Research Article

Comparative Pharmacognosy of *Asclepias curassavica* Used in Ayurvedic Drug "Kakanasa" with its Adulterant *Leptadenia reticulata*

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ABSTRACT

Asclepias curassavica is the original authentified plant used in ayurvedic drug "Kakanasa". Which is formulated with its root, stem and leaf extracts. Because of number of factors this drug is adultered, substituted making it a controversial drug which is also mentioned in Ayurvedic literature and these adulterations can potentially alter the results in clinicals and reports causing low grade quality, efficacy of these botanical supplements. Our investigation relies on uncertain botanical identity therefore we have focused on comparative pharmacognostical studies of original specimen *Asclepias curassavica* which gets changed and substituted with taxon *Leptadenia reticulata* in formulations of kakanasa. Fine powdered parts of Leaf, Shoot, Root in equal proportions of *Asclepias curassavica* and *Leptadenia reticulata* are analyzed for ash value, organoleptic characters, and fluorescence analysis were undertaken. Comparative monograph of selected taxa showed larger variations in microscopy, anatomy and pharmacognostical evaluation. Our investigations depicted that it is imperative and ambiguous in genuineness of botanical materials in ayurvedic drug market and differentiated between original taxa *Asclepias curassavica* and its substituent *Leptadenia reticulata*.

Keywords: *Asclepias curassavica*, Ayurvedic drug, Kakanasa, *Leptadenia reticulata*, Pharmacognostical studies.

INTRODUCTION

Kakanasa is an ayurvedic drug used as immune booster[1], acts as an emetic and controls edema, vitilligo, hemorrhoids, suppresses aggravated disorders and skin diseases.[2] The plant species *Asclepias curassavica* mentioned in the official pharmacopoeias of Ayurveda have been considered as authentic and legitimate substitutes. In this paper we focused on the comparative pharmacognostical studies of "Kakanasa" the original plant *Asclepias curassavica* with *Leptadenia reticulata* which is a morphological fake and has been substituted or adultered with the original taxa due to some misconceptions. Without proper identification at a starting point, the safe use of quality products cannot be guaranteed. Dried products sold in the medicinal plant trade are generally difficult to identify, as many useful diagnostic characters are lost through desiccation.[3] The overall objectives of the present paper investigates comparative Anatomy, Micro and Macroscopic, Flourescence studies have been done to authenticate the raw material of original and substituent which is adultered in the herbal drug market. Other important tools for understanding this drug adulteration include organoleptic methods (personal sensory based information), such as heating and cooling effects, tastes, and physically felt actions.[4] Because of number of factors the botanical specimens were adultered or contaminated and these adulterations can potentially alter the results in clinical and reports causing subtle variations on quality, efficacy of these botanical supplements.[5]

As Kakanasa is described as controversial drug in Ayurvedic literature, we made an attempt on the controversial causes of the drug. This research is a best example in this framework which brings to light the mystifications that exist in requisites of botanical sources allied to Ayurvedic drug adulterations with comparative pharmacognostical studies. The detailed and systematic pharmacognostical evaluation and standards to distinguish the former from the latter gives valuable information on drug adulteration.

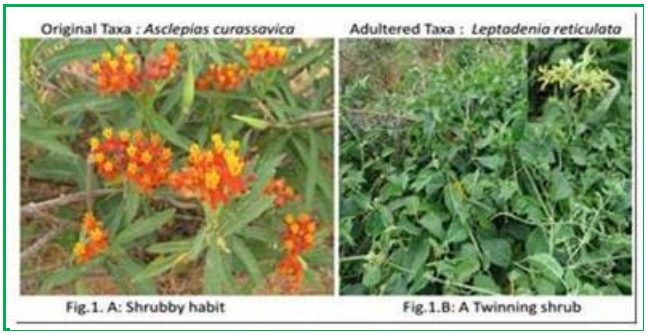
Botanical Description of original and adultered taxa

Asclepias curassavica L.[6] (Family: Asclepiadaceae) is an evergreen perennial sub shrub that grow upto 1 m tall and have pale gray stem. The leaves are lanceolate or oblong-lanceolate shaped ending in acuminate or acute tip. The sap is milky. The flowers are in cymes with 10-20 flowers each. They have purple or red corollas seed ovoid, long dark brown.[7]

Leptadenia reticulata (Retz.) Wight & Arn.[8] (family Asclepiadaceae). is a twinning shrub, stem with corky deeply cracked bark, branches numerous. Leaf is thinly coriaceous, ovate, acute, glabrous. Flowers bisexual, greenish yellow, globose cymes. Calyx pubescent, outside divided to about the middle segments 1.25 mm long. Corolla 5 mm long. Coralline corona of 5 quadrate truncate fleshy lobes at the sinuses, stamina corona minute, annular, close to the stamina column. Seeds 6 mm long, narrowly ovate-oblong acute; coma 3.2-3.8 cm long.[9] The basionym *L. reticulata* is *Cynanchum reticulatum* Retz. In Ayurvedic Literature it is called as Jiwanti.

Previous workers reported the whole plant of *Asclepias curassavica* is used as an emetic, purgative and in the treatment of the piles, gonorrhea, anthelmintic

sudoriffc.[2,10] It has rich source of cardiac glycosides in the Latex.[11] whole plant extract of *Leptadenia reticulata* ameliorates tridosha's and is of great value in general debility, involuntary seminal discharge and as a stimulant.[12]



MATERIALS AND METHODS

Plant specimens (*Asclepias curassavica*- Accs No: SVUTY-ALP/0618; *Leptadenia reticulata*- Accs No: SVUTY-APC/1076) were collected using field visits in Seshachalam Reserve Forest and deposited at Department of Botany, S.V University, Tirupati for further reference. Crude plant raw materials collected from Srinivasa Ayurvedic Pharmacy and local medicinal plant merchants at herbal markets of Tirupati, Chittoor district of Andhra Pradesh for the investigation. Macroscopic, microscopic and chemomicroscopic studies (presence of tannins, lignin, oil and calcium oxalate crystals) on the fresh, powdered and anatomical sections of the plant parts were carried out for the purpose of identification and monograph preparation was done. Descriptive terms of the anatomical features were used as per the terminology found in standard anatomical books of Easu[13] and Fahn.[14]

Macroscopy and Microscopic Study

The morphological characters were reconfirmed by using various Local Floras of Gamble JS[15] and Madhava Chetty *et al.*[16] Permanent mount of plant parts was prepared using saffron in fast green stain by double staining technique following the protocol suggested by Johansen DA.[17] Transverse sections of *Asclepias curassavica* and *Leptadenia reticulata* were taken by using a model MTH-1 (NK System) microtome.[18,19] The light micrographs of photographs were taken by means of an images were obtained with a digital camera (DP x 26, Olympus) attached to a light microscope (BX-50,Olympus). For the study of crystals, starch grains and lignified cells, polarized light was employed. Magnifications of the figures were indicated by the scale bars.

Physicochemical Studies

Physicochemical parameters were determined as per guidelines of WHO.[20] Total ash value, loss on drying, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value were determined by taking the powdered leaf ,stem and root in equal proportions of both the taxa.[21,22]

Fluorescence analysis of the whole plant powder was carried out according to the methods followed by Chace and Pratt[23] and Kokoski *et al.*[24]

RESULTS

Anatomy of *Asclepias curassavica*

Microscopic Features

The midrib is broadly convex and semicircular on the abaxial side and shallow wide concavity on the adaxial side. The vascular strand is 500 μm thick wide and 160 μm thick (Figure 1.1). The marginal part of the lamina is blunt, semicircular and 200 μm thick (Figure 1.2). The epidermal cells are fairly thick walled, their anticlinal walls are highly wavy and the cells appear amoeboid (Figure 2.1). The druses are small and diffuse in distribution (Figure 2.2). They are 10 μm in diameter. The vein-terminations are simple unbranched and curved (Figure 3.1). The vein terminations are branched repeatedly giving rise to dendroid outline of the terminations (Figure 3.2).

The xylem cylinder is 700 μm thick. Secondary xylem includes narrow straight xylem rays, angular vessels and xylem fibers (Figure 4.1). The root is 2.6 mm thick showing well developed periderm, secondary phloem and secondary xylem (Figure 5.1). The cortex includes three to five layers of parenchyma cells including a single layer of cortical fibers (Figure 5.2).

Crystal Distribution

Calcium oxalate crystals of druses are sparsely distributed in the mesophyll cells. The druses are small and diffuse in distribution with 10 μm in diameter. Crystals are sparely distributed in the cortex (Figure 2.2 & 5.3).

Powder Microscopy

Powder or macerated preparation of the plant shows mostly fibers and vessel elements.

Wide fibers: The wide fibers are short and spindle shaped (Figure 6.1). The walls are thin and the lumen is wide. No pits are seen on the walls cells inclusions also absent. The wide fibers are 350 μm long and 20-30 μm wide (Figure 6.2).

Anatomy of *Leptadenia reticulata*

Leaf

The leaf consists of planoconvex midrib and dorsiventral lamina (Figure 1.1). The midrib is flat on the adaxial side and convex on the abaxial side with 600 μm thick and 700 μm wide. The epidermal layers of the midrib have small, squarish, thick walled cells; non glandular, uniseriate unbranched trichomes are common on the midrib (Figure 1.2). The abaxial convex portion of the midrib comprises homogeneous parenchyma cells with flat portion includes small mass of collenchyma cells.

The vascular strand is single, arc shaped and bicollateral (Figure 1.3). It consists of short, parallel lines of thick

walled, angular multiples of xylem elements with 3 or 5 elements in each row. Small nests of phloem elements are located both on the adaxial and abaxial parts of the xylem arc (Figure 1.3).

Epidermal cells and stomata

The adaxial epidermis as seen in surface view consists of polyhedral, thin walled cells with straight anticlinal walls and apostomatic (without stomata). The adaxial epidermis (Figure 2.1).

The abaxial epidermis is stomatiferous with paracytic type consisting of two subsidiary cells, one on either side or parallel to guard cells (Figure 2.2). The guard cells are elliptic in shape measuring 20 x 30 μm in size (Figure 2.3).

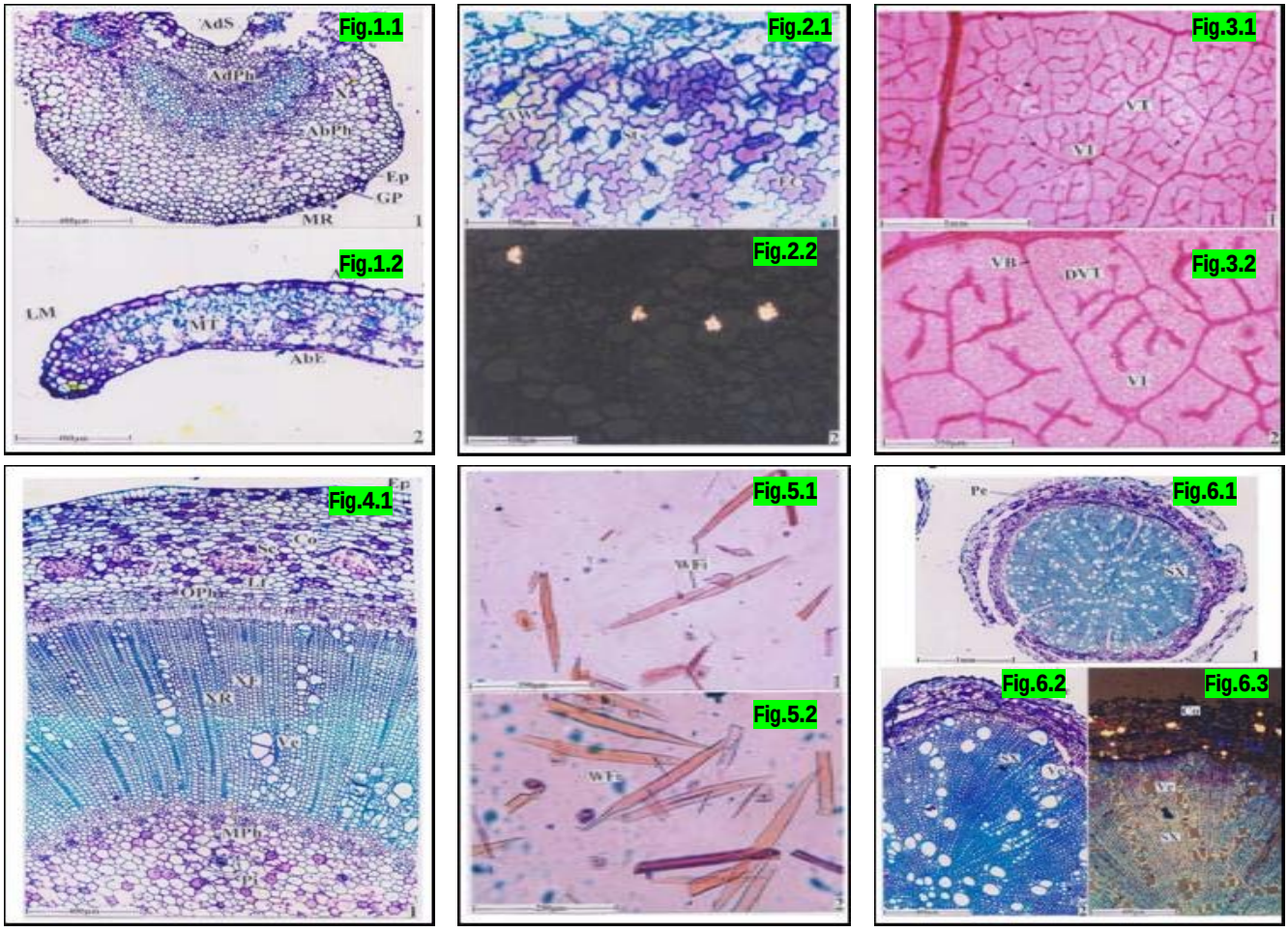


Figure 2: Anatomy And Powder Microscopy of *Asclepias curassavica*

Legend for the Figures

Figure 1.1: T.S. of midrib; Figure 1.2: T.S. of leaf margin; Abph: Abaxial phloem; AdE: Adaxial epidermis; AdS: Adaxial side; Ep: Epidermis; Gp: Ground plan; LM: Leaf margin; MR: Midrib; MT: Mesophyll tissue; X : Xylem; Adph: Adaxial Phloem; AbE: Abaxial epidermis; Figure 2.1: Paradermal view of epidermal cells and stomata; Figure 2.2: Calcium oxalate crystals in the mesophyll tissue as seen under polarized light; Aw: Anticlinal wall; EC: Epidermal cell; St: Stomata; Figure 3.1: Venation pattern; Figure 3.2: Vein-islet and vein terminations enlarged; DvT: Dendroid vein termination; VB: Vein boundary; VI: Vein-islet; VT: Vein-termination; Figure 4.1: T.S. of petiole entire view; Figure 4.2: A sector enlarged; Lf: Laticifer; Vs: Vascular strand; Figure 5.1: T.S. of stem showing cortex, outer of inner phloem and secondary xylem; Lf: Laticifer; Sc: Sclerides; XF: Xylem fibre; XR: Xylem ray; Figure 6.1: T.S. of root entire view; Figure 6.2: A sector enlarged; Figure 6.3: Crystals in the cortex (polarized light);.Sph: Secondary phloem.

Laticifer

Narrow, unbranched laticiferous canal is seen situated within the vascular strand. The laticifer runs all along the veins. It is darkly stained (Figure 2.3).

Crystals

Calcium oxalate crystals are abundant in the epidermal cells. Crystals in the form of thin short needles which aggregated into prominent fan-shaped masses. Fan shaped masses of needles of crystals are again aggregated into large groups which are mostly stellate in appearance (Figure 3.1, 2, 3)

Stem

Stem measuring 1.9 mm thick was studied. The stem is fairly young with limited extent of secondary growth.

The epidermis is thin and intact. Cells are small and squarish. Cortex is heterogeneous. It consists of outer 2

or 3 layers of chlorenchyma and inner entire region of parenchyma. In the median zone of the cortex occur several large masses of sclerenchyma arranged in a regular ring (Figure 4.1, 2).

Root

Thin root, measuring 1 mm in diameter consists of a narrow superficial periderm which is 4 layered. The cells are thin walled and tabular in shape; they are suberised. Secondary phloem elements are in compact radial row's radiating from the xylem core. Inner to the periderm is a narrow zone of 6 or 7 layers of compact cortical parenchyma cells. (Figure 5.1)

The vascular cylinder is circular with central dense core of xylem surrounded by outer zone of secondary phloem. Secondary xylem comprises much wider vessels as well narrow vessels, both type being intermixed. The vessels are circular and thick walled. The vessels are surrounded by highly thick walled and lignified fibers (Figure 5.2).

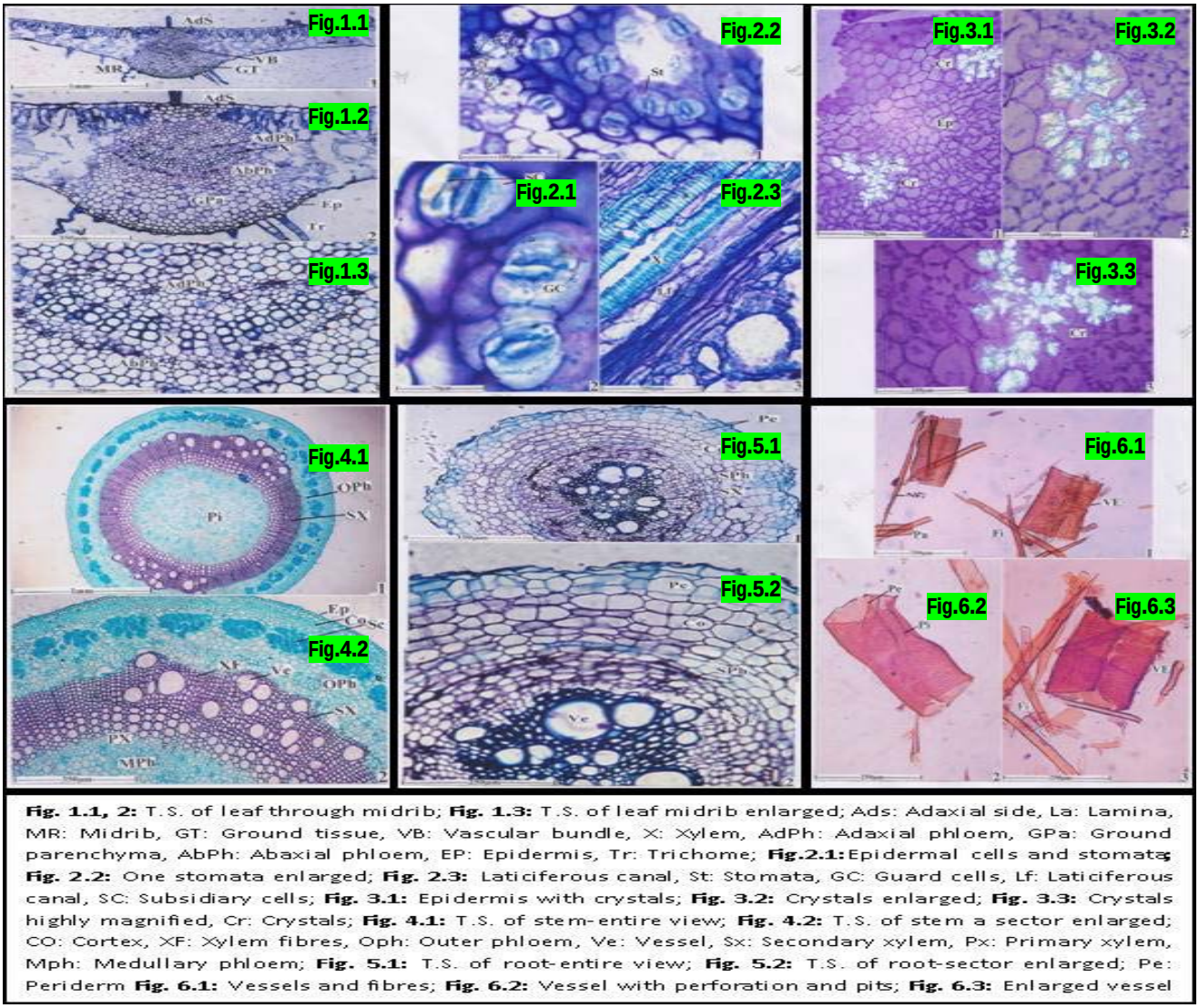


Fig. 1.1, 2: T.S. of leaf through midrib; **Fig. 1.3:** T.S. of leaf midrib enlarged; Ads: Adaxial side, La: Lamina, MR: Midrib, GT: Ground tissue, VB: Vascular bundle, X: Xylem, AdPh: Adaxial phloem, GPa: Ground parenchyma, AbPh: Abaxial phloem, EP: Epidermis, Tr: Trichome; **Fig.2.1:** Epidermal cells and stomata; **Fig. 2.2:** One stomata enlarged; **Fig. 2.3:** Laticiferous canal, St: Stomata, GC: Guard cells, Lf: Laticiferous canal, SC: Subsidiary cells; **Fig. 3.1:** Epidermis with crystals; **Fig. 3.2:** Crystals enlarged; **Fig. 3.3:** Crystals highly magnified, Cr: Crystals; **Fig. 4.1:** T.S. of stem-entire view; **Fig. 4.2:** T.S. of stem a sector enlarged; CO: Cortex, XF: Xylem fibres, Oph: Outer phloem, Ve: Vessel, Sx: Secondary xylem, Px: Primary xylem, Mph: Medullary phloem; **Fig. 5.1:** T.S. of root-entire view; **Fig. 5.2:** T.S. of root-sector enlarged; Pe: Periderm **Fig. 6.1:** Vessels and fibres; **Fig. 6.2:** Vessel with perforation and pits; **Fig. 6.3:** Enlarged vessel

Figure 3: Anatomy and Powder Microscopy of *Leptadenia reticulata*

Table 1: Powder characteristics of the drug

Name of the plant	Colour	Appearance	Odour	Taste
Asclepias curassavica	Green	Fine powder	No characteristic	Bitter
Leptadenia reticulata	Light green	Fine powder	No characteristic	No characteristic

Powder microscopic observations

The powder of the plant exhibits the following inclusions. There are narrow and wide fibers in the powder.

Vessel elements

The vessel elements are wide and cylindrical. They have wide, circular perforations at end walls; the perforation is horizontal in orientation. Dense, multiseriate bordered

pits are abundant on the lateral walls. The vessel elements are 250-300 μm long. (Figure 6.1, 2, 3)

Organoleptic and Powder Microscopy Comparison (Table 1-8)

Comparative organoleptic characters revealed that in both the plants have similar properties since. There is a possibility of adulterations. Table 1 gives the stated character, powder analysis differed when the whole plant powder is treated with 5% aqueous NaOH in coloration.

Ash, solubility values, extractive values showed differences (Table 3, 4, 5). Fluorescence analysis of various extracts of the drug eluted various colors for both the plants which are described in Table 6. In Table 7 & 8, fluorescence analysis of the drug powder of *Asclepias curassavica* and *Leptadenia reticulata* plant powders were tabulated. These two plants showed variation under the fluorescence microscopy.

Table 2: Powder analysis of the drug

Treatment	Observation	
	Asclepias curassavica	***Leptadenia reticulata***
Powder treated with water	Non-sticking	Non-sticking
Powder shaken with water	Foam like froth	Foam like froth
Powder treated with 5% aqueous NaOH	Green	Brown
Powder treated with 60% aqueous sulphuric acid	Black	Green
Powder pressed between filter paper for 24 hours	No oil stain	No oil stain

Table 3: Ash values of the drug

Name of the plant	Total ash (% w/w)	Water soluble ash (% w/w)	Alkalinity of water soluble ash (ml)	Acid in soluble ash (% w/w)
Asclepias curassavica	9.02	35.65	0.5	59.01
Leptadenia reticulata	7.45	22.45	0.4	57.41

Table 4: Solubility values of the drug

Name of the plant	Ethanol (% w/w)	Water aq. (% w/w)	Methanol (% w/w)
Asclepias curassavica	76.11	33.12	83.43
Leptadenia reticulata	69.45	29.74	75.36

Table 5: Extractive values of the drug

Name of the plant	Ethanol soluble extract (% w/w)	Water soluble extract (% w/w)	Hexane soluble extract (ml)	Chloroform soluble extract
Asclepias curassavica	66.89	25.11	7.50	63.01
Leptadenia reticulata	59.74	19.46	6.72	54.13

Table 6: Fluorescence analysis of various extracts of the drug

Extract	Treatment	Observation	
		Asclepias curassavica	***Leptadenia reticulata***
Ethanol	Day light Short U.V Long U.V	Pale green Green Dark green	Green Dull green Red
Water	Day light Short U.V Long U.V	Green Green Blue	Green Pale green Very pale green
Hexane	Day light Short U.V Long U.V	Pale green Pale green Green	Dull green Greenish yellow Pale green
Chloroform	Day light Short U.V Long U.V	Pale blue Light green Green	Very pale green Pale green Green

Table 7: Fluorescence analysis of the drug powder of *Asclepias curassavica*

Experiments	Visible / Day light	U.V. Light	
		254 nm	365 nm
Drug powder	Green	Brown	Black
Drug powder + 1N NaOH (aq.)	Green	Brown	Black
Drug powder + 1N NaOH (alc.)	Dark green	Black	Black
Drug powder + 1N HCl	Green	Black	Black
Drug powder + 50% H_2SO_4	Green	Black	Black
Drug powder + 50% HNO_3	Brown	Black	Black
Drug powder + picric acid	Brown	Black	Black
Drug powder + Ferric chloride	Green	Green	Green
Drug powder + HNO_3 + NH_3	Green	Green	Black

Table 8: Fluorescence analysis of the drug powder of *Leptadenia reticulata*

Experiments	Visible / Day light	UV light	
		254 nm	365 nm
Drug powder	Light green	Black	Black
Drug powder + 1 N NaOH (aq.)	Brown	Black	Black
Drug powder + 1 N NaOH (alc.)	Green	Black	Black
Drug powder + 1 N HCl	Dark green/Brown	Black	Black
Drug powder + 50% H_2SO_4	Pale brown/green	Green	Green
Drug powder + 50% HNO_3	Brown/Green	Dark green	Dark green
Drug powder + Picric acid	Brown	Dark Brown	Dark Brown
Drug powder + Ferric chloride	Brown/green	Brownish black	Brownish black
Drug powder + HNO_3 + NH_3	Brown/Green	Green	Light green

DISCUSSION

The authentified botanical source of the drug Kakanasa is *Asclepias curassavica*.[25] The originality of plant drugs in terms of raw material extraction, preparation and marketability requires proper guide lines according to WHO standards.[20]

Macroscopic and microscopically these two taxa are different *Asclepias curassavica* is an erect herb but *Leptadenia reticulata* is a twinning shrub. The vascular bundles are bowl shaped in *A. curassavica*. But in *L. reticulata* the vascular cylinder consists of crowded clusters of vessels at two opposite poles of the vascular cylinder. Physicochemical constituents like ash, extractive and solubility values are more in *A. curassavica* when compared with *L. reticulata*.

Our previous works on Kakanasa investigated the causes of controversy, and documented comparative pharmacognostical and phytochemical analysis of original taxa *Asclepias curassavica* L. used in kakanasa with its adulterant taxa,*viz*., *Pentatropis capensis* (L. f.) Bullock., (Apocynaceae), *Trichosanthes cucumerina* L (Cucurbitaceous), *Dicliptera paniculata* (Forssk.) I.Darbysh. (Acanthaceae) and *Martynia annua* L. (Martyniaceae) was resolved and authentified.[26-30]

Previous workers reported on *L. reticulata* and proved it as a valuble medicinal plant.[31-37] Though *Leptadenic reticulata* is also an important taxa in medical therapeutics it has been substituted as spurious plant by the unscrupulous manufacturers of Ayurvedic medicine.

It is clear from our research there is quite a bit of uncertainty in terms of the right botanical entity, plant part or substitutes used. It has brought to light issues that need to be resolved in terms of authenticity of correlation of Ayurvedic entities to botanical sources like Kakanasa.[38]

CONCLUSION

In conclusion the present studies have revealed differentiation between the genuine taxa with the pharmacognostical, physicochemical and fluorescence analysis studies which will be useful for deciding the identity, quality, purity, strength and efficacy of the drug Kakanasa if it is prepared with genuine taxa *Aclepias curassavica* and also essential for providing authentic scientific characterization and identification of the other drugs used in Ayurveda and Siddha systems. Our Investigation relies in establishing Pharmacopoeial standardization profiles of phytodrugs.

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