate of *A. napellus*, Watad *et al.* (1995) designed an efficient micropropagation protocol using floating membrane raft (Interfacial membrane raft) on liquid medium, where requirement of various growth components was minimized significantly as compared to that in solidified agar medium.

A. heterophyllum Wall is one of the most important medicinal herb species of this genus. It is presently identified as critically endangered herb and calls for its sustainable utilization and conservation, which can be accomplished in a short duration only through tissue culture.

Sterilization of a material (explant/seeds) before subjecting them for *in vitro* propagation is essential for the production of clean *in vitro* plantlets that ensures the reduction of the contaminants as well as high survival rate of explants. Requirements may differ for different parts of plants depending on their morphological characters like softness /hardness of the tissue etc. A protocol has been standardized for sterilization of nodal segments and seeds of *Aconitum heterophyllum* for its micropropagation intended for its mass propagation and conservation. Results showed that out of three sterilizing agents 0.1% (w/v) $HgCl_2$ for 5 min, was significantly reducing the contamination of explants and 7.5% (v/v) H_2O_2 of seeds *in vitro*, which shows that requirement of sterilization, may vary with the type tissue used for micropropagation (Srivastava *et al*., 2010b).

Previously Giri *et al*. (1993) had standardized the procedure for *in vitro* propagation of *A. heterophyllum* Wall. In this study, they observed that callus induction occurred on MS medium supplemented either with 1 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} Kinetin with 10% coconut water or with 5 mg L^{-1} NAA and 1 mg L^{-1} BAP by maintaining them on MS medium with 1 mg L^{-1} NAA. Whereas, in Jabeen *et al*. (2006), for a second time carried out the *in vitro* propagation of *A. heterophyllum* Wall and reported that callus induction occurred at extremely low concentration of growth regulators NAA (0.5 mg L^{-1}) and BAP (0.25 mg L^{-1}) supplemented in MS medium. Conditions for maintenance of the callus were same. In this report, *in vitro* shoot proliferation experiments revealed that MS medium containing 0.25 mg L^{-1} NAA with 0.5 mg L^{-1} BAP and 1.0 mg L^{-1} IAA was the best blend for shoot multiplication and *in vitro* rooting respectively, while Giri *et al*. (1993) optimized 1.0 mg L^{-1} IBA for *in vitro* rooting. Somatic embryogenesis was attained when callus (induced on 2, 4-D and NAA) was transferred to MS medium with 1 mg L^{-1} BAP and 0.1 mg L^{-1} NAA after 2 subculture passage (Giri *et al*., 1993). Complete plantlet formation from these embryos was obtained after 4 weeks on medium with ¼ MS nutrient containing 1 mg L^{-1} IBA (either supplementing it in medium or by dipping embryos in it for 5 min).

A. balfourii Stapf. is another important medicinal herb specie of alpine region. Its wild habitat needs to be conserve as it is being destroyed for the raw material for herbal drug industries. An effort has been made by Pandey *et al*. (2004) to conserve this specie through micro-propagation. They achieved callus induction from

small leaf segment of *in vitro* sprouted axillary buds on MS medium fortified with 4.5 µM BAP and 26.9 µM NAA and obtained shoot induction on same concentration of BAP with lowered concentration of NAA (1.1 µM). *In vitro* shoot multiplication and rooting was observed at 1.1 µM BAP and 12.3 µM IBA, respectively.

In vitro propagated plants are liable to variation when compared to seed grown or vegetative propagated plants in reference to morphology, genetic make up, phytochemical content or the stages of development. Therefore, Pandey *et al.* (2004) also evaluated the difference in chromosome, protein profile and alkaloid content of *in vitro* tubers raised and seed raised plants of comparable age, which revealed identical profile of all the parameters tested. Comparative analysis of the development of somatic embryos in callus and of sexual embryo *in situ* was done, which showed relative similarity (Batygina, 2004).

Concern for increasing the yield of secondary metabolite production through genetic engineering of plant *in vitro* is growing significantly to cope up with the increasing demand of herbal products. Giri *et al.* (1997) standardized the production of hairy roots in *A. heterophyllum* Wall using *Agrobacterium rhizogenes* for the first time which revealed that total alkaloidal (aconites) content of transformed roots was 3.75% times higher than non transformed roots.

CHEMICAL CONSTITUENTS

Aconitum genus is reputed for its medicinal and pharmaceutical value. The genus *Aconitum* comprises of 400 species, including some ornamental and medicinal plants (Utelli *et al*., 2000). These medicinal plant species are a rich source of diterpene alkaloids and flavanoids, many of which exhibit broad spectrum activities. The roots which have been used mostly as poison than as drugs, are now reported to possess significant antipyretic and analgesic properties and a high therapeutic index (Jabeen *et al*., 2006). Lycaconitine, obtained from several *Aconitum* sp. was found to be effective against multidrug resistant cancers (Kim *et al*., 1998). Various studies have been carried since last two decades on the isolation, identification, structural elucidation of active constituents of *Aconitum* sp. and their pharmacological and biological activity. Alkaloids and Flavanoids both are manifested with wide range of pharmacological and biological activity. This section of the review will focus on the research done on active constituents of *Aconitum* sp.

Alkaloids: The tuberous roots of genus *Aconitum* contains alkaloids benzoylmecasonine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetsinone and benzoylheteratisine (Wang *et al*., 2006; Pelltier *et al*., 1968) and plant contain alkaloids: heteratisine, heterophyllisine, heterophyllidine, atidine, isotisine, hetidine, hetsinone and benzoylheteratisine (Wang *et al*., 2006). These alkaloids are known to be very toxic, but turn easily into less toxic alkaloids such as aconitine transforms into benzylaconine, aconine, pyraconine by heating or alkaline treatment through deacetylation, debenzoylation or oxidation reaction (Kitagawa *et al*., 1984). The tubers of the *Aconitum* spp. have been used as herbal drug only after it was treated by immersion in salt solution or by heating to reduce the toxicity. Currently the processed aconite tubers are widely and safely used for the treatment of pain neuronal disorders and inflammation with no problematic or annoying adverse effects (Murayama *et al*., 1991; Oyama *et al*., 1994; Ameri, 1998; Taki *et al*., 1998). But still there is a need of standardized method for assessing the levels of the toxic alkaloids in aconite roots in order to ensure the safe use of these plant materials as medicinal herbs. Optimization of extraction, separation and measurement condition may generate a reliable, reproducible and accurate method for the quantitative determination of alkaloids (Jiang *et al*., 2005). Data on such methods are scarce but efforts are being made to develop this technology. In one of the study by Jiang *et al.* (2005), three aconitum alkaloids (aconitine, mesaconitine and hypaconitine) in processed and unprocessed tubers were separated by modified HPLC method employing a C18 column gradient eluted with acetonitrile and ammonium bicarbonate buffer. Processed tubers showed lower level of alkaloidal content as compare to unprocessed tubers. The variations obtained were attributed to differences in species, processing methods and places of origin. Csupor *et al.* (2009), also optimized the extraction and analytical conditions using HPLC method coupled with photo-diode array detection (HPLC-DAD) for quantitative and qualitative analysis of aconitine type and lippoalkaloids of *A.carmicaheli* roots. Effects of processing on both type of alkaloids and pure aconitine was evaluated which revealed higher levels of alkaloids in unprocessed one. Various alkaloids are reported as principal component of the processed Aconite tubers that manifest pharmacological activities (Hikino *et al*., 1979; Kitagawa *et al*., 1984; Suzuki *et al*., 1993). Although, there is an abundance of scientific works on chemical constituents of *Aconitum* sp. but meager data is available on specific characterization and standardization of these constituents. New derivatives of different alkaloids and flavanoids have also been isolated

from different species of *Aconitum* along with their structural and medicinal elucidation. Following are some such research reports which have shown isolation of new alkaloids (Fig. 1) and flavanoids and derivatives (Fig. 2) from different *Aconitum* sp. with their structure elucidation and bio-pharmacological activities.

Dzhakhangirov and Bessonova (2002) isolated 11 diterpene alkaloids. Isoatisine and coryphine were found to be most active might be due to presence of oxazolidine rings. Investigation on alkaloidal constituents of *A. jaulense* led to the isolation of seven C-19 norditerpenoids and C-20 diterpene alkaloids (Shim *et al*., 2003). 8-O-Azeloyl-14-benzoylaconine, a new alkaloid from roots of *A. karacolicum* Rapcs. has been reported by Chodoeva *et al.* (2005).

Ahmad *et al.* (2008) isolated two new aconitine type norditerpenoid alkaloids from namely 6-dehydroacetylsepaconitine and 13-hydroxylappaconitine along with three known norditerpenoid alkaloids while Nisar *et al.* (2009) isolated two new diterpene alkaloids heterophylline A and heterophylline B along with two known alkaloids from the roots of *A. heterophyllum* Wall and their structural elucidation was done on the basis of spectral data. In a study by Gao *et al.* (2006), diterpene alkaloids were reported for the first time in *A. spicatum* Stapf. Thirteen norditerpenoids were isolated from the chloroform fraction of 90% ethanol extract of roots, out of which two were new namely spicatine A and spicatine B.

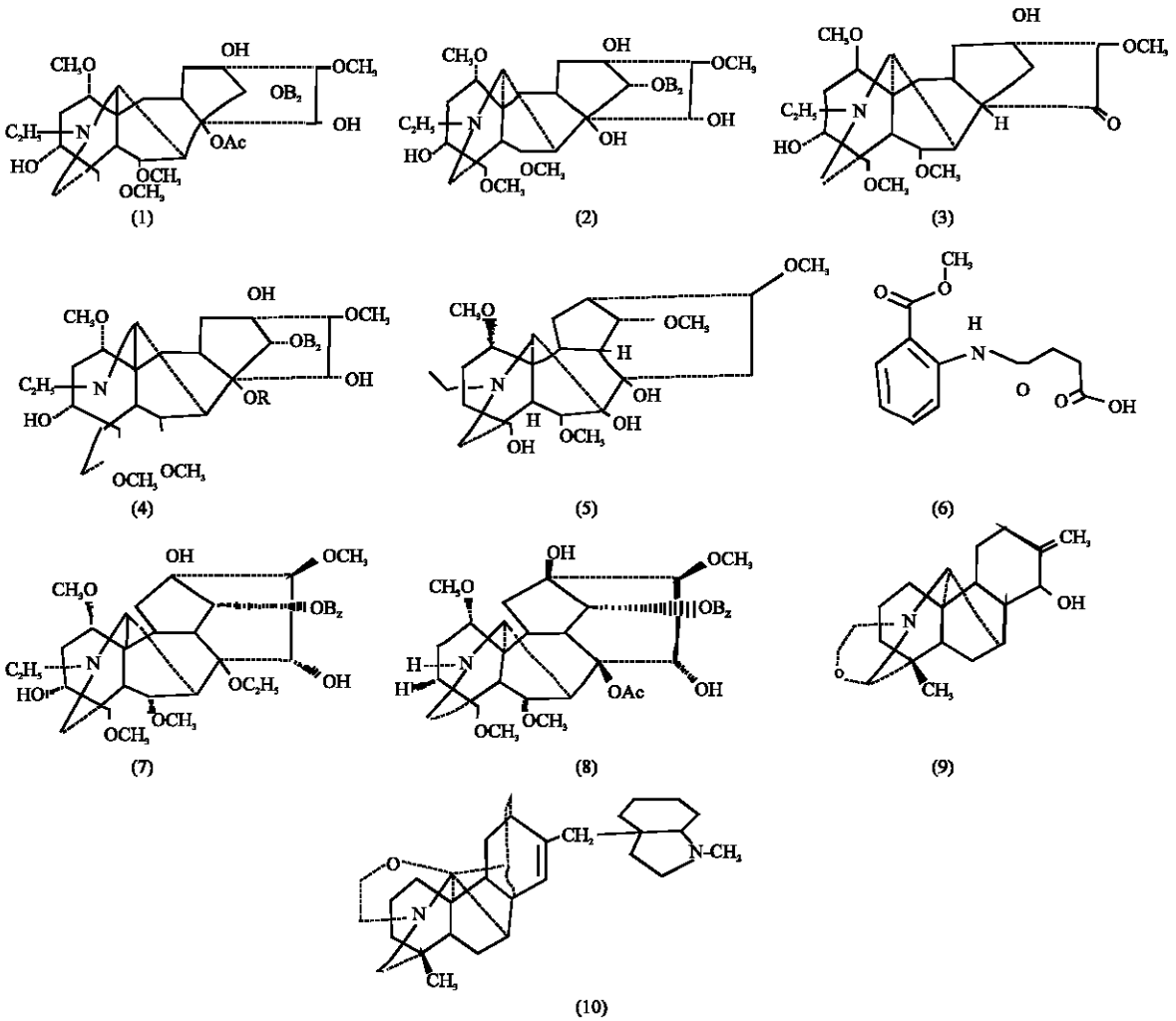

Fig. 1: Alkaloids of different *Aconitum* sp. (1) Aconitine; (2-4) Transformed products of aconitine- Benzoylaconine, Pyraconitine and Lipoaconitine (aconiti tubers, Shoyoma *et al*., 1993); (5-6) Swatinine and benzene derivative (*A.laeve* Royle (aerial part), Shaheen *et al*., 2005); (7-8) Spicatine A and B (*A. spicatum* Stapf. (roots), Gao *et al.* (2006); (9-10) Isoatisine and Coryphine (*A. coreanum* L., Dzhakhangirov and Bessonova (2002)

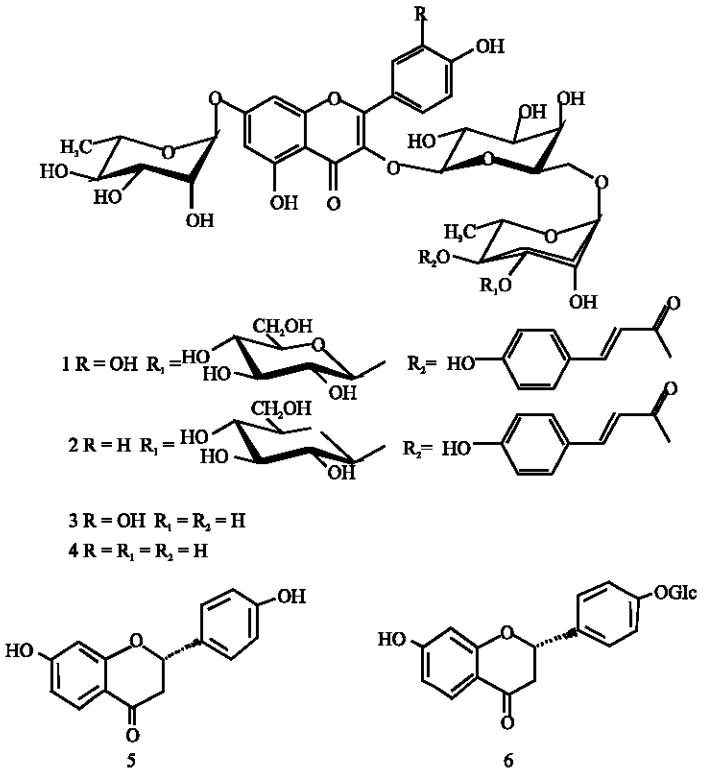

Fig. 2: Flavanoids of different *Aconitum* sp. (1) Chemical structure of flavanoids from *A. anthora* L.; (1-2) new quercitin derivative, (3-4) new kaempferol derivative, clovin and robinin (Mariani *et al*., 2008); (5-6) Liquiritigenin and liquiritin (processed aconiti tubers, Lyu *et al*., 2008)

Isolation of active components from the extracts of medicinal plant species is the initial and vital step for any pharmacological and biological studies; this step decides the purity level of isolated chemical content, the most crucial feature for these studies. As discussed earlier also, Jiang *et al*. (2005) and other researchers also employed new methodology or modified chromatographic and spectrophotometric method for assessing the levels and type of alkaloids. These methods are being affianced in purifying the active content of *Aconitum* sp. Preparative high speed counter current chromatography coupled with evaporative light scattering detection was employed for isolation and purification of alkaloids from the roots of *A. coreanum* (Levl.) Rapaics. The purity of the compounds was found to be in the range of 90-98% as determined by HPLC. Their chemical structure was identified by MS, ^{1}HNMR and ^{13}CNMR (Tang *et al*., 2007). Yue *et al*. (2009) examined aconitine-type alkaloids in the Chinese herb *A. carmichaeli* Debx. by means of HPLC/ESIMS/MS (n) and FTICR/ESIMS in positive ion mode. The novel alkaloids including 1 MDA, 2 DDA and 48 lipo-alkaloids were detected. In addition, 1 DDA, 7 lipo-alkaloids and 2 alkaloids with small molecular weights that possess C19-norditerpenoid skeleton were reported in *A. carmichaeli* for the first time.

Although, the main activity has been reported to transpire in roots or tubers of many *Aconitum* sp. (as per the literature), but many new alkaloids have also been isolated from plant parts other than the roots/ tubers. These compounds are also reported to have pharmacological and biological properties. Bessonova *et al*. (1990) isolated the new alkaloid from the epigeal parts of *A. coreanum* (Levl.) Rapaics. This new alkaloid has the structure of 14-hydroxy-2-isobutrylhetisine N-oxide deduced on the basis of spectral data and chemical transformation.

New norditerpenoid alkaloid, swatinine and benzene derivative 4-[2-(methoxycarbonyl)-anilino]-4oxobutanoic acid along with four known alkaloids, delphatine, lappaconitine, puberanine and N-acetylsepaconitine were isolated from aerial parts of *A. leave* Royle. Biological and pharmacological study had also been done for these compounds (discussed later in this review). Benzene derivative previously has been reported as a synthetic precursor of some heterocyclic compounds (Balasubramaniyan and Argade, 1988) as a substituent in

many isolated for the first time norditerpenoid alkaloids, but it is from a natural source (Shaheen *et al*., 2005).

Alkaloidal content may vary with environmental condition in which the *Aconitum* sp. are growing. Different conditions are conscientious for these discrepancies. Such conditions include temperature, light, stage of vegetative cycle, mineral nutrition, altitude, photoperiod etc. therefore quantification of alkaloids is one of the most critical step for identifying elites for raw material. Colombo *et al*. (1988) suggested that diterpene alkaloids in *A. napellus* sp. *neomontanum* are present in good quantity in all the plant parts during vegetative phase, which is beneficial for early collection of raw material. Evaluation of impact of cultivation and low altitude acclimatization on active constituents of *A. heterophyllum* revealed that this specie can be cultivated at moderate elevation as decrease in active constituents in cultivated form was fairly small (Prasad, 2000), while but no correlation was observed in alkaloidal (aconitine and pseudoaconitine) content in *A. heterophyllum* Wall and *A. balfourii* Stapf. with respect to altitudinal variation (Pandey *et al*., 2008).

In vitro propagation intended for conservation of *Aconitum* sp. may also result in variation in active component. In a plant cell culture secondary metabolite production entirely depends on the composition of the culture medium and all environmental conditions (Stafford *et al*., 1986). In a report by Shoyama *et al*. (1993), it was observed that relation between temperature and tuber production in *A. carmichaeli* was inversely proportional. Temperature clearly effected the production of aconitine type alkaloids; levels of aconitine and mesaconitine were higher at 25°C than at 15°C. It was concluded that this variation in active constituents occurs in relation to the harvest time of the tubers.

Flavanoids: The genus *Aconitum* comprises of many European and Asian species. The plants of this genus are tall, erect, stem being crowned by racemes of large and eye-catching blue, purple, white, yellow or pink zygomorphic flowers. The interest on this genus is based on the diterpene alkaloids, used in oriental medicine (Bisset, 1981) and flavanoids studied in the last ten years as chemotaxonomic markers (Lim *et al*., 1999). These alkaloids and flavanoids are manifested with different biological and pharmacological properties (Anwar *et al*., 2003; Braca *et al*., 2003; Di Carlo *et al*., 1999; Williams *et al*., 2004) which make this genus most imperative among all other genuses. Ample amount of published data are available on alkaloidal content and their pharmacological properties, but only few data have been reported on other pharmacologically active component e.g., flavanoids, these are the class of low molecular weight phenolic compounds widely distributed in the plant kingdom. They exhibit different biological functions that allow interaction between plants and their environment (Treutter, 2005). They give color to flowers which attracts the pollinating animals. Besides pigmentation, flavanoids also exhibit phenomenon of co-pigmentation (Koes *et al*., 1994) which imparts different shades to the flower. Flavanoids are mainly involved in photo protection from sunlight ultraviolet and are also potent scavengers of reactive oxygen species which prevent lipid peroxidation (Treutter, 2005). Thus, flavanoids are as important as alkaloids for various functioning in the plant body. Only little information is available on the flavanoid composition of the *Aconitum* sp. but the research is being done on this aspect of genus *Aconitum* as well. In a research manuscript by Mariani *et al*. (2008), two flavanoids quercitin and kaempferol derivatives with two known flavanol glycosides clovin and robinin (reported first time in Ranunculaceae) had been reported to be isolated and identified (TLC, ^{1}HNMR, ^{13}CNMR) from the methanol extract of aerial parts of *A. anthora* L. *In vitro* antioxidant activity of 4 isolated flavanoids were also screened.

Two another flavanoids, liquiritigenin and liquiritin were also isolated and identified (NMR, MS and IR) from processed Aconiti tuber of *A. carmichaeli* Debx. by Lyu *et al*. (2008).

Other than alkaloids and flavanoids, there are other chemical components which have been reported to be present in *Aconitum* sp. and are beneficial for human health e.g., Free Fatty Acids (FFA), which have been attracting increased attention in food nutrition value (Tomaino *et al*., 2001; Skonberg and Perkins, 2002; Martin *et al*., 2005; Sajid *et al*., 2008; Phillip and Matt, 2008), diagnosis of certain diseases and in pharmacology (Stoddart *et al*., 2008) due to their biological and environmental importance (Wallace *et al*., 2000; Cherif *et al*., 2008). Yue *et al*. (2010) reported a complete FFA profile of *A. taipeicum* Hand.-Mazz. using alcohol extraction and esterification followed by GC-MS, which showed abundancy of three types of FFA including two essential fatty acids to human body. Linoleic acid (play vital role in reducing cholesterol, decreasing arterio sclerosis, cancer prevention etc.) content was higher than the other two.

A water-soluble polysaccharide named as FPS-1 was isolated from the root of *Aconitum carmichaeli* Debx. by hot-water extraction, anion-exchange and gel-permeation chromatography and tested for its pharmacological activities. FPS-1 showed potent stimulating effects on murine lymphocyte proliferation induced by concanavalin

A or lipopolysaccharide and on splenocyte antibody production both *in vitro* and *in vivo* (Zhao *et al*., 2006).

There is paucity of published data on alkaloidal content, but, scanty of data on flavanoids and other active component in *Aconitum* sp. is available. However, flavanoids exhibit strong antioxidant activity and other pharmacological properties. Other components like free fatty acids which may be beneficial in other way, further studies on these aspects of chemical composition of *Aconitum* may prove be a better way to utilize these very important species more efficiently and wisely in medicinal materials.

BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES

Biological and Pharmacological activity of any compound corresponds to its both adverse and beneficial effect on living. The tubers of the *Aconitum* sp. have been widely used as a poison since ancient time but with the advancement in technology, its medicinal aspect was explored and a therapeutic dose has been set. Proper processing (Park *et al*., 1990) and multiherb formulation (Wang *et al*., 2009) can reduce the level of toxicity. It has been known that herbs like *Aconitum*, *Ephedra* are never used as single herb for intervention and aconite is only used when it is processed and in combination with specific matched herbs (Wang *et al*., 2009). The active components of *Aconitum* have been reported to have significant pharmacological and biological features.

Different species of this genus exhibit antipyretic, anti-inflammatory, analgesic, astringent and anti-diarrhoeal activities. Besides, they also show strong antioxidant and antimicrobial activity (Table 1).

This segment of the review portrays some of the research work done on biological and pharmacological activities of chemical constituents isolated from various *Aconitum* sp. as follows:

Anti-oxidant and Anti-inflammatory activity: Swatinine and a benzene derivative along with four known alkaloids, delphatine, lappaconitine, puberanine and N-acetylsepaconitine isolated from aerial parts of *A. leave* Royle were subjected to biological studies. Swatinine and delphatine, alkaloids isolated from aerial parts of *A. leave* Royle showed fair good antioxidant activity of 54.1% and 55.4%, respectively, when evaluated by DPPH radical scavenging activity and comparing with 1 mM BHA (92.1%) as standard. Anti-inflammatory activity was exhibited by all the isolated compounds but lappaconitine and puberanine showed best and significant anti-inflammatory activity among all the other alkaloids (Shaheen *et al*., 2005). In another study, anti-oxidant activity of flavanoids, quercitin and kaempferol derivatives was screened via three *in vitro* assays namely DPPH radical scavenging, total antioxidant capacity and lipid peroxidation assay using quercitin as standard. All the analyzed compounds revealed meek activity towards lipid peroxidation assay but ten folds lesser than the standard. Quercitin derivative showed values higher than

Table 1: Some important species of *Aconitum* with their distribution pattern in Himalayas and medicinal properties

Species	Remarks	Location (*Himalayan range*)	Medicinal properties
A. chasmanthum	Vatsanabha of W. Himalayas /Endemic	NW-Kashmir Himalayas (Pakistan J and K Him.)	Antirheumatic, useful in heart diseases, neurasthenic and fever, diaphoretic, diuretic, anodyne, anti diabetic
A. heterophyllum	Atees	NW-Cent. Himalayas (Pakistan-Nepal Him.)	Anti-inflammatory, Antipyretic, Diarrhea, Vomiting, Cough, Cold, Astringent
A. falconeri	Vatsanabha Subst./ Endemic	W. Himalayas (Punjab- Uttaranchal Him.)	Antipyretic, Paralysis, Sciatica, Gout, Diarrhea etc.
A. moschatum	Vatsanabha Subst.	NW Himalayas of J and K	Medicinal properties of *A. chasmanthum*
A. deinorrhizum	Common. Vatsanabha Subst.	West.-East. Himalayas (J and K-Bhutan Him.)	Said to be medicinal, extremely poisonous.
A. violaceum	Common.Vatsanabha Subst.	NW-Cent. Himalayas (Pakistan-Nepal Him.)	Antipyretic, Abdominal Pain, Antidote, Anti-inflammatory, Febrifuge
A. laeve	Adult. of Atees	NW-Cent. Himalayas (Pakistan-Nepal Him.)	Anti-inflammatory, Antipyretic, Diarrhea, Vomiting, Cough, Cold, Astringent
A. rotundifolium		Cent.NW- Cent. Himalayas (Pakistan-Nepal Him.)	Roots used as tonic in Jammu and Kashmir
A. ferox	Vatsanabha of the East. Him.	Cent. - East. Himalayas (Nepal-Arunachal Pradesh Him.)	Antipyretic, Anti-rheumatic, Paralysis, Snake bite etc.
A. bisma	Vatsanabha	East. Himalayas (Sikkim-E.S.(Myanmar) Him.)	Medicinal properties of *A. chasmanthum*
A. spicatum	Subst. of Vatsanabha	Cent. - East. Himalayas (Nepal-Tibetan Him.)	Medicinal properties of *A. chasmanthum*
A. laciniatum	Subst. of Vatsanabha and arrow poison	Cent. - East. Himalayas (Nepal-Tibetan Him.)	Medicinal properties of *A. chasmanthum*
A. kashmiricum	Adult. of Atees/ Endemic	NW-Kashmir Himalayas (Pakistan -J and K Him.)/	Anti-inflammatory, Antipyretic, Diarrhea, Vomiting, Cough, Cold, Astringent

kaempferol derivatives in other two assays, which suggests that quercitin derivative was the most active amongst all and can be capably utilized in various anti-oxidant medications (Mariani *et al*., 2008).

Neurological studies: Effect of several *Aconitum* alkaloids on central nervous system was screened by Ameri (1998) after dividing them in three different groups comprising of highly toxic, less toxic and reduced toxic alkaloids on the basis of their structure. The pharmacology and therapeutic v/s toxic potential of these alkaloids has been highlighted and discussed which is of great importance.

It has been reported that aconitine cause persistent activation of Na^+ channels in heart, skeletal muscles, CNS by blocking their inactivation (Wang and Wang, 2003). Ameri and Simmet (1999) further observed extracellular recordings of stimulus evoked population spikes and field excitatory postsynaptic potential (EPSP) in rat hippocampal slices for the evaluation of antagonistic activity of aconitine (activates voltage dependent Na^+ channel) against lappaconitine (blocks voltage dependent Na^+ channel) and ajacine which revealed that the inhibitory and antiepileptiform effect of ajacine and lappaconitine is mediated by a frequency-dependent inhibition of the voltage-dependent sodium channel, thereby decreasing the excitability.

Songorine, a diterpene alkaloid from the genus *Aconitum* was showed to enhance the excitatory synaptic transmission in rat hippocampus and concluded as a novel non-competitive antagonist in the GABAA receptor in rat brain (Zhao *et al*., 2003). Biological activity of 11 alkaloids isolated from *A. coreanum* illustrated myorelaxant activity with Isoatisine and coryphine to be the most active (Dzhakhangirov and Bessonova, 2002).

Antiproliferative activity: Little information is available regarding the antiproliferative properties of *Aconitum* alkaloids against human tumor cells despite of their intense toxicities.

8-O-azeloyl-14-benzoylaconine, a new alkaloid isolated from the roots of *A. karacolicum* Rapcs. was purified using scheme based upon its antiproliferative properties against 3 human tumor cell lines in culture. It's IC_{50} of about 10-20 μM, when investigated through *in vitro* cytotoxicity test (Chodoeva *et al*., 2005). Hazawa *et al*. (2009) investigated anti-tumor properties and radiation sensitizing effect of novel derivatives prepared from C-20 diterpenoid and C-19 norditerpenoid alkaloids of *Aconitum*. The anti-tumor activities studied against human tumor cell lines A172, A549, HeLa and Raji demonstrated that C-20 diterpenoid derivatives were the significant suppressor of these tumor cell lines. However, only novel derivatives of *Aconitum* alkaloid smothered these cell lines but not natural alkaloids.

Anti-diarrhoeal and anti-microbial activity: *A. heterophyllum* Wall has been previously reported to have anti-diarrhoeal activity along with astringent and tonic properties (Singh and Chaturvedi, 1982). It is now used as a key ingredient of anti-diarrhoeal medicine Diarex Vet. Mitra *et al*. (2001) investigated the activity of Diarex Vet against lactose induced diarrhoea in wistar rats and found that it as a potent anti-diarrhoeal agent at the dose of 750 mg kg^{-1} of b.wt.

Antimicrobial activity of crude extracts of *A. chasmanthum* along with brime shrimp lethality and insecticidal activity was evaluated. Antibacterial activity tested against gram negative and gram positive bacteria using methanolic extracts (100 and 200 μg) showed negligible activity when compared to standards. All the fractions exhibited strong antifungal activity against *Trichophyton mentagrophyte*. Ethyl acetate extract was found to be most effective antifungal agent. Insecticidal activity of methanolic extract was comparable to standard atropine at 500 ppm, while 1000 μg mL^{-1} of LD_{50} was observed with brime shrimp lethality test (Anwar *et al*., 2003). *A. tanguticum* showed strong inhibition against *Fusarium semitectum* and methicillin resistant *Staphylococcus aureus* and low activity against *Escherichia coli*. The results suggested that the essential oil can be used as antimicrobial agents for treatment of infectious diseases especially for antibacterial agents against MRSA (Zhang *et al*., 2009).

Enzyme inhibition activity: Enzyme inhibitors are clinically very useful. Tyrosinase inhibitors are involved in treating dermatological disorders associated with melanin hyper pigmentation and also important in cosmetics for whitening and de-pigmentation after sunburn (Shiino *et al*., 2001). Shaheen *et al*. (2005) tyrosinase inhibition studies on 5 alkaloids showed that only lappaconitine and puberanine exhibited mild inhibition against the enzyme with IC_{50} of 93.33 and 205.21 μM, respectively. Nisar *et al*. (2009) isolated heterophylline A and heterophylline B alkaloid from *A. heterophyllum* Wall which inhibited muscle contracting enzyme acetylcholineatrase and butyrylcholinestrase responsible for Alzheimer disease. But both compounds were 13 times more specific to butylcholinestrase.

DIVERSITY PROFILE AS QUALITY CONTROL TOOL

Aconitum sp. provides wide range of alkaloids, flavanoids and other active constituents which are responsible for their medicinal properties. These are used in many herbal drug formulations as a key ingredient. Within the context of increased herbal medicines use and lack of effective regulatory control, the safety of herbal medicines has become a key priority issue. Scientifically validated and technologically standardized herbal medicines may be derived using a safe path of reverse pharmacology approach based on traditional knowledge database. This may play a vital role in drug discovery, development and therapeutics, in addition to dealing with a typical Western bias against Ayurveda [1]. Correct identification and quality assurance of the starting material is, therefore, an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy.

There are various methods for the identification and authentication of plant species viz., organoleptic inspection (morphological and histological identification), Biochemical markers and DNA based markers. Macroscopic identity of botanical materials is based on parameters like shape, size, color, texture, surface characteristics, fracture characteristics, odor, taste and such organoleptic properties that are compared to a standard reference material. Microscopy involves comparative microscopic inspection of broken as well as powdered, crude, botanical materials. However, these parameters are judged subjectively and substitutes or adulterants may closely resemble the genuine material. Mainly, markers are categorized in to two classes: Biochemical markers generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids. Chemical profiling establishes a characteristic chemical pattern for a plant material, its fractions or extracts. Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) are routinely used as valuable tools for qualitative determination of small amounts of impurities. DNA markers are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors. DNA can be extracted from fresh or dried organic tissue of the botanical material.

Research studies on diversity profiling that is fingerprinting of *Aconitum* sp. has been done by using both the means. Following are some of the recent work done on the chemical and molecular profiling.

Biochemical markers: Besides phytochemical analysis, chromatographic or spectrophotometric techniques also provide a contrivance for quality control management. With some or advanced modification in chromatographic techniques, a profile of chemical constituents for a particular species can be generated. This chromatographic fingerprinting authenticates and identifies the herbal medicines accurately and also demonstrates the sameness and differences between various samples successfully (11, 14). Qiao *et al.* (2009) developed sensitive and Reliable Rapid Resolution Liquid Chromatographic (RRLC) method coupled with diode array detection for the fingerprint analysis of raw and processed *Aconitum kusnezoffii.* The method provided the comparison profile of the RRLC fingerprints of processed and raw *Aconitum kusnezoffii* which indicated that the major constituents changed during processing.

Three pairs of aconite alkaloid isomers from *Aconitum nagarum* var. *lasiandrum* were differentiated based on their fragmentation pathways as revealed by ESI-MS and tandem Mass Spectroscopy (Li *et al.*, 2006).

HPLC-DAD method aided by similarity and Hierarchical Clustering Analysis (HCA) was applied for identification of four species of the roots of Aconitum (*Aconitum kusnezoffii* Rchb. samples and related herbs including *Aconitum karacolicum* Rapcs., *Aconitum austroyunnanense* W.T.Wang and *Aconitum contortum* Finet and Gagnepain). The results revealed that the chromatographic fingerprint combining similarity measurement and hierarchical cluster analysis could efficiently identify and distinguish *Aconitum kusnezoffii* Rchb. samples from its related species (Zhao *et al.*, 2009)

DNA-based markers: Though the biochemical markers can be employed for the characterization based on the diversity profile of the active components, but the biochemical markers are governed by multi-gene family and are liable to deviated by environmental factors, which may lead to false outcomes. This problem can be surmounted by using DNA-based markers or molecular markers (RFLP/AFLP/RAPD/SSR/ISSR). DNA markers have now become a well-liked means for identification and authentication of medicinal plants. This is because, using DNA markers with the techniques like PCR and molecular cloning can provide every minute detail about the subject sample. Also these DNA-based markers are less affected by age, physiological condition and other environmental factors, can be detected at any phase of organism development as they are not tissue specific. Only a small amount of sample is adequate for analysis. These flexible possessions of DNA markers are particularly relevant for medicinal plants that are expensive or in limited supply

e.g., some vital species of *Aconitum* are quite expensive and also their availability is limited to a large extent. DNA-based molecular markers have proved their utility in fields like taxonomy, physiology, embryology, genetics and many more.

Fico *et al*. (2003) used RAPD technique and phytochemical analysis, based on the investigation of flavonoid composition, to study the sameness and differences in *Aconitum vulparia*, *A. paniculatum*, *A. napellus* subsp. *Tauricum* (from two different localities) and *A. napellus* subsp. *Neomontanum* and patterns of relatedness observed in chemical profiles appear to correspond with the genetic profiles generated by RAPDs of *Aconitum* species, suggesting that there may be a genetic basis for the chemical profiles observed. Also, polymorphic micro satellites were designed as efficient tool for examination on population genetic structure of *Aconitum napellus* L. (Cadre Le *et al*., 2005).

ITS topology demonstrated that subgrouping of the subgenus *Aconitum* based on the morphology of seeds and of petals, suggesting that seed and petal morphology may reflect well the phylogenetic relationships within the subgenus, but other morphological characters might be unreliable (Luo *et al*., 2005). Investigation on cytogenetic (Giemsa C-banding) and molecular ISSR and RAPD markers in Sudetic and Western Carpathian high-mountain *Aconitum* sect. Aconitum, was carried. The results obtained were also compared with predictions of species relationships based on biogeographical evidence, which suggested that combined analysis of hypervariable PCR ISSR+RAPD and more conservative cytogenetic markers can prove to be an effective way to elucidate taxonomic problems, as some results argued biogeographical evidences (Mitka *et al*., 2007).

Introgressions on diversity of *Aconitum* sp. have been carried out but still there are scarcity of published data on *Aconitum* sp. under this area, may be due to expensive and limited sample availability. Although, DNA analysis in genus *Aconitum* is considered to be revolutionary technology presently, however it also has certain limitations (costly and requires high degree of skills) due to which its use has been limited to academia. Important issue is that DNA fingerprint will not vary with plant part used, while the phytochemical content will vary with the plant part used, physiology and environment. DNA fingerprinting ensures presence of the correct genotype but does not reveal the contents of the active principle or chemical constituents. Neither technique solely can provide complete details of the samples of *Aconitum* sp. Hence, both the DNA analysis and pharmacognostic techniques for chemoprofiling such as TLC, HPTLC, etc should be employed synergistically to obtain much effective results for quality control and assurance of drugs prepared by different species of *Aconitum*.

FUTURE PROSPECTS

Aconitine and other phytochemical content of this genus Aconitum have received a special value in scientific society. These phytochemical contents have been bestowed with the number of medicinal compassion which includes antibacterial (Anwar *et al*., 2003; Zhang *et al*., 2009), antioxidant (Shaheen *et al*., 2005; Mariani *et al*., 2008), antiproliferative (Chodoeva *et al*., 2005; Hazawa *et al*., 2009), enzyme inhibition activities (Shaheen *et al*., 2005). Many species of this genus are now identified at different level of threat. Tissue culture is useful for multiplying and conserving the species, which are difficult to regenerate by conventional methods and save them from extinction (Tripathi and Tripathi, 2003). Genetic transformation may provide increased and efficient system for *in vitro* production of secondary metabolites and for engineering the metabolic pathways for enhancement of active component. But so far this has been confined only to *A. heterophyllum* Wall; therefore it still needs lots of attention for various other species of this genus exploited for pharmaceutical purposes.

Proper standardization of any medicinal herb is very important as per the WHO guidelines before any herb can truly find its budding market on the worldwide scale (WHO, 2000). Organisation of proper conservation programmes and analysis of phytochemical content thoroughly is the need of the time. Good quality DNA is always a prerequisite in all molecular biology experiments especially PCR (Sharma *et al*., 2010), therefore standardization of DNA isolation from such valuable and threatened medicinal plants should be standardised aptly. No technology solitarily can make valuable medicinal plants of this genus successful in the international market. Therefore, present scenario of herbal market suggests that concoction of the various techniques viz., conservation via cultivation or micropropagation, phytochemical analysis and its authentication (through biochemical and DNA- based markers both) and their pharmacological activities with adequate process of advertising and subsequent globalization may prove advantageous in upgrading the market of *Aconitum* sp. efficiently and lucratively.

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