Pharmacognosy of aconite sold under the name *Vatsanabha* in Indian market

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Aconite is one of the lethal poisons used in therapeutics of Ayurveda. It is sold under the name of *Vatsanabha* in Indian market. The aconite is generally found as mixture of different species of *Aconitum*, due to the close morphological similarity of the different species of aconites. The aims of the study were to identify different species of *Aconitum* present in the market samples of *Vatsanabha* and to find out the authentic species of *Vatsanabha*, i.e. *Aconitum chasmanthum* Stapf. ex Holmes in the market sample. *Vatsanabha* was sold from Indian markets and was identified by means of macroscopic and microscopic studies and by TLC profile. It was found that ‘*Vatsanabha*’ of Indian market is mixture of 8 species of *Aconitum*, 6 of these were identified as *A. spicatum* Stapf., *A. falconeri* Stapf., *A. chasmanthum* Stapf. ex Holmes, *A. laciniatum* Stapf., *A. balfourii* Holmes ex Stapf. and *A. deinorrhizum* Holmes ex Stapf. *A. chasmanthum* Stapf. ex Holmes species is rarely available in market samples.

**Keywords:** Aconite, *Vatsanabha*, Macroscopy, Microscopy, Isolation, TLC

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Aconite has long been considered as one of the most poisonous plants, but its use as medicine in Ayurvedic system of medicine since time immemorial after proper treatment called *Shodhana*. They are used in cases of arthritis, inflammation, heart disease, fever, neuralgia, externally in the form of liniment or ointment to reduce pain and inflammation in muscles and joints. In Tibetan medicine, where aconite is considered an important herb, is referred as the king of medicine. Aconite is a fast acting poison, having a very small dose causes numbness of lips, tongue followed by vomiting, even death also reported by taking aconite containing medicines.

Different species of aconites have widely been used for medicinal purposes in various parts of the world and 28 plant species have been reported to grow in India at higher altitude of 3000 to 4000 ft in the Himalayas, out of which the existence of few species like *Aconitum balfourii* Holmes ex Stapf., *A. bisma* Rap., *A. chasmanthum* Stapf. ex Holmes, *A. deinorrhizum* Holmes ex Stapf., *A. elewesii* Stapf., *A. ferox* Wall ex Secinge, *A. falconeri* Stapf., *A. heterophyllum* Wall ex Royle, *A. leave* Royle, *A. lethale* Stapf., *A. laciniatum* Stapf., *A. luriderm* Munz., *A. moschatum* Stapf., *A. palmatum* Rap., *A. spicatum* Stapf. and *A. violaceum* Jack. have been reported. Most of which contain the therapeutically active alkaloids. *A. chasmanthum* and *A. ferox* have been found to be respectively seven and three times more potent than the European variety, *A. napellus* and hence the Indian varieties have a great demand in the world markets. Unfortunately, the drug has been subjected to extensive adulteration with less active species. *A. chasmanthum* Stapf., which is the richest in potent alkaloidal content, and which grows plentifully in wild state in Kashmir, Lahul and Assam, has been included in IPL, IPC and IP, and also included in Schedule E(1) of the Drugs and Cosmetics Act, 1940 as poisonous plant. No standard procedure of assay of the crude drug has been given in official monographs.

Some species of aconite sold under the name of *Vatsanabha* or *Visha* in the Indian market, have been studied by Dutta, Dutta and Mukerji, and Mehra and Puri. IPL and IP give short description of the roots of *A. chasmanthum*. Review of Indian Medicinal Plants, published by ICMR has compiled the previous works on aconite species. Due to the close morphological similarity of the different species of aconites, the pharmacognostic study of the drug has
been considered to be important and in the present study raw aconite, which was sold under the name ‘Vatsanabha’, was identified on the basis of macroscopic and microscopic studies by using latest testing equipments and techniques.

**Methodology**

For the present study, aconites were procured from various markets all over India like from Kolkata market, West Bengal, Nasik market, Maharashtra, Mangalore market, Karnataka, Sakut market, Rajasthan and Jamnagar market, Gujarat, Pharmacy, Gujarat Ayurved University, Jamnagar, Gujarat, Pharmacy, SVSP Hospital, Kolkata, West Bengal and from Yogindernagar District, Himachal Pradesh.

Identification of the collected root tubers was done by studying their macroscopic and microscopic characters and histology of roots and comparing them with the characters mentioned in various Floras, Texts and published articles. Help was also taken from eminent Botanists of Gujarat Ayurved University, Jamnagar, and National Botanical Research Institute (NBRI), Lucknow, for confirming their further identity. Voucher specimens have been deposited in the department of Rasashastra and Bhaishajya Kalpana (NBRI), Lucknow, for confirming their further identity.

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For macroscopic observations few pieces of dried roots were kept as such, while for microscopic observation few pieces of root were preserved in the solution of 50 % ethyl alcohol, glacial acetic acid and formaldehyde in the proportion of 90 : 5 : 5, respectively.  

Forty mesh powder was prepared from the remaining root pieces and stored well in air tight glass bottle for microscopic investigation.

Organoletic features of the root tubers were noted, taste was perceived carefully by taking very minute quantity of powder because aconites are lethal poison. Length and diameter at crown and middle was measured. Fractures were observed by breaking the root at the middle.

Anatomical characters of the root tubers were studied by preparing various slides under the microscope at different magnifications. Preserved samples in alcohol, acetic acid and formalin solution were taken and free hand sections were cut and taken on glass slides. The section were cleared with chloral hydrate by heating and then stained with phloroglucinol and hydrochloric acid. For the detection of starch grains the transverse sections were stained with iodine solution.

The forty mesh powder of aconite roots was spread on glass slides and observed under microscope at different magnifications. For the detection of lignified tissues (stone cell, sclereids, xylem vessel, etc.) the powder was stained with phloroglucinol and hydrochloric acid and to observe the starch grains the powder was stained with iodine solution.

Isolation of tissues is done by Schultz’s maceration process. Some small fragments of the root were taken in a test tube and 5 mL of 50 % nitric acid (HNO₃) was added, then the test tube was heated till the pieces become very soft. The tube was removed from the flame and pinch by pinch the powder of potassium chlorate (KClO₃) was added in the hot HNO₃ solution till the effervescence was subsided. Gently the soften pieces of the root were removed in another test tube and was thoroughly washed with fresh water and then was shaken vigorously by adding few drops of water to get all the separated lignified elements. The elements were allowed to settle down and taken on glass slides and observed under microscope at different magnifications.

Accurately weighed 5 gm of drug powder was exhausted with 75 % 50 mL of methanol. After removing much of methanol at a low temperature, the remaining was treated with 30 mL dil. sulphuric acid and then washed with petroleum ether. The acid portion containing the alkaloids fraction was made alkaline (pH 9) with ammonia solution. It was washed thrice with ether (20 mL in each time) to extract the alkaloids in ether in a separating flux. The total alkaloid was obtained by evaporating ether at a low temperature.

The total alkaloids obtained were dissolved in 5 mL diethyl ether; and was used as test solution for TLC identity test. The aluminium TLC plates precoated with Silica Gel 60F₂₅₄ (E. Merck) (10 cm × 10 cm) was used as stationary phase. The mobile phase used for developing the plate was Toluene: Ethyl acetate: Diethyl amine (7 : 2 : 1). Ascending mode was used for development of thin layer chromatography. TLC plate was developed up to 7.5 cm. The plate was scanned under UV light at 254 nm and 366 nm and the fingerprint profiles were recorded. After that the plate was sprayed with Dragendorff’s reagent, heated at 50°C and brought to room temperature for spot development. The Rf values and colours of the resolved bands were noted.

**Results**

The results revealed that the procured aconite under the name of Vatsanabha was mixture of 8 different
samples (Fig. 1). Roots of sample 1 were 7.5 to 9.5 cm in length and 1.2 to 1.5 cm in thickness, plenty of stone cells present outside the endodermis, cambium was continuous and stellate, starch grains were numerous in number; simple or compound and vessel elements were reticulately thickened or simple pitted.

Root tubers of sample 2 were 6.5 to 7.5 cm in length and 1.5 to 2.0 cm in thickness, plenty of stone cells present both outside and inside of the endodermis, cambium was continuous and stellate, starch grains were numerous in number; simple or compound and vessel elements were reticulately thickened or simple pitted.

Root tubers of sample 3 were much shorter with 3.0 to 4.5 cm in length and 0.6 to 1.0 cm in thickness, stone cells were absent in this species, cambium was continuous and stellate, starch grains were numerous in number; simple or compound and vessel elements were reticulately thickened or simple pitted.

Root tubers of sample 4 were short; 3.5 to 4.5 cm in length and 1.0 to 1.2 cm in thickness, sparsely distributed; isolated stone cells were present outside the endodermis, cambium was continuous and stellate, starch grains were numerous in number; and mostly simple and vessel elements were reticulately thickened or simple pitted.

Root tubers of sample 5 were 5.5 to 8.0 cm in length and 1.2 to 1.5 cm in thickness, plenty of stone cells present outside the endodermis, cambium was discontinuous and arranged in a circular fashion, starch grains were numerous in number; and mostly simple and vessel elements were reticulately thickened or simple pitted.

Root tubers of sample 6 were short and broad; 3.7 to 4.3 cm long and 1.5 to 2.2 cm thick, with plenty of stone cells present both outside and inside of the endodermis. Cambium was discontinuous and arranged in irregular fashion, starch grains were numerous in number; simple or compound, vessel elements were reticulately thickened.

Root tubers of sample 7 were much branched from the base; 9.0 to 10.0 cm in length and 1.2 to 1.3 cm in thickness, with plenty of stone cells present outside the endodermis. Cambium was continuous and circular, starch grains were numerous in number; simple or compound, vessel elements were reticulately thickened or simple pitted.

Root tubers of sample 8 were short, thin, branched from the base; 4.0 to 4.5 cm in length and 0.6 to 0.7 cm in thickness, stone cell was absent in this species, cambium was discontinuous and arranged in irregular fashion, starch grains were numerous in number; and mostly simple and vessel elements were reticulately thickened or simple pitted (Figs. 2,3,4,5,6,7).

Total alkaloid content of sample 3 was 3.0923 %. 2.426 % total alkaloid was obtained from sample 6. The total alkaloid extracted from sample 1, 2, 5 and 7 were 1.372 %, 1.218 %, 1.394 % and 1.204 % respectively. Sample 4 contains lowest total alkaloid, i.e. 1.096 %.

TLC profiles of different samples vary from each other. In TLC study of sample 1, 4 spots were found on Rf of 0.09, 0.13, 0.30 and 0.88 under 254 nm; 3 spots on Rf value of 0.09, 0.13 and 0.35 under 366 nm; and 2 spots at 0.14 and 0.35 after spraying by Dragendorff’s reagent were observed. 10 spots at Rf of 0.09, 0.16, 0.28, 0.35, 0.45, 0.53, 0.65, 0.78, 0.88 and 0.94 under 254 nm; 1 spot at 0.09 under 366 nm and 6 spots at 0.35, 0.58, 0.65, 0.72, 0.83 and 0.90 after spraying by Dragendorff’s reagent were found in TLC profile of sample 2. Sample 3 showed 6 spots at Rf of 0.09, 0.13, 0.40, 0.48, 0.54 and 0.66 under 254 nm; 4 spots at Rf of 0.13, 0.40, 0.85 and 0.90 under 366 nm and 5 spots at 0.11, 0.35, 0.63, 0.74 and 0.94 after spraying by Dragendorff’s reagent. In TLC of sample 4, 4 spots were found on Rf of 0.09, 0.12, 0.38 and 0.78 under 254 nm; 1 spot at Rf of 0.09 under 366 nm and 2 spots at 0.15 and 0.35 after spraying by Dragendorff’s reagent were observed. 4 spots at Rf of 0.07, 0.12, 0.19 and 0.37 under 254 nm; 2 spots at 0.07 and 0.37 under 366 nm and 2 spots at 0.17 and 0.42 after spraying by Dragendorff’s reagent were observed in TLC profile of sample 5. Sample 6 showed 5 spots at Rf of 0.07, 0.12, 0.19, 0.37 and 0.63 under 254 nm; 2 spots at 0.07 and 0.37 under 366 nm and 2 spot at 0.17 and 0.42 after spraying by Dragendorff’s reagent. Sample 7 exhibited 4 spots at Rf of 0.07, 0.12, 0.38 and 0.63 under 254 nm; 2 spots at Rf of 0.10 and 0.38 under 366 nm and 2 spots at 0.10 and 0.35 after spraying by Dragendorff’s reagent (Fig. 8).

Discussion

The macroscopic, microscopic and anatomical characters of different species of aconite sold under the name of Vatsanabhaa studied in the present study were compared with the findings of the previous studies. Characters of sample 1, i.e. long root tubers with broad base, continuous stellate cambium and presence of scattered stone cells outside the endodermis are almost similar to the characters of A. spicatum. Continuous stellate cambium and presence
Fig. 1 (a-h) – Photograph of different samples of aconite (a) Photograph of sample 1; (b) Photograph of sample 2; (c) Photograph of sample 3; (d) Photograph of sample 4; (e) Photograph of sample 5; (f) Photograph of sample 6; (g) Photograph of sample 7; (h) Photograph of sample 8
Fig. 2 (a-h) – Diagrammatic transverse section of different samples (a) Diagrammatic transverse section of sample 1; Mt: Metaderm; Ct: Cortex; St: Stone cell; En: Endodermis; Ph: Phloem; Xy: Xylem; Pi: Pith; Ca: Cambium; Ss: Sieve strands (Note: Stone cells outside of endodermis, continuous stellate cambium.); (b) Diagrammatic transverse section of sample 2 (Note: Stone cells outside and inside of endodermis, continuous stellate cambium.); (c) Diagrammatic transverse section of sample 3 (Note: Stone cell absent, continuous stellate cambium.); (d) Diagrammatic transverse section of sample 4 (Note: Isolated stone cells outside of endodermis, continuous stellate cambium.); (e) Diagrammatic transverse section of sample 5 (Note: Stone cells outside of endodermis, discontinuous circular cambium.); (f) Diagrammatic transverse section of sample 6 (Note: Stone cells outside and inside of endodermis, discontinuous irregular cambium.); (g) Diagrammatic transverse section of sample 7 (Note: Stone cells outside of endodermis, continuous circular cambium.); (h) Diagrammatic transverse section of sample 8 (Note: Stone cell absent, discontinuous irregular cambium.)
Fig. 3 (a-h) – Diagrammatic histological transverse section of different samples (a) Diagrammatic histological transverse section of sample 1 (Mag.: ×100); Mt: Metaderm; Ct: Cortex; St: Stone cell; En: Endodermis; Ss: Sieve strands; Ph: Phloem; Ca: Cambium; Xy: Xylem; Pi: Pith (Note: Stone cells outside of endodermis, continuous stellate cambium); (b) Diagrammatic histological transverse section of sample 2 (Mag.: ×100) (Note: Stone cells outside and inside of endodermis, continuous stellate cambium.); (c) Diagrammatic histological transverse section of sample 3 (Mag.: ×100) (Note: Stone cell absent, continuous stellate cambium.); (d) Diagrammatic histological transverse section of sample 4 (Mag.: ×100) (Note: Isolated stone cells outside of endodermis, continuous stellate cambium.); (e) Diagrammatic histological transverse section of sample 5 (Mag.: ×100) (Note: Stone cells outside of endodermis, discontinuous circular cambium.); (f) Diagrammatic histological transverse section of sample 6 (Mag.: ×100) (Note: Stone cells outside and inside of endodermis, discontinuous irregular cambium.); (g) Diagrammatic histological transverse section of sample 7 (Mag.: ×100) (Note: Stone cells outside of endodermis, continuous circular cambium.); (h) Diagrammatic histological transverse section of sample 8 (Mag.: ×100) (Note: Stone cell absent, discontinuous irregular cambium.)
Fig. 4 (a-h) – Histological transverse sections of cortex, endodermis and stone cells (a) Photomicrograph of representative section of cortex, stone cells and endodermis of sample 1 (Mag.: ×100). Mt: Metaderm; Ct: Cortex; St: Stone cell; En: Endodermis. (Note: Scattered stone cells outside of endodermis.); (b) Photomicrograph of representative section of cortex, stone cells and endodermis of sample 2 (Mag.: ×100). (Note: Multiple stone cells outside and inside of endodermis.); (c) Photomicrograph of representative section of cortex and endodermis of sample 3 (Mag.: ×100). (Note: Stone cell absent.); (d) Photomicrograph of representative section of cortex, stone cells and endodermis of sample 4 (Mag.: ×100). (Note: Isolated stone cells outside of endodermis.); (e) Photomicrograph of representative section of cortex, stone cells and endodermis of sample 5 (Mag.: ×100). (Note: Stone cells outside of endodermis.); (f) Photomicrograph of representative section of cortex, stone cells and endodermis of sample 6 (Mag.: ×100). (Note: Numerous stone cells outside and inside of endodermis.); (g) Photomicrograph of representative section of cortex, stone cells, endodermis of sample 7 (Mag.: ×100). (Note: Stone cells outside of endodermis.); (h) Photomicrograph of representative section of cortex and endodermis of sample 8 (Mag.: 1×100). (Note: Stone cell absent.)
Fig. 5 (a-h) – Histological transverse section of phloem, cambium and xylem (a) Photomicrograph of representative section of phloem, cambium and xylem of sample 1 (Mag.: ×100). Ph: Phloem; Ca: Cambium; Xy: Xylem. (Note: Continuous stellate cambium.); (b) Photomicrograph of representative section of phloem, cambium and xylem of sample 2 (Mag.: ×100). (Note: Continuous stellate cambium.); (c) Photomicrograph of representative section of phloem, cambium and xylem of sample 3 (Mag.: ×100). (Note: Continuous stellate cambium.); (d) Photomicrograph of representative section of phloem, cambium and xylem of sample 4 (Mag.: ×100). (Note: Continuous stellate cambium.); (e) Photomicrograph of representative section of phloem, cambium and xylem of sample 4 (Mag.: ×100). (Note: Continuous stellate cambium.); (f) Photomicrograph of representative section of phloem, cambium and xylem of sample 6 (Mag.: ×100). (Note: Discontinuous irregular cambium.); (g) Photomicrograph of representative section of phloem, cambium and xylem of sample 7 (Mag.: ×100). (Note: Continuous circular cambium.); (h) Photomicrograph of representative section of phloem, cambium and xylem of sample 8 (Mag.: ×100). (Note: Discontinuous irregular cambium.)
Fig. 6 (a-h) – (a) Powder microscopy of sample 1 (Mag.: ×400); Mt: Metaderm; PC: Parenchyma cells; SG: Starch grains; St: Stone cells; Vs: Vessels; Sc: Sclereids. (b) Powder microscopy of sample 2 (Mag.: ×400); (c) Powder microscopy of sample 3 (Mag.: ×400); (d) Powder microscopy of sample 4 (Mag.: ×400); (e) Powder microscopy of sample 5 (Mag.: ×400); (f) Powder microscopy of sample 6 (Mag.: ×400); (g) Powder microscopy of sample 7 (Mag.: ×400); (h) Powder microscopy of sample 8 (Mag.: ×400).
Fig. 7 (a-h) – Isolated tissues of different samples (a) Isolated tissues of sample 1 (Mag.: ×400); (b) Isolated tissues of sample 2 (Mag.: ×400); (c) Isolated tissues of sample 3 (Mag.: ×400); (d) Isolated tissues of sample 4 (Mag.: ×400); (e) Isolated tissues of sample 5 (Mag.: ×400); (f) Isolated tissues of sample 6 (Mag.: ×400); (g) tissues of sample 7 (Mag.: ×400); (h) Isolated tissues of sample 8 (Mag.: ×400). [St: Stone cells; Vs: Vessels, Fb: Fibre & Sc: Sclereids]
Fig. 8 (a-b) – TLC profile of different samples (a) TLC profiles of different samples ofaconite under 254 nm. (1: Sample 1; 2: Sample 2; 3. Sample 3; 4: Sample 4; 5: Sample 5; 6: Sample 6; 7: Sample 7; 8: Sample 8.); (b) TLC profiles of different samples ofaconite under 366 nm. (1: Sample 1; 2: Sample 2; 3. Sample 3; 4: Sample 4; 5: Sample 5; 6: Sample 6; 7: Sample 7; 8: Sample 8.)

of numerous stone cells in both outside and inside of the endodermis of sample 2 are almost similar to the characters of A. falconeri. Characters of sample 5, i.e. discontinuous circular cambium, presence of stone cells outside the endodermis and vessels with reticulately thickened walls are nearer to the characters of A. deinorrhizum, and characters of sample 6, i.e. comparatively shorter root tubers, discontinuous irregularly arranged cambium, presence of plenty of stone cells in both outside and inside of the endodermis and vessels with reticulately thickened walls are almost similar to that of A. balfourii (A. atrox).

Characters of sample 4, i.e. shorter and thinner root tubers, continuous stellate cambium and scattered stone cells present outside the endodermis are similar to the findings of Dutta and Mukerji regarding A. laciniatum. But the characters differ with the findings of Mehra and Puri regarding A. laciniatum, because they have mentioned that it is devoid of stone cells.

Previous literatures regarding identification of A. chasmanthum are most controversial. So, in this present study authentic sample of A. chasmanthum was collected and studied in details. The distinguishing characters found are short and thin roots, continuous stellate cambium, absence of stone cells and distinct sieve strands lying above the xylem poles. The characters of sample 3, i.e. shorter and thinner root tubers, absence of stone cells, continuous stellate cambium, presence of prominent sieve strands above the xylem poles are almost similar to that of the authentic sample of A. chasmanthum.

The characters of sample 7, i.e. branched roots from the base, continuous circular cambium and presence of stone cells outside the endodermis and characters of sample 8, i.e. discontinuous irregularly arranged cambium and absence of stone cells could not match with identification characters of any species of Aconitum, mentioned in the previous literatures.

So, by taking consideration of the above discussion, regarding identification of different samples it may be inferred that sample 1 is A. spicatum, sample 2 is A. falconeri, sample 3 is A. chasmanthum, sample 4 is A. laciniatum, sample 5 is A. deinorrhizum and sample 6 in A. balfourii (A. atrox).

Alkaloids are the main chemical component of the aconite roots. Qualitative and quantitative variations of these alkaloids are found to be identification character of different species. Guha RC16 examined different varieties of aconite usually available in the Indian market for their total alkaloidal content and reported that total alkaloid content of A. chasmanthum is 2.85 to 3.11 %, total alkaloid content of A. napellus is 1.05 to 1.83 %. Total alkaloid content of other species of aconite like A. spicatum, A. ferox, A. palmatum, A. balfourii, A. falconeri, A. deinorrhizum and A. laciniatum are 1.75 %, 1.85 %, 1.48 %, 2.04 %, 1.00 %, 1.50 % and 1.06 %, respectively.

A huge variation in total alkaloids content in different samples found in the present study is indicative of their identity too. Total alkaloids of sample 1 (1.372 %) indicates similarity with total alkaloids content of A. spicatum. Likewise total alkaloids content of sample 2 (1.218 %), sample 3 (3.092 %), sample 4 (1.096 %), sample 5 (1.394 %) and sample 6 (2.426 %) are suggestive of their similarity with A. falconeri, A. chasmanthum, A. laciniatum, A. deinorrhizum and A. balfourii, respectively.

Results of the present study reveals that the drug sold under the name of ‘Vatsanabha’ are mixture of eight different species of Aconitum. Six of these are identified as A. spicatum, A. falconeri, A. chasmanthum, A. laciniatum, A. deinorrhizum and A. balfourii, respectively. The differentiation in the species of Aconitum could be made by presence and position of stone cells and structure of cambium. It is also to be observed that the authentic species of Vatsanabha, i.e. A. chasmanthum, which is considered as most toxic species of Aconitum4, is rarely available in the market samples.

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