

Pharmacognostic and phytochemical studies on Ayurvedic drugs *Ativisha* and *Musta*

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Abstract: The study include the establishment of pharmacognostic and phytochemical characters of *Ativisha* (*Aconitum heterophyllum* Wall. ex Royle, Ranunculaceae) and to compare them with its substitutes, *Cyperus rotundus* L. (Musta), *C. scariosus* R. Br., Cyperaceae, and *Cryptocoryne spiralis* (Retz.) Fisch. ex Wydler, Araceae (Country *Ativisha*). Morphology of the four species was compared in authentic samples collected from the field. We performed histological, histochemical, phytochemical tests, using standard protocols. HPLC studies were done on aqueous extracts of samples in a Shimadzu HPLC system and the peaks were observed at 254 nm. Pharmacognostic characterization of *Ativisha* and others was done as completely as possible. On basis of histochemical analyses revealed the presence of alkaloid, terpenoid-alkaloid complex, lipids and calcium oxalate majorly. There was less than 50% similarity between *Ativisha* and the other three species in microscopic characters. There was greater similarity (87%) between the two *Cyperus* species. The phytochemical studies, on the other hand, showed less similarity (79.2%) between the two *Cyperus* species. There was greater phytochemical similarity (84.6%) between *Aconitum* and *Cryptocoryne*, which justifies the name “Country *Ativisha*” for the latter. Based on anatomical and histochemical analysis, structural as well as chemical parameters helpful in distinguishing *Ativisha* from the other three species were established. The phytochemical profiles showed that *A. heterophyllum* and *Cyperus* species have five common HPLC peaks which may explain some of their common therapeutic activities. *Ativisha* and *Cryptocoryne* show greater phytochemical similarities to one another and this explains why the latter is used in Siddha system of medicine as country *Ativisha*.

Introduction

The Himalayan endemic *Aconitum heterophyllum* Wall. ex Royle, Ranunculaceae, is the genuine *Ativisha* of Ayurvedic (The Ayurvedic Pharmacopoeia of India, 1999) and *Athividayam* of Siddha literature (Anandakumar et al., 1982). Charaka described this drug, as per its therapeutic actions, under *Lekhaniya* (tissue scraping action) *Arsoghna* (haemorrhoids-curing), *Sirovirecana* (errhines, nasal therapy) Gana (groups) and *Tikta skandha* (bitter tasting) (Pandeya, 1997), while Sushruta placed it under *Mustadi* and *Vacadi*. *Ativisha* is broadly used for its antipyretic, anti-inflammatory, anti-diarrheal activities and prevention of vomiting, cough and cold (Sharma, 2006). On account of its high demand, *A. heterophyllum* has now become critically endangered (Srivastava et al., 2004).

Musta, also an Ayurvedic drug, is placed by Charaka under *Lekhaniya*, *Trsnanigrahana* (anti-dyspic),

Kandughna (destroying itching), and *Sanyasodhana* (galacto-depurant) (Sharma, 2006). There is some controversy regarding its botanical identity largely because early Ayurvedic texts mentioned two varieties “*Nagara musta* and *Bhadra musta*” which were later identified respectively as *Cyperus scariosus* R. Br. and *Cyperus rotundus* L. (Sastry, 2005). The work of Kaviraj Govind Das Sen of 17-18th centuries entitled “*Bhaisajya Ratnavali*”, states that “*Musta ca Ativisha abhave*”, which literally means that *Musta* can be used as a substitute of *Ativisha* (Mishra, 2007). In fact it has been accepted by Ayurveda that *Musta* is a substitute (*Abhava Pratinidhi* Dravya) for *Ativisha* (Venkatasubramanian et al., 2010). However their bioequivalence has not been scientifically validated or published. It was also indicated that the roots of *Cryptocoryne spiralis* (Retz.) Fischer ex Wydler can be used as a substitute of *Ativisha* (Anandakumar et al., 1982), although it was recommended to the contrary (Sastry, 2005). Since the annual demand for *Ativisha* has reached over 200 t with a cost of US\$ 73.62/kg, (Ved

& Goraya, 2008) markets, especially in south India, substitute *Ativisha* with known and unknown substitutes and adulterants, which include among others *C. scariosus*, *C. rotundus*, and *Cryptocoryne spiralis*. Hence, it is necessary to study the pharmacognosy of authentic field collections to establish quality standards to distinguish the genuine from substitute/adulterants of *Ativisha*. It is also necessary to find out the extent of similarity between the genuine and the substituted/adulterated samples, in order to understand the legitimacy behind substitution.

Fragmentary pharmacognostic studies have been made earlier on *A. heterophyllum* (The Ayurvedic Pharmacopoeia of India, 1999; 2001; The Siddha Pharmacopoeia of India, 2008), *C. rotundus* (Rehman, 2007; Rai et al, 2010; Sharma & Singh, 2011) and of *Cryptocoryne spiralis* (Anandakumar et al., 1982; Shantha, 1995) but none on *C. scariosus*.

The phytochemistry of *Ativisha* has been studied extensively for its alkaloid profile (Srivastava et al., 2004; Csupor, 2007). The phytochemistry of *C. rotundus* (Paknikar et al., 1977; Sonwa & Koenig, 2001; Lawal & Oyedeji, 2009; Meena et al., 2010), and of *C. scariosus* (Nerali et al., 1965; Nerali & Chakravarti, 1967; 1970; Hikino et al., 1967; Neville et al., 1968; Gopichand et al., 1978; Uppal et al., 1984; Garg et al., 1989; Pandey & Chowdhury, 2002) have been studied with greater focus on essential oils. The phytochemistry of *Cryptocoryne* has also been studied (Anandakumar et al, 1982; Gupta et al, 1983; 1984; Gupta & Shukla, 1986). The comparative phytochemical aspects studies of *A. heterophyllum* and *C. rotundus* have done. Except for this study (Venkatasubramanian et al, 2010), none of the above investigations focus on comparing the candidate species. Quality standards for *Ativisha* (*A. heterophyllum*) and *Musta* (*C. rotundus*) including pharmacognosy and alkaloid content in *Ativisha* and volatile oil content in *Musta* (The Ayurvedic Pharmacopoeia of India, 1999; 2001). Histochemical details are totally lacking in this account. Hence, the present studies have been undertaken with a view to establish the diagnostic pharmacognostic characters of *Ativisha* and *Musta* as completely as possible, as well as to compare the similarities and differences between the original and substituted botanical drugs of these two in terms of morphological, histological, histochemical and phytochemical parameters.

Material and Methods

Collection and authentication of samples

Fresh mature underground parts, used as raw drugs, were collected from various places in India, identified and authenticated by our institute botanists. The voucher specimens were deposited in FRLH herbarium and assigned identification numbers.

Morphology

The collected samples were subjected to morphological and taxonomical analyses using features such as the shape, size, color, odor and taste.

Histology

The samples were preserved either in formalin-acetic acid-alcohol (40% formalin: 5 mL: 50% ethanol: 90 mL; glacial acetic acid: 5 mL) or as such after room drying. Transverse sections, taken by using razor blade, were stained with toluidine blue O 0.05% in benzoate buffer (benzoic acid 0.25 g in 200 mL water pH 4.4) (Krishnamurthy, 1988), washed with water, observed under a microscope (Olympus BX 41, Tokyo, Japan) and the photographic images were captured using a digital Olympus camera fixed with the microscope. The images were processed on Image Pro Express 6.0.

Histochemistry

Specimens (at least ten numbers from each collection) were soaked in water and transverse sections were taken using sharp razor blades. The sections (at least twenty for each of the procedures) were stained using specific procedures for localizing starch, lignin, calcium oxalate, tannins and total lipids (Krishnamurthy, 1988). Dragendorff reagent was used for localizing alkaloids (Yoder & Mahlberg, 1976; Joger, 1998) while terpenoids were localized using vanillin in acetic acid and perchloric acid (Abraham et al., 1988; Narayanan et al., 2002; Khatun et al., 2011) Photographic images were captured as above.

Autofluorescence images were captured for lipid and indole alkaloid-terpene complex (Mabroug et al., 2007) using Olympus BX 41 microscope fitted with epifluorescence unit. These results were correlated with results obtained through light microscope images of histochemistry studies for lipids, terpenoids and alkaloids. Calcium oxalate crystals were localized using polarizing optics.

Powder microscopy

The prepared powder of 1 mm size (Bureau of Indian Standards, Mesh no 16) was examined after further maceration with Jeffery's maceration fluid (1:1 of 10% nitric acid and 10% chromic acid mixture and heated in water bath until a bleaching effect was observed) (Marimuthu, 2008). The residual acid in the bleached powder fragments, after repeated water wash, was neutralized by adding a few drops of ammonium hydroxide. The macerated powder was then stained with TBO and observed under Olympus BX 41 microscope for powder characters.

Phytochemical screening

The phytochemical analysis was performed using standard procedures (Kokate, 2000; Raaman, 2006). Dried, powdered (mesh size 85) samples were successively extracted, using a soxhlet apparatus, with solvents of increasing polarity, namely *n*-hexane, ethyl acetate, chloroform, methanol and water at 60-70 °C for three complete cycles. All extracts were concentrated at 40-45 °C using a rotary evaporator (Rotavapor R-3, Buchi, Switzerland) to 50 mL and tested for presence of various chemicals.

HPLC studies

Dried and finely powdered (2 g, mesh size 85) specimens were refluxed with distilled water for 1 h. The extracts were filtered through coarse filter paper, concentrated on a water bath at 100 °C and made up to 25 mL in a volumetric flask. These extracts were then used for the HPLC analysis after filtration through 0.25 µ nylon membrane filters (Ultipor N66 Nylon 6, 6 membrane filter, Pall India Pvt. Ltd).

A Shimadzu HPLC system (Model No: SCL-10AVP, Japan) consisting of LC-10AVP pump, a rheodyne injector, and LC Solutions software was used for HPLC analysis. The stationary phase was a Purosphere Hibar (Purospher STAR columns, MERCK, German) RP-18, 250 mm x 4.6 mm, 5 µm column. HPLC grade solvents were used as received from suppliers, after degassing using a sonicator (Servewell Instruments Pvt. Ltd, Bangalore). The injection volume was 20 µL. A flow rate at 1 mL/min was maintained and the column was equilibrated at the initial ratio of 10% methanol-90% water for 60 min. The

initial solvent ratio of 10% methanol was raised to 100% methanol over 25 min, maintained for 10 min, reduced back to 10% methanol instantaneously and maintained for 10 min, for a total run time of 45 min. The chromatogram was observed at 254 nm.

Results

The general morphological characters and taxonomic status of the four species are listed in Chart 1, while those of the raw drugs are given in Table 2. They differ in the nature of the underground organs. *A. heterophyllum* has an ovoid-conical taproot/ root modification (Figure 1a), while all the rest are underground stems (rhizomes and corm in *Cyperus* species and *Cryptocoryne* respectively) (Figure 1b-d).

Anatomical and histochemical characters

Aconitum heterophyllum

The structure of the root tubers of *Ativisha* (*A. heterophyllum*) is very peculiar and unlike that of any other Ranunculaceae member. The tuber has 4-6 “vascular strands” embedded in the ground tissue which is indistinguishable into pith and cortex. These “strands” are caused by the splitting of one vascular “strand”, as seen at the basal tip of the tuber. Each exarch vascular strand has 2-6 radiating xylem strands, the gaps between which are filled with parenchyma cells. Phloem occurs in patches just outside the xylem strands in a more or less discontinuous ring. The parenchyma cells of vascular strands continue into the ground tissue through spaces between the phloem patches. A number of lateral root vascular strands arise

Chart 1. General characters of four species used /sold as *Ativisha*-*Musta*.

Sample N ^o	Features	<i>Aconitum heterophyllum</i>	<i>Cyperus rotundus</i>	<i>Cyperus scariosus</i>	<i>Cryptocoryne spiralis</i>
1	Family	Ranunculaceae	Cyperaceae	Cyperaceae	Araceae
2	Habit	Herb	Herb	Herb	Aquatic Herb
3	Leaves	Heteromorphous	Whorled	Whorled, Lanceolate	Whorled, Lanceolate, Acute at apex
4	Inflorescence	Raceme or a laxy panicle	Spike	Spike	Spadix
5	Flower Color	Blue or Violet	Brown	Red - Brown	Yellow - Orange

Chart 2. Morphological characters of the underground organs used as raw drug.

Sample N ^o	Features	<i>Aconitum heterophyllum</i>	<i>Cyperus rotundus</i>	<i>Cyperus scariosus</i>	<i>Cryptocoryne spiralis</i>
1	Shape	Ovoid- conical, tapering downwards to a point	Oval , tapering towards points of the both the end	Oblong	Cylindrical, the body was differentiated into nodes and internodes.
2	Color	Externally light ash-grey, white or grey – brown, internally starch white	Dark brown or black externally, creamish- yellow internally	Initially white in color, eventually turning brown or black	Yellowish brown in color
3	Type	Tuberous root	Rhizome	Rhizome	Corm
4	Odor	No odor	Muddy odor	Muddy odor	Herbaceous
5	Taste	Bitter and astringent	Bitter	Bitter	Bitter



Figure 1. External morphology of samples traded as *Ativisha-Musta* A. *Aconitum heterophyllum* x 1.5; B. *Cyperus rotundus* x 0.75; C. *Cyperus scariosus* x 0.5; D. *Cryptocoryne spiralis* x 0.75.

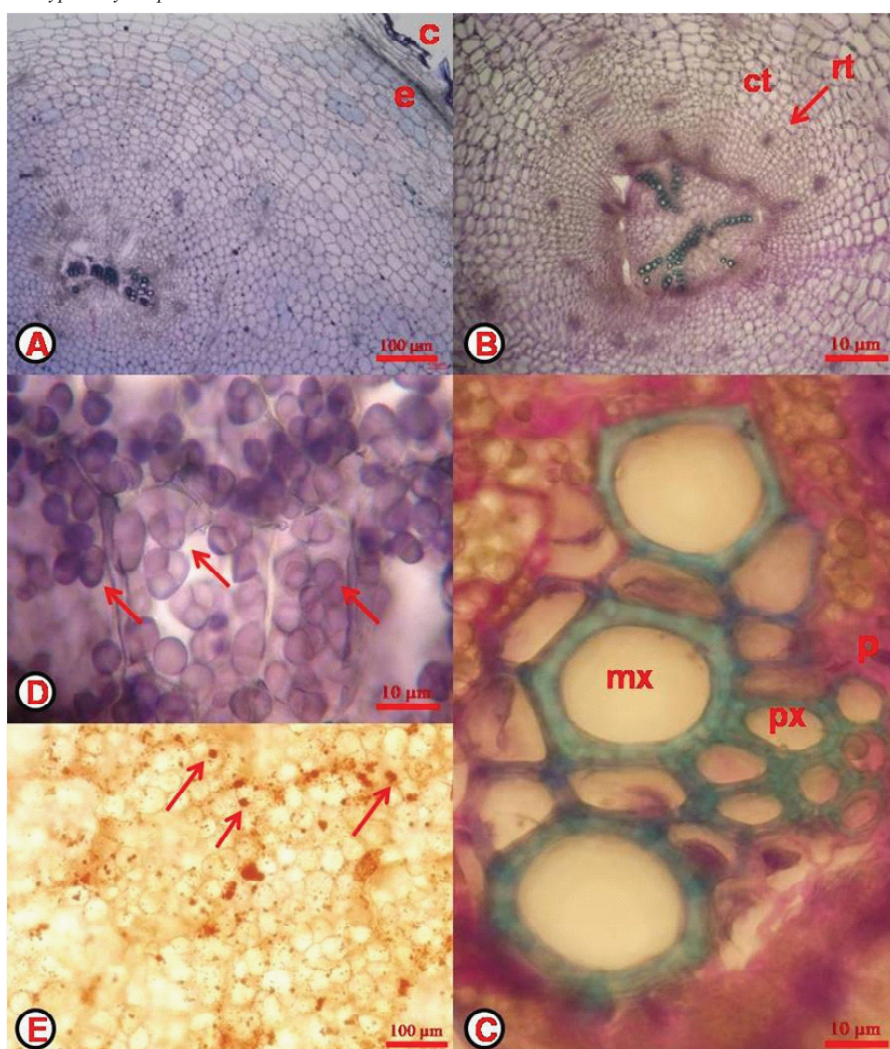


Figure 2. *Aconitum heterophyllum* (*Ativisha*) A. T.S. of portion of root tuber showing cork (c), endodermis (e) and broad stele with one of the 4-7 strands; B. structure of vascular strand with 5 protoxylem poles in the xylem (stained green). Note also the cambiform tissue (ct) surrounding the vascular strands and the scattered root traces (rt); C. a portion of vascular strand enlarged to show exarch protoxylem (px) and large metaxylem elements (mx), Phloem occurs outside xylem (p); D. starch grains (arrow); E. lipids droplets (arrow) stained with Sudan III.

opposite to protoxylem points. Immediately around each vascular strand, the parenchyma cells are in very regular radiating files (cambiform), which has been mistaken by some earlier workers as cambium. There is a typical single layered endodermis extending throughout the entire perimeter, the inner tangential walls and radial wall of whose cells are thickened with both suberin and lignin. Outside endodermis are a few layers of cells divided into an outer zone of 4 to 5 “cork” layers and an inner zone of 4 to 5 layers of thin-walled cells. Most parenchyma cells are fully filled with simple and compound, round to oval starch grains of 14-25µm width (larger grains) and 5-6 µm (smaller grains). Alkaloids and lipids were detected histochemically (Figure 2).

Cyperus rotundus

The rhizome has an outermost 3-6 layered “cork” showing ridges and furrows; its cells have suberized,

very thick walls and lumen filled with dark brown tannin content, The “cork” is followed on the inside by 2-3 layered sclerenchyma of tangentially elongated cells whose cell walls are heavily lignified with tanniferous lumen. Inner to this is the wide cortex of thin-walled parenchyma, the cell walls of which are impregnated with phenolic materials. Most cells are filled with elliptical starch grains (10-12 µm broad at their broadest and 4-6 µm at their narrowest regions). A few cells here and there are tanniferous or with lipid droplets while a number of cells are filled with a complex mixture of terpenoids and alkaloids. The last ones are positive to both Dragendorff reagent and vanillin-perchloric acid. The mixed contents are also demonstrated using autofluorescence. There is a single (occasionally two here and there) layer of tangentially elongated uniformly thick-walled, lignified cells forming the boundary between the cortex and the inner part of the rhizome (false endodermis). The region inside this layer may be called stele as it contains vascular tissue in the form of scattered

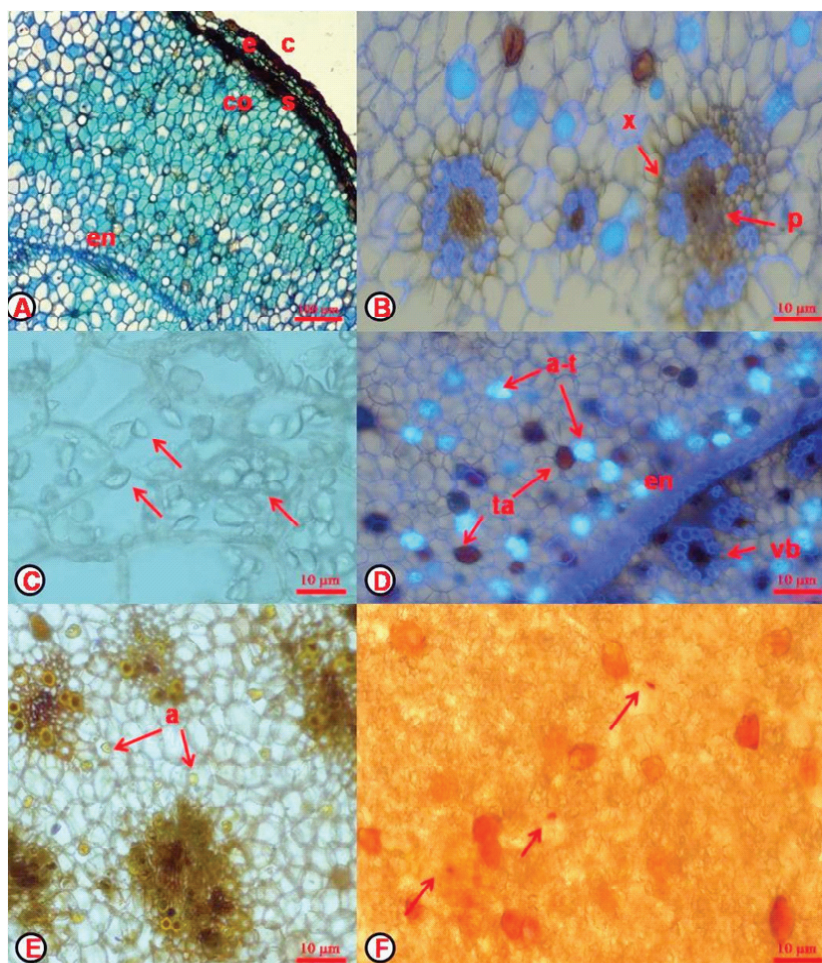


Figure 3. *Cyperus rotundus* (*Musta*) A. T.S. showing portion of rhizome; B. portion of stele under fluorescence microscope; C. cells showing starch grains; D. portions of cortex stele junction; E. portion of stele enlarged to show vascular bundles and alkaloid-terpenoid containing cells stained for alkaloids (arrow); F. portion of cortex with cells filled with lipid droplets stained with Sudan III (arrow). a: alkaloid; a-t: alkaloid-terpenoid complex; c: “cork”; co: cortex; e: “endodermis”; en: endodermis; l: lipid droplets; p: phloem; s: sclerenchyma; ta: tannin; vb: vascular bundle; x: xylem.

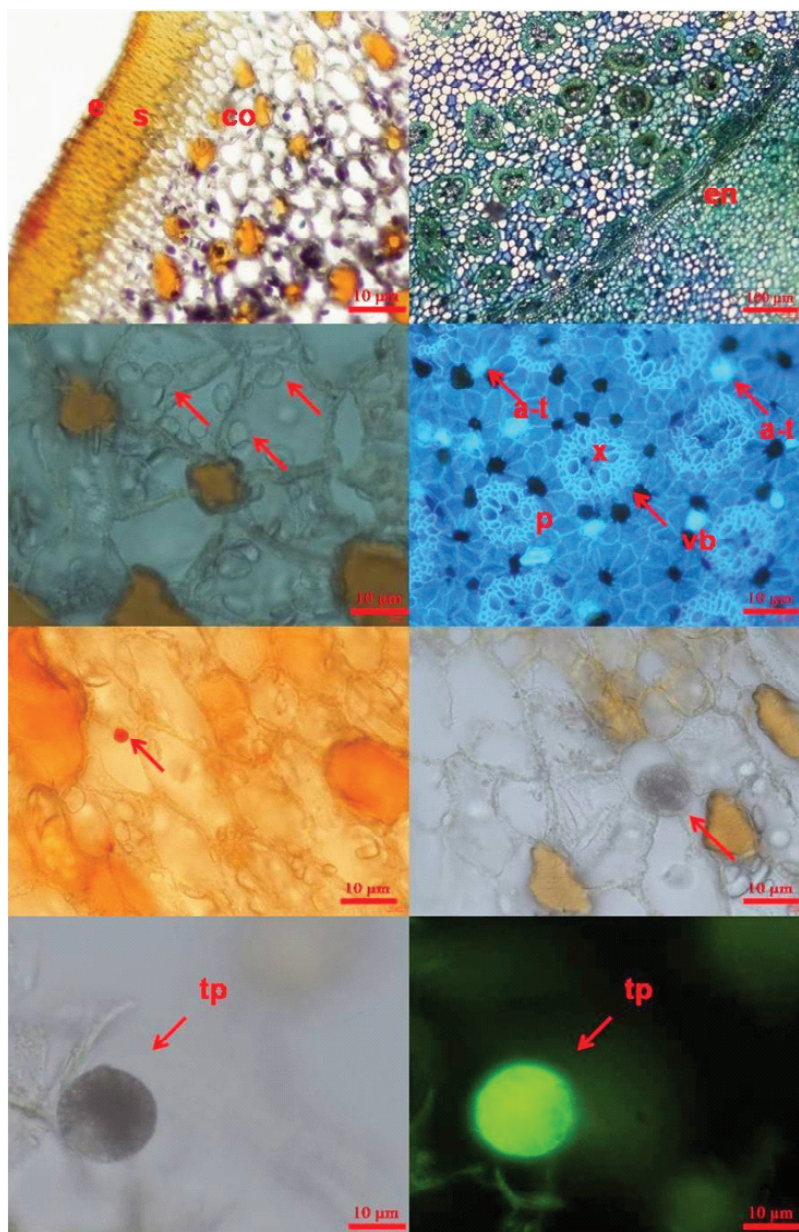


Figure 4. *Cyperus scariosus* A. T.S. showing peripheral part of the rhizome, note the absence of “cork” and the presence of inclusions in cortical cells; B. cortex-stele junction region showing large number of vascular bundles; C. portion of cortex showing starch grains and tannin (arrow); D. portion of stele enlarged under fluorescence microscope showing concentric vascular bundles and parenchyma cells filled with tannin (black content) and alkaloid-terpenoid complexes (light blue autofluorescence under UV incident light); E. parenchyma cells filled with lipid droplets stained with Sudan III (arrow); F. parenchyma cells stained with vanillin-perchloric acid to show terpenoid in the alkaloid-terpenoid complex (arrow); G,H. portion of cortex enlarged respectively in bright field and fluorescence microscope to show the same alkaloid-terpenoid complex. a: alkaloid; a-t: alkaloid-terpenoid complex; co: cortex; e: “endodermis; l: lipid droplets; p: phloem; s: sclerenchyma; st: starch grains; ta: tannin; tp: terpenoid; vb: vascular bundle; x: xylem.

bundles embedded in ground parenchymatous tissue which shows all features described for the cortical region. The bundles are all concentric with an almost continuous patch of xylem tissue enclosing centrally placed phloem tissue. There is no distinguishable pith (Figure 3).

Cyperus scariosus

The rhizome shows a characteristic undulating outline in transverse sections. There is no cork; there is an outer layer of epidermis made up of small tabular cells followed by a hypodermal sclerenchyma (3-4 layers) filled

with brownish or blackish tannin deposits. Inside this sclerenchymatous zone is a broad zone of inner cortex whose parenchyma cells are filled with starch grains which are elliptical and having 12-15 μm width in the broadest region and 4-6 μm in the narrowest region. Many cortical parenchyma cells are also filled with condensed tannin which shows a natural reddish-brown color or with a mixture of terpenoids and alkaloids as in the case of *C. rotundus*. The innermost cortex is separated from the stele by a two layered false endodermis with lignified and tangentially elongated cells. The stele is a mixture of scattered vascular strands and ground parenchyma tissue, the latter showing all features of cortical parenchyma. Each vascular strand is concentric with phloem in the center and xylem surrounding it. Some of the phloem cells also contain tannin, but rarely alkaloid-terpenoid complex (Figure 4).

Cryptocoryne spiralis

The underground organ, a corm, is used as country *Ativisha* in Siddha system of medicine. It consist of an outer "cork" of 2-3 layers of tangentially elongated

suberized cells but the cork producing meristem is not cork cambium. Some cork cells are filled with tannin. Inner to the cork producing cells are the cortical parenchyma cells, many of which are filled with round to elliptical starch grains with a maximum size of 7-13 μm . Many parenchyma cells are fully filled with orangish-red colored tannin. Yet others are loaded with a yellowish brown substance of terpenoid nature, not filling the entire cell lumen. Some cells show lipids droplets. Alkaloids could not be detected histochemically. A number of root vascular traces are also seen in the cortex. Large intercellular spaces in the cortex often contain bundles of needle shaped (raphide) crystals of calcium oxalate. The innermost zone of the cortex is the 2-3 layered false endodermis, with fully lignified cells. False endodermis is interrupted by broad zones of non-sclerified parenchymatous passage cells. Inner to the false endodermis is the stele in which are scattered concentric vascular bundles. The parenchyma cells in the stelar region are similar to those of cortex in nature and contents. Pith is not distinguishable (Figure 5).

The comparison of anatomical features of four species is provided in Chart 3. The powders of all four raw drugs were organoleptically and microscopically analyzed and the results are presented in Chart 4.

Chart 3. Comparison of the features seen in the transverse sections of the four species used as *Ativisha-Musta*.

Sample N°	Features	<i>Aconitum heterophyllum</i>	<i>Cyperus rotundus</i>	<i>Cyperus scariosus</i>	<i>Cryptocoryne spiralis</i>
1	Cork	4-5 layers, cells without tannin	3-6 layers of "cork" showing ridges and furrows; cells filled with tannin	No "cork"	2-3 layers of "cork"; some cells filled with tannin
2	Hypodermis	No hypodermis	2-3 layers of sclerenchyma cells below cork	Semi-circular patches of hypodermal sclerenchyma	No specific hypodermis
3	Cortex	Narrow, 4-5 layers of thin walled parenchyma cells, filled with starch, alkaloids, and lipids	Wide, several layers of thin walled parenchyma cells filled with starch grains, tannins, lipids, and a mixture of terpenoids and alkaloids	Broad, with thin walled parenchyma cells filled with starch grains, tannins, lipids, and a mixture of terpenoids and alkaloids	Broad, with thin walled parenchyma cells filled with Starch, tannins, lipids, terpenoids, raphide crystals present in intercellular spaces
4	Endodermis	Present, Single layered, suberized	Single layer (in some places two layered) of tangential elongated uniformly thick walled cells, not typical of endodermis	2 layered tangential elongated cells, lignified, not typical of endodermis	2-3 layers of lignified cells, not typical endodermis
5	Stele	4-6 "vascular strands" embedded in ground parenchyma tissue, Protoxylem exarch, phloem outside xylem strands in discontinuous ring, pith present, parenchyma cells possess content same as cortex	Many scattered vascular bundles embedded in ground parenchyma tissues bundle concentric with xylem enclosing central phloem tissues, pith absent, parenchyma cells possess content same as cortex	Many scattered vascular bundles embedded in ground parenchyma tissues bundle concentric with xylem enclosing central phloem tissues, pith absent, parenchyma cells possess content same as cortex	Many scattered vascular bundles embedded in ground parenchyma tissues bundle concentric with xylem enclosing central phloem tissues, pith absent, parenchyma cells possess content same as cortex

Chart 4. Powder study of four species used as *Ativisha-Musta*.

Sample N°	Features	<i>Aconitum heterophyllum</i>	<i>Cyperus rotundus</i>	<i>Cyperus scariosus</i>	<i>Cryptocoryne spiralis</i>
1	Color	White	Brown	Reddish brown	Muddy white
2	Odor	No odor	Muddy odor	Muddy odor	Herbaceous
3	Taste	Bitter and astringent	Bitter	Bitter	Bitter
4	Parenchyma cells	Cells with starch grains	Cells with starch grains	Cells with starch grains	Cells with starch grains
5	Vessel elements	Present	Present	Present	Present
6	Fibers	Libriform	Libriform	Libriform	Libriform
7	Starch	Simple and compound, round and oval	Simple, elliptical	Simple, elliptical starch	Simple, Round and elliptical
8	Raphide	Not detected	Not detected	Not detected	Detected

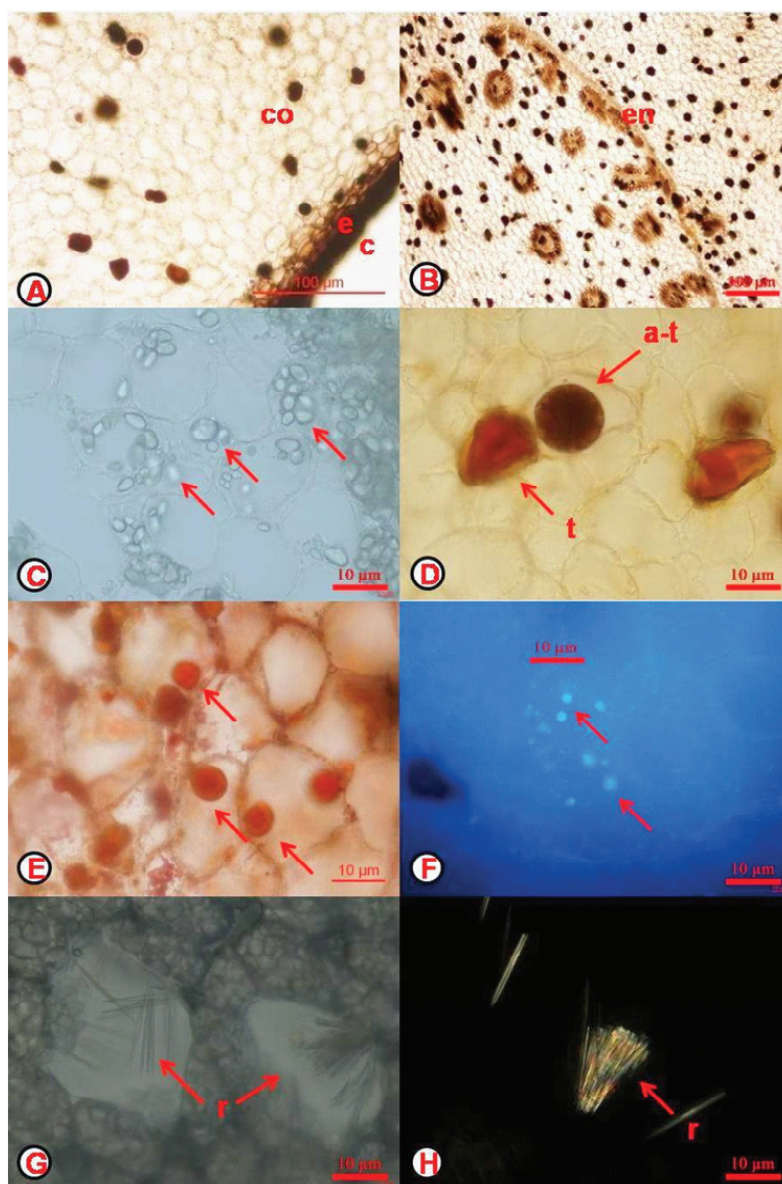


Figure 5. *Cryptocoryne spiralis*: A. T.S. portion of corm showing cortex and “cork”; B. T.S. portion of corm showing cortex-sterle junction. Note many scattered vascular bundles and inclusions in parenchyma cells; C. parenchyma cells showing starch grains (arrow); D. same with alkaloid-terpenoid complex and tannin; E, F. lipid droplets under bright field and fluorescence microscope respectively (arrow); G. raphide calcium oxalate crystals in air spaces; H. the same under polarizing microscope. e: “endodermis”; vb: vascular bundle; co: cortex; c: “cork”; a-t: alkaloid-terpenoid complex; ta: tannin; r: raphide crystals as air spaces.

Based on Charts 1 to 4, a similarity matrix was worked out between all the four species taking into consideration both positive and negative similarities in features (Figure 6). It is evident from this figure that the two *Cyperus* species share a maximum similarity of 87%, followed by *C. rotundus* and *Aconitum* (50%), both *Cyperus* species and *Cryptocoryne* (46%) and *Aconitum* and *Cryptocoryne* (33%).

	<i>Cyperus rotundus</i>	<i>Cyperus scariosus</i>	<i>Aconitum heterophyllum</i>	<i>Cryptocoryne spiralis</i>
<i>Cyperus rotundus</i>				
<i>Cyperus scariosus</i>				
<i>Aconitum heterophyllum</i>				
<i>Cryptocoryne spiralis</i>				
100% similarity	87% similarity	50% similarity	47% similarity	33% similarity

Figure 6. Similarity matrix between *Ativisha-Musta* candidates based on microscopy studies.

Phytochemical characteristics

Alkaloids were detected in all four species. Saponins were found in *C. scariosus* and *Cryptocoryne* but not in *Aconitum* and *C. rotundus*. Phenols and tannins were detected only in *C. rotundus*. However, it was detected in *C. scariosus* and *Cryptocoryne* histochemically). *A. heterophyllum* shares more or less the same similarity, with reference to phytochemical parameters tested, with other three species (82.3% with *C. rotundus*, 81.5% with *C. scariosus* and 84.6% with *Cryptocoryne*). Interestingly, the two *Cyperus* species share the least similarity of 79.2% while *C. scariosus* shares the highest similarity of 93.85% with *Cryptocoryne spiralis*. This is at great variance with similarity percentage worked out on histochemical, anatomical and powder studies.

HPLC

The results of the HPLC studies of the water extracts (prepared as described in Material and Methods) of the four species are shown in Figure 7. Two portions of the chromatogram are of particular interest, the first from beginning till about a retention time of 15 min. and a second, ranging from 20 to 25 min. For purposes of comparison, the chromatograms of the extracts were normalized by aligning the first peak of each of the chromatograms as it happens to be

the most intense peak.

As can be seen from Figure 7, many common peaks are observed in the region between 3 and 15 min amongst the four species. However, in the region between 20-25 min, common peaks are seen only for *C. rotundus* and *C. scariosus*. The peaks 1, 2, 3, 4 and 6 are common to all four species. Additionally, *C. rotundus* and *C. scariosus* also display four more common peaks (peak nos. 8, 9, 10, 11). Peak 5 is present in all except *C. scariosus* while peak 7 occurs in *A. heterophyllum* and *Cryptocoryne*. These preliminary results suggest that common constituents may be present in the four species. Nine peaks are common between *C. rotundus* and *C. scariosus* while seven peaks are seen in both *A. heterophyllum* and *C. spiralis*. A more detailed study is necessary to draw definite conclusions in this regard.

Discussion

The natural populations of medicinal plants are often unable to meet the demands for many herbal drugs, often due to the very limited geographical distribution of these sources of drugs (Ved & Goraya, 2008) or due to highly reduced population size and consequent threatened status of the taxa. This has led to genuine or arbitrary substitution by other plants (Venkatasubramanian, 2010). While the concept of genuine substitution was in vogue even during the period of Charaka Samhita (Pandeya, 1997) due to the non-availability of the required original drug in specific regions, arbitrary substitution is on the increase in more recent times due to market-driven demands. One such drug complex where substitution (both genuine and arbitrary) is prevalent to a greater extent is the *Ativisha-Musta* complex studied here.

Two aspects of this study merit discussion at length. One pertains to the parameters that need to be employed to identify and authenticate the true ayurvedic *Ativisha* and *Musta*, as different from their substitutes. The other pertains to the therapeutic efficacy of the substituted taxa.

There appears to be no doubt about the botanical correlation as per (The Ayurvedic Pharmacopoeia of India, 1999; 2001) of ayurvedic *Ativisha* and *Musta*, with *A. heterophyllum* and *C. rotundus*, respectively. In the ayurvedic raw drug markets, *Ativisha* is substituted in total or in part by the three other species. *C. rotundus* is relatively very cheap and easily available as it is a weed. *Cryptocoryne*, although not as common as *C. rotundus*, is preferred as a substitute because in South India, it is considered as country *Ativisha* (Nattu Atividayam). The substitution by *C. scariosus* in raw drug markets is more due to ignorance of raw drug collectors to distinguish it in the field from *C. rotundus*. Although there is less than 50% similarity in non-phytochemical parameters studied at present between genuine *Ativisha* and the other three

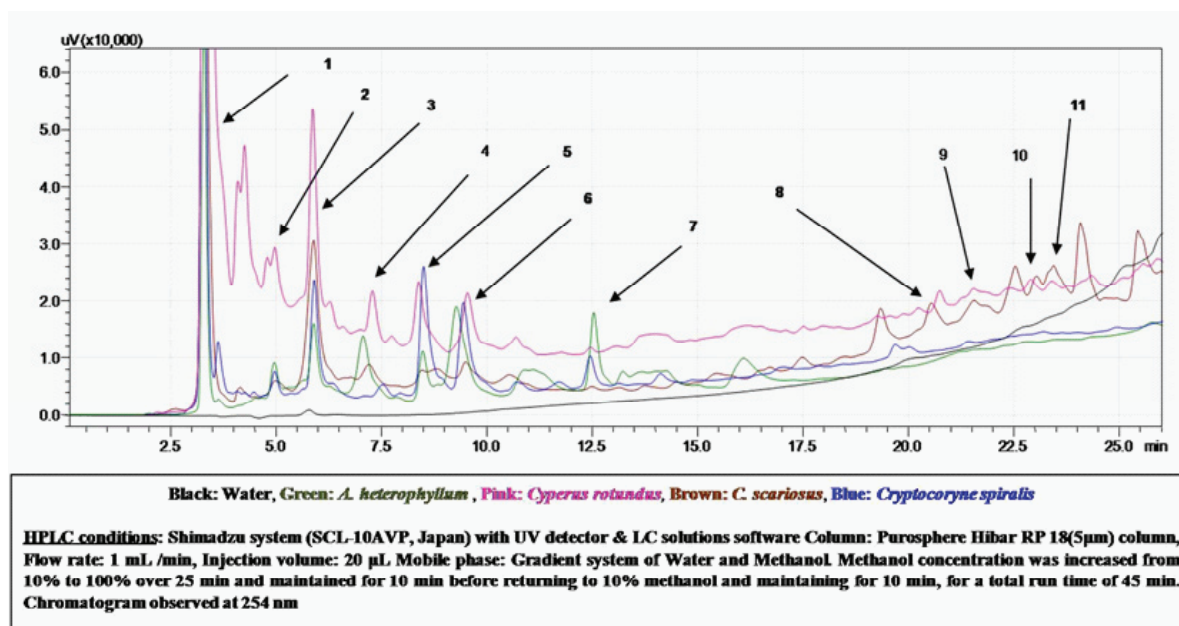


Figure 7. Aligned HPLC chromatograms of *Aconitum heterophyllum*, *Cyperus rotundus*, *Cyperus scariosus* and *Cryptocoryne spiralis* (see text for explanation). Black: water; green: *Aconitum heterophyllum*; pink: *Cyperus rotundus*; brown: *Cyperus scariosus*; blue: *Cryptocoryne spiralis*.

substitutes, lay people are not aware of these differences. Hence, the distinguishing parameters between *Ativisha* and the other three substitutes can serve as characters for authentication of genuine *Ativisha*. These include the tuberous root nature (in others the raw drug is an underground stem), lack of odor, and astringent taste of the raw drug of *Ativisha* while the other three do not have these properties. At the laboratory level, the following structural parameters are helpful in authenticating *Ativisha*: presence of true cork and endodermis, extremely broad parenchymatous stelar region with fewer (4-7) vascular strands (not bundles) embedded in it and the non-detection of terpenoids. *Ativisha* can be distinguished from country *Ativisha* not only by the above characters but also by the lack of raphide crystals. *C. rotundus* can be distinguished from country *Ativisha* by the lack of raphide crystals and also by the different odor and absence of alkaloids. It is often very difficult to distinguish the raw drugs obtained from the two species of *Cyperus* based on internal structure.

Venkatasubramanian et al. (2010) have summarized the similarities in the ayurvedic properties and actions (*Raspanchakas*) of *A. heterophyllum* and *C. rotundus*. There are similarities in taste or *Rasa* (pungent, bitter), property or *Guna* (light, dry) taste after digestion or *vipaka* (pungent), actions or *Karma* (reducing *kapha-pitta*, digesting undigested material, absorbing after digestion, anti-pyretic, and anti-diarrheal) etc. However, they could not definitely attribute these similarities to specific common chemicals in both the taxa. It is, however, likely that the

presence of similar chemicals as detected by the present phytochemical and HPLC studies could be the reason for the similarities in properties and actions recorded by the above authors between these two taxa. It is also likely that different chemicals present in these taxa may cause the same therapeutic actions. Hence, the commonalities and their chemical basis need to be explored at greater depth before making any definite conclusion on this aspect.

The high degree of phytochemical similarity in all four candidates is probably the reason for the high degree of similarity in their therapeutic activities as recorded by many earlier investigators. Whatever differences are there in phytochemical parameters may account for the more specific therapeutic actions exhibited by each one of the taxa. These also need to be ascertained by more detailed individual chemical based therapeutic action studies. The presence of 84% similarity (based on phytochemical profile) and presence of seven common peaks (based on comparative HPLC studies) between *Aconitum* and *Cryptocoryne* may in all likelihood justify the vernacular name “country *Ativisha* (*Nattu Atividayam*)” for *Cryptocoryne spiralis*.

Ativisha, one of the very important raw drugs used in *Ayurveda* and *Siddha*, is often substituted in raw drug markets with substitute and adulterants such as *Cyperus rotundus* (*Musta*), *C. scariosus* and *Cryptocoryne spiralis* (Country *Ativisha*). The present study not only establishes the diagnostic pharmacognostic characters of *Ativisha* as completely as possible but also the differences between it and its substitutes. All the three show less

than 50% similarity to genuine *Ativisha* based on non-phytochemical features, while the two species of *Cyperus* show among themselves a maximum of 80% similarity. Phytochemically, however there was greater similarity between *Aconitum* and *Cryptocoryne* than between either of them and the two *Cyperus* species, justifying the Tamil name Country *Ativisha* for *Cryptocoryne spiralis*. The similarities and differences between the four taxa probably explain, respectively, the observed similarities and uniqueness in therapeutic activities of the four taxa. There appears to be some justification in the substitution of *Ativisha* with the other three species in *Ayurveda/Siddha* medical systems.

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Authors' contributions

SJA contributed in anatomy, histology and histochemistry studies of *Ativisha-Musta* complex under the guidance of KVK. GRK has done the phytochemical and HPLC analyses of *Ativisha-Musta* candidates under the supervision of MN and the research problem was designed by PV. All authors have read and approved of the manuscript.

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