

## Morpho-phenological and Antibacterial Characteristics of *Aconitum* spp.

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### Abstract

*Aconitum* species have been traditionally used as ethnomedicine to cure various ailments. The present study reveals the morpho-phenology and antibacterial property of alkaloid extracts of the two *Aconitum* species. The morpho-phenological characteristics will be helpful for determining the resource availability. *Aconitum nazarum* is erect type, whereas, *Aconitum elwesii* is a climber. *Aconitum elwesii* grows in advance of *A. nazarum* in terms of growth, flowering and senescence. Towards the end of the year, when the fruits have ripened, the parent tuber dies off. As a result, the daughter tuber becomes independent and in the following spring, takes over the function of the parent tuber. *Aconitum nazarum* and *A. elwesii* were found to contain 4-5 aconitine equivalent (AE) mg/g of alkaloid. These alkaloids showed antibacterial activity against different bacterial species including human pathogens, namely, *Staphylococcus aureus*, *Salmonella typhimurium*, *Bordetella bronchiseptica*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescense* and *Xanthomonas campestris*. However, the extent of antibacterial activity varied among different bacterial species. The antibacterial activity against *S. aureus*, *B. bronchiseptica*, and *B. subtilis* was bactericidal in nature, whereas, against other tested bacterial species was bacteriostatic. Efficacy of the antibacterial activity of these alkaloids was evaluated by comparing with that of standard antibiotics. Differential localization of the antibacterial principle was observed among the *Aconitum* species studied.

**Keywords:** *Aconitum*, alkaloid, antibacterial, bactericidal, bacteriostatic, morpho-phenology

### Introduction

*Aconitum* comprising about 400 species is a circumboreal arctic and alpine genus that extends into lower latitudes where there is suitable mesic habitat at high elevations along the north-south chains of mountains. The greatest distribution of *Aconitum* species is in Asia, with smaller groups in Europe and North America (Brink and Wood, 1997). About 28 species have been reported in India from sub-alpine and alpine regions between the altitudes of 2000 - 4800 m above mean sea level (Sharma *et al.*, 1993). Botanical Survey of India reported two species namely, *Aconitum nazarum* Stapf. and *Aconitum elwesii* Stapf. from Manipur, India (Sharma *et al.*, 1993; Singh *et al.*, 2002). *Aconitum*, called Nuishiwon in the local dialect, named after a migratory bird, locally called Nuishi which migrates into Shirui peak and has the same bluish colour of *Aconitum* flower. The extracts of *Aconitum* species are traditionally being used at diluted concentrations for curing, fever, gastro-intestinal dysfunction, inflammation, cough and asthma. Moreover, its paste is also used for the external applications such as treatment of neuralgia and other painful infections (Mori and Murayama, 1989). Some *Aconitum* species are also reported to possess antifungal, insecticidal and rodenticidal properties (Deshmukh and Borle, 1976;

Smith and Secoy, 1981). The rhizomes of *Aconitum* species are used by the tribes of Sikkim, India for curing different ailments (Pradhan and Badola, 2008). Although, the alkaloids of *Ranunculaceae* family are reported to have antimicrobial properties, there are only a few reports available on antimicrobial activity of alkaloids from *Aconitum* species (Samy and Gopalakrishnakone, 2008). Ukhrol, the present study site is in one of the five hill districts of Manipur and falls under group 11 (Sub-Group 11 BC<sub>1</sub>) of Eastern Himalayan Wet Temperate Forest type (Champion and Seth, 1968). Understanding the morpho-phenological characteristics of a medicinal plant is as much important as exploring its biochemical characteristics which inform us about the timing and duration of resource availability in the ecological communities. The species reported from this region have not been explored thoroughly for their clinical potential. With the recent trends showing preference for the traditional medicines, the plant-derived compounds are considered a better alternative. Therefore, in the present study, alkaloids from *A. nazarum* and *A. elwesii* were extracted and tested for antibacterial activity against a wide range of bacteria including important pathogens. Study also aimed to determine the mode of their antibacterial action, either bactericidal or bacteriostatic and minimum inhibitory concentration (MIC) against these test

bacteria. Further, the efficacy of antibacterial activity of extracts was also compared with that of several common antibiotics such as rifampicin, kanamycin, streptomycin and spectinomycin. Therefore, as an integrated approach, we carried out the present investigation on the morpho-phenological and antibacterial characteristics of *Aconitum* species available in this part of Indo-Himalayan region. To the best of our knowledge this is the first study of its kind elucidating the potential clinical significance of *Aconitum* from this region.

## Materials and methods

### *Morpho-phenology*

The field survey was carried out during 2007-2010 on monthly basis. Generally, several specimens of each species were collected after making necessary observations on habit, habitat, and morphological characteristics. Specimen collection and herbarium preparation was done according to the method described by Jain and Rao (1958). Specimens were authenticated by comparing with the deposit at Central National Herbarium, Indian Botanic Garden, BSI Howrah, Kolkata, India, namely *Aconitum nagarum* Stapf, Sirohee: 19.9.1948, Mukerjee, 3515, and *Aconitum elwesii* Stapf., Sirohee: 19.9.1948, Mukerjee, 3518. Voucher specimens SYM-002201, *Aconitum nagarum*; SYM-002202, *Aconitum elwesii*, were also deposited at the herbarium of Department of Life Sciences, Manipur University, Imphal, Manipur, India. Morpho-phenological study was carried out according to the method described earlier (Chowdhury et al., 2006; Sharma, 2000).

### *Extraction of alkaloids*

*Aconitum* alkaloid was extracted by the method described earlier (Ohta et al., 1997). Dried leaves and roots were ground separately into fine powder using a mixer grinder. The fine powder was homogenized with 1M HCl in the ratio of 1:10 (w/v) in a mortar and pestle and filtered through double-layered cheesecloth. The filtrate was centrifuged at 12,500 g for 20 min at ambient temperature ( $26 \pm 2^\circ\text{C}$ ) and the supernatant was adjusted to pH 10 with ammonia solution (25%). The resulting suspension was extracted thrice with equal volumes of chloroform. The organic layer was pooled together and washed thoroughly with distilled water to remove ammonia. The organic phase was dried over anhydrous sodium sulfate to remove traces of water molecules and later evaporated with  $\text{N}_2$  gas.

### *Detection of alkaloid*

Mayer's reagent ( $\text{K}_2\text{HgI}_4$ ) was prepared by dissolving mercuric chloride (1.36 g) and potassium iodide (5 g) in 100 ml of milli Q water. It was used for the qualitative detection of alkaloid in the herbal extracts (Wangchuk, 2004). One ml of extract was transferred to a petri dish and Mayer's reagent was added. The presence of alkaloid

resulted in milky appearance due to the formation of alkaloid salt precipitate (Wangchuk, 2004).

### *Quantification of alkaloid using HPLC*

High performance liquid chromatography (HPLC) was performed for the quantification of alkaloids from different samples and compared with a standard curve prepared using aconitine (Sigma-Aldrich, Inc, St. Louis, Mo., U.S.A). The alkaloid residue was dissolved in acetonitrile and was analyzed using HPLC system (PU-980 HPLC pump, UV-975 UV/Vis detector and Rheodyne injector 7725, Jasco, Tokyo, Japan). Octa-decyl silane (ODS) Hypersil ( $250 \times 4.6$  mm internal diameter, Thermo Hypersil-Keystone, PA, USA) was used as the stationary phase. The alkaloid peaks were detected at 235 nm. Flow rate was 1 ml/min and the gradient system consisted of solvent A (0.2%, Trifluoroacetic acid) and solvent B (Tetrahydrofuran). A gradient elution of mobile phase, 20 to 40% solvent B in 20 min followed by decreasing gradient of 40 to 0% solvent B in 5 min, then re-equilibrating the column with 20% solvent B in 5 min was used for resolving the alkaloids in the extract. The alkaloid content (mg/g) was expressed in terms of aconitine equivalent.

### *Analysis of antibacterial activity*

Antibacterial activity of the alkaloid extracts was tested against different bacterial species including certain common human pathogens including *Staphylococcus aureus*, *Salmonella typhimurium* (MTCC 98), *Bordetella bronchiseptica* (NCIM 2267), *Escherichia coli* (MG 1655), *Bacillus subtilis* (NCIM 2063), *Pseudomonas putida* (NCIM 2847), *Pseudomonas fluorescense* (NCIM 2059) and *Xanthomonas campestris* using disc diffusion method (An et al., 2004). In brief, the test bacterium was grown overnight in 10 ml of Luria Bertani broth. The pathogenic bacteria including *S. aureus*, *S. typhimurium*, *B. bronchiseptica* and *E. coli* were incubated at  $37^\circ\text{C}$  and other microbes at  $28^\circ\text{C}$ . Cultures were diluted to  $\sim 10^5$  to  $10^6$  cfu / ml. A 100  $\mu\text{l}$  of this dilution was spread plated on a Luria Bertani agar plate and the plates were dried under laminar airflow for 20 min. The test was conducted using alkaloid extracts at four different concentrations (12.5, 25, 50 or 100  $\mu\text{g}$  / disc) by diluting from the stock of 10 mg/ml prepared in dimethylsulfoxide (DMSO). DMSO without any extract served as a control. Sterile paper discs (6 mm in dia.) were placed aseptically on the inoculated plate. A 20  $\mu\text{l}$  aliquot of the sample was transferred on the disc and the plates were later incubated overnight at appropriate incubation temperature as mentioned above. The diameter of the zone of inhibition was measured.

Ten different concentrations ranging from 0.001 to 50  $\mu\text{g}$ /disc of standard antibiotics, namely, rifampicin, streptomycin, kanamycin and spectinomycin were used to evaluate the efficacy of aconitum alkaloid for their antibacterial property against above mentioned microbes.

### Analysis of mode of antibacterial activity

To determine the possible mode of antibacterial activity, whether bactericidal or bacteriostatic, twenty random points from the zone of inhibition were re-spotted on a fresh Luria Bertani agar plate without having any antibiotics and observed for growth of any viable colony present, up to 72 h of incubation. No growth from the spot was indicative of bactericidal action, whereas, growth from the spot indicated bacteriostatic action. The experiment was repeated at least twice to confirm the observations.

### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined as described earlier (Deviene and Raddi, 2002). The overnight grown culture was appropriately diluted to obtain  $\sim 10^5 - 10^6$  cfu / ml. A 200  $\mu$ l of Luria-Bertani (LB) medium was incubated with 10  $\mu$ l of this diluted culture in 96-well microtitre plate and various concentrations of alkaloids prepared in DMSO (Dimethyl sulfoxide) were added to the wells, separately. DMSO alone was taken as control. The pathogenic bacteria namely *S. aureus*, *S. typhimurium*, *B. bronchiseptica* and *E. coli* were incubated at 37°C for and other test bacteria at 28°C. Absorbance at 600 nm was measured at different time intervals up to 72 h of incubations using ELISA reader.

## Results

Morphological Characteristics of *Aconitum* spp. in Ukhrul District, Manipur, India

### *Aconitum nagarum* Stapf.

The morphological and inflorescence characters of *Aconitum nagarum* are presented in Fig.1 I, 2 a and b. Roots: paired, tuberous with a length of 1.7-2.5 cm; penetrate up to a depth of 14-20 cm below the soil surface. Stem: erect, with diameter up to 1 cm at the base, reaches up to 1.4 m in height, simple, green in colour, terete, finely pubescent with adpressed reversed hairs. The basal leaves soon-withering, upper scattered and few, lowest petioles up to 49 cm long. They are winged, sulcate and amplexicauline. Lamina: cordate-reniform, 3-partite to the base, glabrous with dimension of 6.5-23  $\times$  4.5-15.5 cm, with 4.5-10 cm deep sinus and 3-lobed, lateral lobes unequally two lobuled: lobules acute, 1-2 dentate, sharply and coarsely dentate. Flowers: terminal and axillary over 20-140 cm long racemes; pedicels up to 1.5 - 5 cm long, erect or sub erect, bracts foliaceous. 3 partite; sepals blue, upper sepal helmet shaped, 0.5 - 2.8 cm long semi orbicular, lateral sepal 0.4-1.5 cm long, sub orbicular, oblique lower sepals deflexed, 1.1 cm long elliptic sub acute: clawed 1.4 cm long, very slender; carpels 5, oblong, pubescent. Follicles oblong, divergent towards the apex, truncate minutely pubescent.

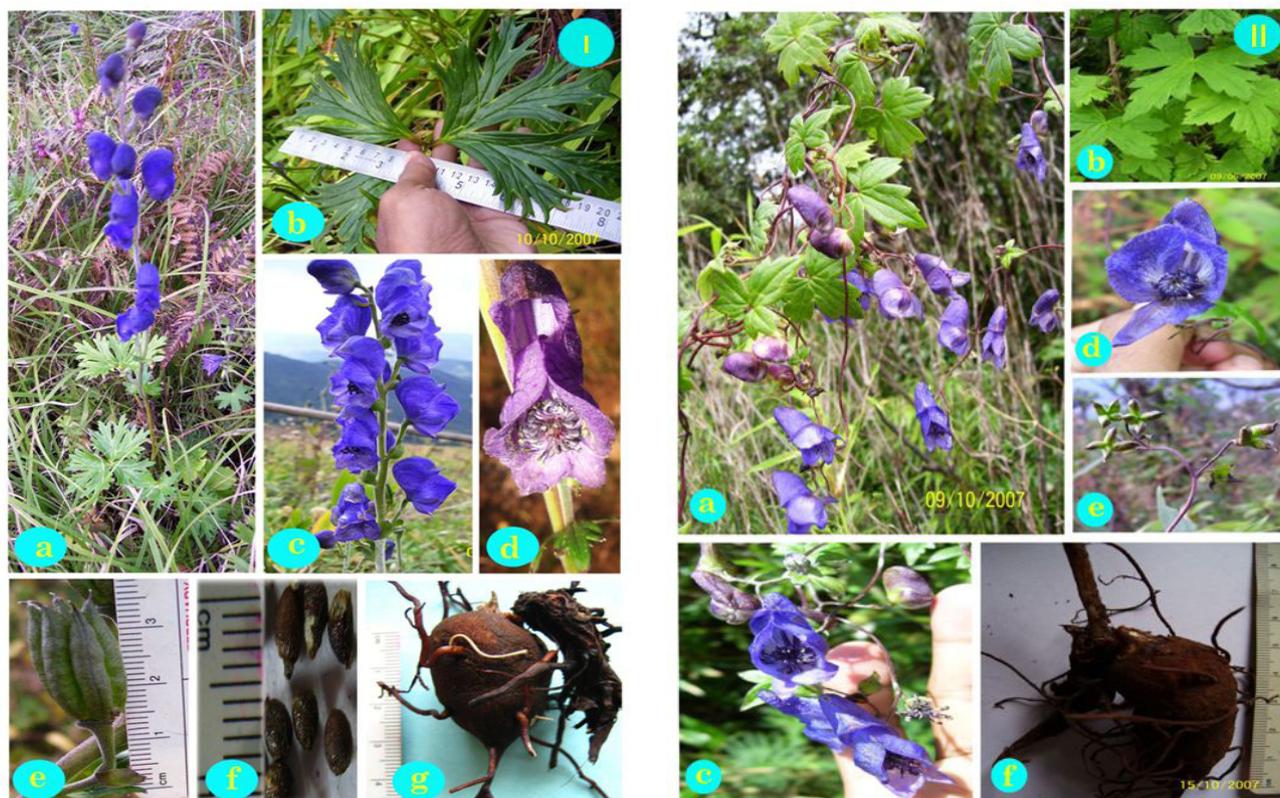


Fig. 1. I. Morphological features of *Aconitum nagarum*, a: Habit, b: Leaf, c: Inflorescence, d: Single flower, e: Fruit, f: Seed, g: Root; II. Morphological features of *Aconitum elwesii*, a: Habit, b: Leaf, c: Inflorescence, d: Single flower, e: Fruit, f: Root.

Seeds: transversely lamellate. Flowering and fruiting occurs between September to December.

#### *Aconitum elwesii* Stapf.

The morphological and inflorescence characters of *Aconitum elwesii* are presented in Fig.1 II, 2 a and b. Roots paired tuberous with a length of 1.9-4.3 cm; they penetrate up to a depth of 16-25 cm below the soil surface. Stem scrambling, very long, sparingly branched scabrous or puberulous with diameter up to 0.17-0.3 cm at the base, reached up to 2 m high, simple, green in colour, terete, finely pubescent with adpressed reversed hairs. Leaves cauline, scattered petioled with a length of 3.2-6 cm, pubescent; lamina ovate-cordate or rotund with wide sinus, palmately 3-5 partite; lobes cuneate-ovate, finely toothed. Flowers few to many blue or violet in axillary and terminal racemes or or sub-paniculated, over 3-8 cm long; pedicels recurved up to 1.5-2 cm long. Sepals blue or violet, sparingly hairy; uppermost sepal narrowly helmet-shaped, arched, 0.5-2.8 cm, beaked; upper lateral sepals 0.7-1.1 cm long; lower lateral sepals 0.5-1.2 cm; spur reflexed; petals glabrous. Follicles 5, glabrous; testa of seeds plaited. Flowering and fruiting occurs between August to November.

#### Phenology of *Aconitum* spp.

The sprouting of root stock and germination of seeds in both the species starts during the month of March and continue their vigorous growth in the later part of the year. Six phenological stages are noted and the same are diagrammatically represented by the lines radiating from different angles of a hexagonal figure for each month in regard to individual species (Tab. 1). Both the species be-

ing perennial, the plants in matured phase generally have one mother root with one to two daughter roots to be the successor of the mother root for the upcoming generation. Tab. 1 displays the phenophases of both the *Aconitum* species. In both the species, sprouting of the daughter root and seeds starts by March and up to July is the vegetative phase. The plants start flowering from the first week of August and September for *A. elwesii* and *A. nagarum*, respectively, flowering starts a month earlier in *A. elwesii*. With onset of second week of September and November fruiting starts in *A. elwesii* and *A. nagarum*, respectively, and last up to November in *A. elwesii*, whereas, in *A. nagarum* some of the flowers are still in bloom, with initiation of fruiting phase. Plants of both the species get matured by the month of January and December and subsequently the areal parts of the plants become dry by January and last up to February.

The connection of the daughter tuber with the mother plant is maintained by a usually very short neck through which the food material wanders from the stem into the new tuber and gets deposited there mainly as reserve starch and alkaloids. Therefore, it is these daughter tubers which are principally collected, and they should, of course, not be taken up before they have attained maturity.

#### Alkaloid detection and quantification

In a qualitative assay, the alkaloid extracts from *A. nagarum* and *A. elwesii* turned milky upon addition of Mayer's reagent which confirmed the presence of alkaloid in the samples.

The alkaloid content was determined using HPLC and expressed in terms of aconitine equivalent (AE) mg/g.

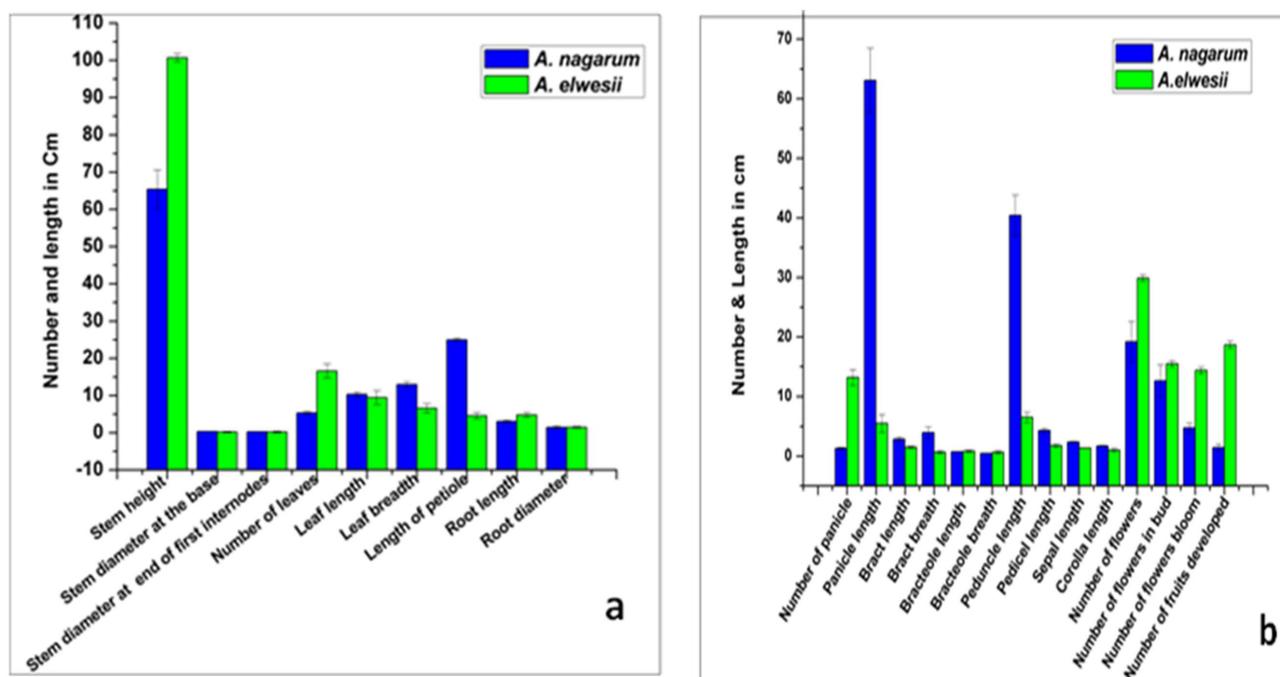


Fig. 2 a. Morphological characteristics of *A. nagarum* and *A. elwesii*; b. Characteristics of *A. nagarum* and *A. elwesii* inflorescence

Tab. 1. Phenogram of *Aconitum* spp.

Month	Species	
	<i>A. nagarum</i>	<i>A. elwesii</i>
January		
February		
March		
April		
May		
June		
July		
August		
September		
October		
November		
December		

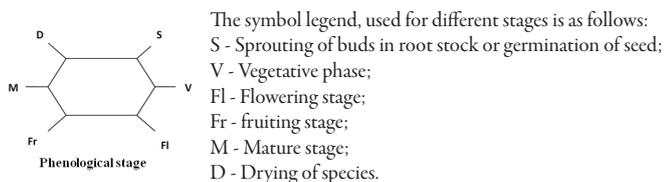


Fig. 3 displays the HPLC profile of standard aconitine as well as alkaloid contents including aconitine in *A. nagarum* root and *A. elwesii* root. The standard aconitine peak was observed at a retention time of 10 min. The alkaloid content in *A. nagarum* root and leaves were 4.3 and 5 AE mg/g, while in *A. elwesii* root and leaf it was 4.7 and 4.8 AE mg/g, respectively.

*Antibacterial activities of alkaloids and possible mode of activity*

The antibacterial activity of the alkaloid extract was tested against different bacteria including certain human pathogens, namely, *S. aureus*, *S. typhimurium*, *B. bronchiseptica* and *E. coli* (Tab. 2). The efficacy of antibacterial potency of aconitum alkaloid was evaluated by comparing with the antibacterial activity displayed by certain standard antibiotics, namely, rifampicin, kanamycin, streptomycin and spectinomycin against some of these test organisms (Tab. 3). For a comparative characterization, the antibacterial activity was categorized as weak, moderate and strong depending upon zones of inhibition (weak: 7-8 mm, moderate: 9-11 mm and strong:  $\geq 12$  mm). Alkaloid extract from *A. nagarum* root showed moderate to strong level of bacterial inhibition against *S. aureus* *B. bronchisept-*

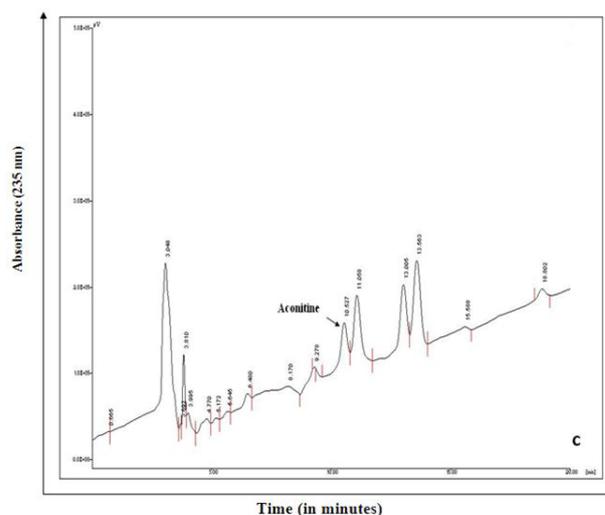
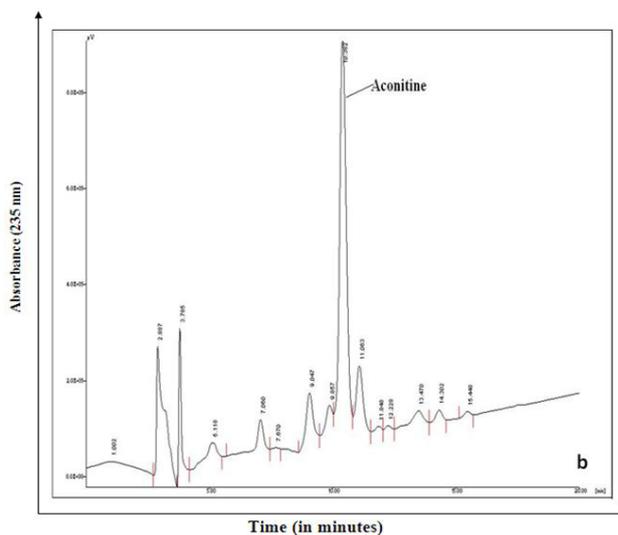
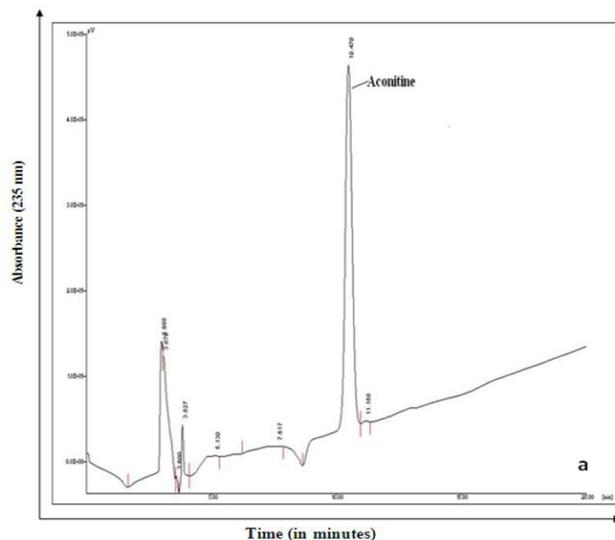


Fig. 3. HPLC profile of a. standard aconitine; b. *A. nagarum* root; c. *A. elwesii* root

Tab. 2. Antibacterial activity of alkaloid extract from *Aconitum* spp.

Source of alkaloid	Alkaloid concentration (µg/disc)	S.a <sup>a</sup>	S.t <sup>b</sup>	B.b <sup>c</sup>	E.c <sup>d</sup>	B.s <sup>e</sup>	P. p <sup>f</sup>	P. f <sup>g</sup>	X.c <sup>h</sup>
		Zone of inhibition (mm)							
<i>Aconitum nagarum</i> (root)	12.5	-	-	8	-	8	8	-	7
	25	-	-	8	-	10	9	-	8
	50	11	-	9	-	11	9	-	11
	100	12	7	10	7	11	10	7	12
<i>Aconitum nagarum</i> (leaf)	12.5	-	-	7	-	8	-	-	7
	25	-	7	7	-	8	-	-	8
	50	-	7	8	-	9	7	-	8
	100	7	8	10	7	10	8	8	9
<i>Aconitum elwesii</i> (root)	12.5	-	-	7	-	8	-	-	8
	25	-	-	8	-	9	-	-	9
	50	-	7	9	7	9	-	-	10
	100	-	8	10	7	10	-	7	11
<i>Aconitum elwesii</i> (leaf)	12.5	-	-	8	-	9	-	-	9
	25	-	-	8	-	10	-	-	10
	50	8	-	10	-	11	-	7	11
	100	9	-	12	-	11	7	8	12

<sup>a</sup>*Staphylococcus aureus*; <sup>b</sup>*Salmonella typhimurium*; <sup>c</sup>*Bordetella bronchiseptica*; <sup>d</sup>*Escherichia coli*; <sup>e</sup>*Bacillus subtilis*; <sup>f</sup>*Pseudomonas putida*; <sup>g</sup>*Pseudomonas fluorescense*; <sup>h</sup>*Xanthomonas campestris*; -: No activity

Tab. 3. Analysis of antibacterial activity of standard antibiotics against different bacteria including pathogens

Antibiotics	Antibiotics concentration (µg/disc)	S.a <sup>a</sup>	S.t <sup>b</sup>	B.b <sup>c</sup>	E.c <sup>d</sup>	B.s <sup>e</sup>
		Zone of inhibition (mm)				
Rifampicin	0.001	13	-	10	-	7
	0.01	16	-	12	-	10
	0.05	20	-	14	-	12
Kanamycin	0.1	8	-	7	-	9
	1	13	7	20	10	22
Streptomycin	0.1	7	-	-	-	9
	1	12	-	12	10	22
	5	14	7	24	17	30
Spectinomycin	5	8	7	10	12	8
	10	9	8	12	15	11
	20	11	9	18	19	14

<sup>a</sup>*Staphylococcus aureus*; <sup>b</sup>*Salmonella typhimurium*; <sup>c</sup>*Bordetella bronchiseptica*; <sup>d</sup>*Escherichia coli*; <sup>e</sup>*Bacillus subtilis*; -: No activity

*tica*, *B. subtilis*, *P. putida* and *X. campestris* at the higher concentration of 100 µg/disc. The inhibition was found to be weak in case of *S. typhimurium*, *E. coli* and *P. fluorescense* at the same concentration. Antibacterial activity of alkaloid (100 µg/disc) from *A. nagarum* root was found to be quite close to the activity displayed by 0.001 µg/disc of rifampicin, 1 µg/disc of kanamycin and streptomycin and 10 µg/disc of spectinomycin (Tab. 2 and 3).

Alkaloid from *A. nagarum* leaf was found to have comparatively less antibacterial activity than that of alkaloid from *A. nagarum* root. A weak to moderate level of bacterial inhibition was observed at the higher concentration (100 µg/disc). Interestingly, *S. aureus*, *P. putida* and *X. campestris* were found to be less sensitive to alkaloid extract from leaf, however, the sensitivity of other tested bacteria was almost similar to that of root. Antibacterial activity of

alkaloid from *A. nagarum* leaf (100 µg/disc) was closer to activity displayed by 0.001 µg/disc of rifampicin, 0.1 µg/disc of kanamycin, 0.1 to 1 µg/disc of streptomycin and 5 µg/disc of spectinomycin (Tab. 2 and 3).

Alkaloid extract from *A. elwesii* root showed weak to moderate inhibition to *B. bronchiseptica*, *B. subtilis* and *X. campestris* at the concentration of 100 µg/disc. It showed relatively weak inhibition to *S. typhimurium*, *E. coli* and *P. fluorescense* even at the higher concentration of 100 µg/disc. No inhibition was observed for *S. aureus* and *P. putida*. Alkaloid extract from *A. elwesii* leaf showed higher antibacterial activity as compared to *A. elwesii* root. The extent of antibacterial activity was moderate to strong against *B. bronchiseptica*, *B. subtilis* and *X. campestris* at the higher concentration of 100 µg/disc. Level of inhibition was weak to moderate at same concentration for *P. fluores-*

Tab. 4. Mode of antibacterial action of *Aconitum* alkaloid extracts

Source of alkaloid	S.a <sup>a</sup>	S.t <sup>b</sup>	B.b <sup>c</sup>	E.c <sup>d</sup>	B.s <sup>e</sup>	P. p <sup>f</sup>	P. f <sup>g</sup>	X.c <sup>h</sup>
	Mode of antibacterial action							
<i>Aconitum nagarum</i> (root)	C <sup>i</sup>	S <sup>i</sup>	C	S	C	S	S	S
<i>Aconitum nagarum</i> (leaf)	C	S	C	S	C	S	S	S
<i>Aconitum elwesii</i> (root)	-	S	C	S	C	-	S	S
<i>Aconitum elwesii</i> (leaf)	C	-	C	-	C	S	S	S

<sup>a</sup> *Staphylococcus aureus*; <sup>b</sup> *Salmonella typhimurium*; <sup>c</sup> *Bordetella bronchiseptica*; <sup>d</sup> *Escherichia coli*; <sup>e</sup> *Bacillus subtilis*; <sup>f</sup> *Pseudomonas putida*; <sup>g</sup> *Pseudomonas fluorescence*; <sup>h</sup> *Xanthomonas campestris*; <sup>i</sup> bactericidal; <sup>j</sup> bacteriostatic; -: No activity

Tab. 5. MIC value of alkaloid extract from *Aconitum* spp. against different bacteria

Source of alkaloid	S.a <sup>a</sup>	S.t <sup>b</sup>	B.b <sup>c</sup>	E.c <sup>d</sup>	B.s <sup>e</sup>	P. p <sup>f</sup>	P. f <sup>g</sup>
	MIC value ( $\mu\text{g/ml}$ )						
<i>Aconitum nagarum</i> (root)	125	500	62.5	500	62.5	62.5	500
<i>Aconitum nagarum</i> (leaf)	250	500	62.5	500	62.5	188	500
<i>Aconitum elwesii</i> (root)	-	500	62.5	250	62.5	-	500
<i>Aconitum elwesii</i> (leaf)	94	-	31	-	31	375	250

<sup>a</sup> *Staphylococcus aureus*; <sup>b</sup> *Salmonella typhimurium*; <sup>c</sup> *Bordetella bronchiseptica*; <sup>d</sup> *Escherichia coli*; <sup>e</sup> *Bacillus subtilis*; <sup>f</sup> *Pseudomonas putida*; <sup>g</sup> *Pseudomonas fluorescence*; -: No activity

*cence* and *P. putida*. There was no inhibition observed for *S. typhimurium* and *E. coli* (Tab. 2 and 3).

Antibacterial activity displayed by 100  $\mu\text{g}$ /disc alkaloid from *A. elwesii* root and leaf was quite similar to that displayed by 0.001  $\mu\text{g}$ /disc of rifampicin, 0.1 to 1  $\mu\text{g}$ /disc of kanamycin, 1  $\mu\text{g}$ /disc of streptomycin and 10  $\mu\text{g}$ /disc of spectinomycin (Tab. 2 and 3).

Among the bacteria tested for sensitivity against different antibiotics, *B. bronchiseptica* was found to be significantly sensitive to kanamycin, streptomycin and spectinomycin, whereas, *B. subtilis* was observed to be more sensitive to kanamycin and streptomycin. *E. coli* was found to be more sensitive to streptomycin and spectinomycin. *S. aureus* was found to be more sensitive to rifampicin (Tab. 3).

The mode of antibacterial activity (whether bactericidal or bacteriostatic) was also determined for these alkaloid extracts against different bacteria including pathogens (Tab. 4). The alkaloid extracts showed bactericidal effect against *S. aureus*, *B. bronchiseptica* and *B. subtilis*, whereas, for other bacterial species the effect was bacteriostatic even at 100  $\mu\text{g}$ /disc concentration.

#### Minimum inhibitory concentration (MIC)

A wide variation in the MIC was observed for the bacterial species studied (Tab. 5). The antibacterial potency of alkaloid from *Aconitum* spp. was categorized as high, where MIC was less than 100; medium, where MIC was 100-250; and low, where MIC was more than 250. The antibacterial potency of all the alkaloid extracts was found to be high against *B. bronchiseptica* and *B. subtilis* while low for *S. typhimurium*, *E. coli* and *P. fluorescence*. For *S. aureus*, the alkaloid extract from *A. elwesii* leaf showed high potency, whereas, other samples showed medium potency. In case of *P. putida*, alkaloid extract from *A. nagarum* root

showed high potency and other alkaloid samples showed low to medium potency.

#### Discussion

*Aconitum* in this part of Indo-Himalayan region is yet to explore fully, researchers like Deb (1961) and Sinha (1996) reported *Aconitum* under the family *Helleboraceae*, however in recent study, Singh *et al.* (2002) reported the plant under the family *Ranunculaceae*, which is accepted worldwide. Singh *et al.* (2002) reported *Aconitum nagarum* and *Aconitum elwesii* from Manipur and mentioned the flowering and fruiting period to be July-October and July-September for *A. nagarum* and *A. elwesii*, respectively. However, in the present study after a detailed investigation and observing the phenophases, it was found that flowering and fruiting period is September-December, for *A. nagarum* and August-November for *A. elwesii* respectively, with a slight variation in the period from what Singh has reported. However, Rao (1993) reported the plant from Manipur and mentioned the flowering and fruiting period as April-May for *A. nagarum* and May-September for *A. elwesii* this might be misapplied, as April-May being the vegetative stage for *A. nagarum*, as shown in the phenogram Tab. 5.

The alkaloid content of (4-5 AE mg/g) reported in the present study is in concurrence to what Giri *et al.* (1997) reported in *A. heterophyllum* root. The overall antibacterial activity of the alkaloid extracts from *A. nagarum* root and *A. elwesii* leaf were found to be high and quite similar. The extent of activity was found to be less in the alkaloid from *A. nagarum* leaf and *A. elwesii* root. The pattern observed for alkaloid from *A. nagarum* was opposite to that of *A. elwesii* due to higher alkaloid content from *A. elwesii* leaf than *A. elwesii* root. Therefore, data showed more lo-

calization of bioactive alkaloids in root of *A. nagarum* and leaf of *A. elwesii*. Previously, root was reported as a major site of alkaloid accumulation in *Aconitum* species (Pandey et al., 2005), however in the current study, leaf of an *Aconitum* species was found to contain potent bioactive alkaloid. The antibacterial activity is known to vary significantly among different plant parts (Al-Bayati and AL-Mola, 2008). Interestingly, the Gram-negative bacteria, *S. typhimurium*, *E. coli* and *P. fluorescens* were found to be comparatively less susceptible to the most of the alkaloid extracts tested than the Gram-positive bacteria, *S. aureus* and *B. subtilis*. Similarly, total alkaloids extract from *Mitragyna inermis* have been found to possess comparatively higher antibacterial activity against Gram-positive bacteria than that of Gram-negative bacteria (Zongo et al., 2009). A higher resistance in the Gram-negative bacterial species may be due to the presence of outer membrane, which acts as barrier to the penetration of several antibiotics and the enzymes present in the periplasmic space, which degrades exogenous molecules (Angenot et al., 1991; Tanaka et al., 2006). However, sensitivity of some of the tested Gram-negative bacteria to the alkaloid extracts indicated the wide spectrum of antibacterial activity for these alkaloids. Some alkaloids from *Aconitum* species are known to cause toxic effects at higher concentrations (Bisset, 1981). Similarly, alkaloids from other plants are also reported to be toxic to vertebrates and insects, and can also inhibit the growth of bacteria and plant seedlings (Wink and Latz-Brüning, 1998). Moreover, the presence of such broader activity spectrum alkaloids in plants may be meant for their multi-purpose defense (Wink and Latz-Brüning, 1998). Though the mechanism for antibacterial activity of alkaloids is not yet known. Recent study has shown that alkaloids could inhibit DNA synthesis through topoisomerase inhibition and DNA intercalation (Karoul et al., 2005). Among the human pathogen tested all showed bactericidal in nature except *S. typhimurium*. A study conducted with alkaloid extract from bark of *Holarrhena pubescens* showed highest bactericidal activity against *S. aureus*, followed by *P. aeruginosa*, *E. coli* and *B. subtilis* (Chakraborty and Brantner, 1999). The MIC ranged between 31-500 and is found to be in accordance with the antibacterial activity and differential distribution of bioactive alkaloid noticed in *Aconitum* species. Chakraborty and Brantner, (1999) reported the MIC value of alkaloid extract from bark of *Holarrhena pubescens* as 95, 600, 550 and 420 µg/ml for *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, respectively and these values are quite close to our observation.

## Conclusion

From the present study we conclude that in the spring, vigorous growth takes place and the parent tuber sends out (the future flowering) stem and leave. At the same time, the daughter tuber starts to develop, and it continues to grow throughout the summer and autumn. Towards the

end of the year, when the seeds have ripened, the parent tuber dies. As a result, the daughter tuber becomes independent and in the following spring takes over the function of the parent tuber. Further, our study established the antibacterial property of alkaloid extracts of *A. nagarum* and *A. elwesii* against different bacteria including human pathogens, namely, *Bordetella bronchiseptica* and *Staphylococcus aureus*. Present work also shows the species specific differential localization of bioactive compounds in different plant parts, which could be favourable for the plant to survive from the predators. Moreover, the *Aconitum* alkaloids provide an alternate ethnomedicine having wider spectrum of antibacterial activity against different human pathogens. These alkaloids may also provide clues to find out the mechanism of inhibitory action against different bacteria and to synthesize new potential variants.

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## References

- Al-Bayati FA, AL-Mola HF (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. J Zhejiang Univ Sci 9:154-159.
- An BJ, Son JH, Kwak JH, Park JM, Lee JY, Jo C, Byun MW (2004). Biological and antimicrobial activity of irradiated green tea polyphenols. Food Chemistry 88:447-451.
- Angenot L, Quentin-Leclercq J, Phillipson DJ, Warhurst DC, O'Neill MJ, Bray DH, Wright CW (1991). Antiamoebic and antiplasmodial activities of alkaloids isolated from *Strychnos usambarensis*. Planta Medica 57:337-340.
- Bisset NG (1981). Arrow poisons in China. Part II. *Aconitum*-Botany, Chemistry, and Pharmacology. J Ethnopharmacol 4:247-336.
- Brink DE, Woods JA (1997). *Aconitum*, 191-195. In: Flora of North America Editorial Committee (Ed.). Flora of North America North of Mexico. Oxford University Press, New York.
- Chakraborty A, Brantner AH (1999). Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. J Ethnopharmacol 68:339-344.
- Champion HG, Seth SK (1968). The revised forest types of India. Government of India, New Delhi.
- Chowdhury D, Bolin KK (2006). Morphophenology and karyotype study of Patidoi (*Schumanianthus dichotomus* (Roxb.) Gagnep. Synonym *Clinogyne dichotoma* Salisb.) a traditional plant of Assam. Current Sci 91:648-651.
- Deb DB (1961). Dicotyledonous plants of Manipur territory. Bull Bot Surv India 3:115-138.
- Deshmukh SD, Borle MN (1976). Studies on the insecticidal properties of indigenous plant products. Ind J Entomol

- 37:11-18.
- Devienne KF, Raddi MS (2002). Screening for antibacterial activity of natural products using a microplate photometer. *Braz J Microbiol* 33:166-168.
- Giri A, Banerjee S, Ahuja PS, Giri CC (1997). Production of hairy roots in *Aconitum heterophyllum* Wall. using *Agrobacterium rhizogenes*. *In Vitro Cell Develop Biol Plant* 33:280-284.
- Jain SK, Rao RR (1958). A handbook of field and herbarium methods. Today and Tomorrow's Publishers New Delhi
- Karoul D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Colizzi V, Traore AS (2005). Antibacterial activity of alkaloids from *Sida acuta*. *Afr J Biotechnol* 4:1452-1457.
- Mori T, Oshawa T, Murayama M (1989). Studies on *Aconitum* species. *Components of 'Kakobushi-matsu'*. *Heterocycles* 2:873-884.
- Ohta H, Seto Y, Tsunoda N (1997). Determination of aconitum alkaloids in blood and urine Samples. I. High-performance liquid chromatographic separation, solid-phase extraction and mass spectrometric confirmation. *J Chromatography B* 691:351-356.
- Pandey S, Kushwaha R, Prakash O, Bhattacharya A, Ahuja PS (2005). *Ex situ* conservation of *Aconitum heterophyllum* Wall.- an endangered medicinal plants of the Himalaya through mass propagation and its effect on growth and alkaloid content. *Plant Genetic Res* 3:127-135.
- Pradhan BK, Badola HK (2008). Ethnomedicinal plant use by Lepcha tribe Dzongu valley, bordering Khangchendzonga biosphere reserve, in northern Sikkim, India. *J Ethnobiol Ethnomed* 4:4-22.
- Rao MA (1993). Botanical Survey of India. In: Sharma et al. Fl. India, 1:1-23.
- Samy RP, Gopalakrishnakone P (2008). Therapeutic potential of plants as anti-microbial for drug discovery. *eCAM* 1-12.
- Sharma PD (2000). Ecology and Environment. Rastogi Publications. Shivaji Road, Meerut, India 179-183 p.
- Sharma BD, Balakrishnan NP, Rao RR, Hajra PK (1993). Flora of India. BSI, Calcutta.
- Singh NP, Chauhan AS, Mondal MS (2002). Flora of Manipur 1. BSI, Calcutta.
- Sinha SC (1996). Medicinal plants of Manipur, Mass and Sinha, Publication, Imphal, Manipur.
- Smith AE, Secoy DM (1981). Plants used for agricultural pests control in Western Europe before 1850. *Chemistry and Industry* 12-17 p.
- Tanaka JC, da Silva AC, de Oliveira AJ, Nakamura CV, Filho BP (2006). Antibacterial activity of indole alkaloid from *Aspidosperma ramiflorum*. *Braz J Med Biol Res* 39:387-391.
- Wangchuk P (2004). Bioactive alkaloids from Medicinal Plants of Bhutan. Th. MSc of Science University of Wollongong, Australia.
- Wink M, Schmeller T, Latz-Brüning B (1998). Modes of action of allelochemical alkaloids: interaction with neuroreceptors, DNA, and other molecular targets. *J Chemical Ecol* 24:1881-1937.
- Xiao PG, Wang FP, Gao F, Yan LP, Chen DL, Liu Y (2006). A pharmacophylogenetic study of *Aconitum* L. (*Ranunculaceae*) from China. *Acta Phytotax Sinica* 44:1-46.
- Zongo CE, Akomo O, Savadogo A, Obamc LC, Koudou Traore JA (2009). *In vitro* antibacterial properties of total alkaloid from *Mitragyna inermis* (Willd.) O. Kuntze, a West African traditional medicinal plant. *Asian J Plant Sci* 8:172-177.