Vol III Issue VII August 2013
Impact Factor : 0.2105 ISSN No : 2230-7850

Monthly Multidisciplinary
Research Journal

Indian Streams Research Journal

IMPACT FACTOR : 0.2105

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RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

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Indian Streams Research Journal
ISSN 2230-7850
Volume-3, Issue-7, August-2013

Comparative Pharmacognostical Studies Of Asclepias Curassavica Used In Ayurvedic Drug " Kakanasa " With Its Adulterant Martynia Annua

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Abstract:"Kakanasa" an Ayurvedic drug used as emetic, and controls edema, haemorrhaids, vitiligo and other skin diseases is formulated with plant (root, stem, leaves) extracts of Asclepias curassvica. Because of number of factors the botanical specimens were adultered or contaminated and these adulterations can potentially alter the results in clinicals and reports causing suitable variations to have effects on quality, efficacy of these botanical supplements: we focused pharmacognostical studies of original specimen Asclepias curassavica which gets changed and substituted with other taxon Martynia annua in formulations of Ayurvedic drugs. Comparative pharmacognostic evaluation of fine powdered whole parts of Asclepias curassavica and Martynia annua for ash analysis, organoleptic characters, fluorescent analysis were undertaken. Comparative monograph of selected taxa depicted that showed larger variations in plant (root, stem, leaf, powder microscopy) anatomy and pharmacognostical evaluation. Our investigations showed that it is vital that authenticity of botanical materials in ayurvedic drug market should be focused.

Keyword: Asclepias curassavica, Martynia annua, anatomy.

INTRODUCTION:

Medicinal plants research is an integral component focusing on isolation, extraction, formulation of natural metabolites which is a crucial development for pharmaceutical industries to yield its synthetic form and its analogues in the development of active drugs and compounds against different ailments. It is well known that in course of time, drug-materials get changed or substituted with other plant species. "Kakanasa" as Ayurvedic drug used as a emetic and controls edema, haemorrhaids, vitiligo and other skin diseases is a best example in this context. In this paper we focused on the comparative pharmacognostical studies of Asclepias curassavica the original plant used in ayurvedic drug "Kakanasa" with its adulterant Martynia annua which is a morphological fake.

The pharmacognostical study is the major and reliable criteria for identification of plant drugs. Accurate plant identification is the foundation of the safe use of plant based natural health products in pharmaceutical sciences. 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. Many herbs are sold through brokers where the material can change hands several times. The originality of herbal drugs in terms of raw material extraction, preparation and marketability requires proper guide lines according to WHO standards (WHO, 2003). It is well known that in course of time, drug materials get changed to or substituted with other plant species (Muhammad Zafar, 2011). The overall objectives of the present paper is the use classical and modern techniques of chemotaxonomy to authenticate the original raw material of correct species marketed. The detailed and systematic pharmacognostical evaluation gives valuable information for future studies.

BOTANICAL DESCRIPTION

Asclepias curassavica L. (Genus Asclepias, family Asclepiadaceae) plants are evergreen perennial sub shrubs that grow upto 1 m tall and have pale gray stems. The leaves are lanceolate or oblong-lanceolate shaped ending in acuminate or acute tips. 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. Kakanasa (Asclepias curassavica) is a common medicinal plant used in the ayurvedic system of medicine to treat various ailments Madhava Chetty et al. (2013).

Martynia annua L. (Genus Martynia, family Martyniaceae) carpoceras anqualata A. Rich. is a synonym of Martynia annua L. plant is a stout herb with purplish green glandular hairy and sticky stem. Flowers are purple yellowish with one dark spot on each lobe. The fruit is a drupe with two apical curved beaks. This plant leaves are used for healing wounds and also apply to tuberculous glands of the neck.

ISSN 2230-7850
Volume-3, Issue-7, August-2013

Asclepias curassavica **L.**

Fig. A: Shruby habit

Martynia annua **L.**

Fig. B: Stout herby habit

MATERIALS AND METHODS

Plant speciemens were collected using field visits crude plant raw materials collected from Srinivasa Ayurvedic Pharmacy and local medicinal plant vendours at herbal markets of Tirupati, Chittoor district of Andhra Pradesh. 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.

MICROSCOPIC STUDY

Transverse sections of Asclepias curassavica and Martynia annua were taken by using a microtome. Permanent mount of plant parts was prepared using saffron in fast green stain by double staining technique (Johansen DA 1940). The morphological characters were reconfirmed by using various Floras of Gamble (1957), Thammanna et al. (1994) and Madhava Chetty et al. (2013). 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. Total ash value, loss on drying, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value were determined (Anonymous 1996 and 2009).

Fluorescence analysis of the whole plant powder drug was carried out according to the methods followed by Chace and Pratt (1949). The fluorescence property of the powder is observed both in visible and ultraviolet for their fluorescence characters short wave length 254 nm and long wave length 365 nm after treatment with various chemical reagents.

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 m thick wide and 160 m thick (Fig. 1.1). The marginal part of the lamina is blunt, semicircular and 200 m thick (Fig. 1.2). The epidermal cells are fairly thick walled, their anticlinal walls and highly wavy and the cells appear amoeboid (Fig. 2.1). The druses are small and diffuse in distribution (Fig. 2.2). They are 10 m in diameter. The vein-terminations are simple unbranched and curved (Fig. 3.1). The vein terminations are branched repeatedly giving rise to dendroid outline of the terminations (Fig. 3.2). The petiole is circular in sectional view with steep, V-shaped groove on the adaxial side (Fig. 4.1).

The ground tissues are small thick walled darkly stained laticifers (Fig. 4.2). The thin stem has thin, continuous intact epidermis; periderm at isolated region of the epidermis. The cortex is 400 m thick (Fig. 5.1, 2). The xylem cylinder is 700 m thick. Secondary xylem includes narrow straight xylem rays, angular vessels and xylem fibres (Fig. 6.1). Medullary phloem are in circular units, the cells being small and compact (Fig. 7.2). The vessels lines are separated from each other by wide gaps of xylem fibres (Fig. 7.1, 2). The root is 2.6 mm thick; shows well developed periderm and secondary phloem and secondary xylem (Fig. 8.1). The cortex includes three to five layers of parenchyma cells including a single layer of cortical fibres (Fig. 8.2). Secondary phloem consists of outers zone collapsed phloem elements and inner zone of intact non-collapsed phloem elements (Fig. 9.1). The xylem fibres are thick walled and lignified (Fig. 9.2, 3).

CRYSTAL DISTRIBUTION (Fig. 2.2 & 8.3)

Calcium oxalate crystals of druses are sparsely distributed in the mesophyll cells. The druses are small and diffuse in distribution (Fig. 2.2). They are 10 m in diameter. Crystals are sparely distributed in the cortex. The crystals are druses (Fig. 8.3).

POWDER MICROSCOPY

Powder or macerated preparation of the plant shows mostly fibres and vessel elements. The fibres are two types:

(i)Narrow fibres (Fig.10.1, 2): The narrow fibres are long thick walled with narrow lumen. Some of the narrow fibres have prominent simple pits on their walls (Fig. 10.1). They are 400-500 m long and 12 m wide.
(ii)Wide fibres (Fig. 10.1; 11.1, 2): The wide fibres are short and spindle shaped (Fig. 10.1). The walls are thin and the lumen is wide (Fig. 11.2). No pits are seen on the walls cells inclusions also absent. The wide fibres are 350 m long and 20-30 m wide.

VESSEL ELEMENTS (Fig. 12.1, 2)

The vessel elements characteristically short wide and barrel shaped (Fig. 12.1). They have district circular bordered pits which are multiseriate and dense. The perforation is simple, wide and horizontal. The vessel elements are 190-260 m long (Fig. 12.2).

ANATOMY OF MARTYNIA ANNUA

The midrib hangs down from the thin lamina. It is 1.4 mm wide and 1.5 mm thick on the abaxial part also there are 2 or 3 layers of collenchyma layers (Fig. 1.1). Phloem occurs in small groups along the periphery of the xylem strand (Fig. 2). There is a small collateral vascular strand

ISSN 2230-7850
Volume-3, Issue-7, August-2013

placed in the central part of the vein (Fig. 1.2). The marginal part of the lamina is slightly bulged measuring 180 m thick (Fig. 1.3). The epidermal cells are amoeboid in outline (Fig. 3.1). The guard cells are circular with wide stomatal pore (Fig. 3.2). The veinlets with wide stomatal pore (Fig. 3.2). The veinlets are thin, yet they are district (Fig. 4.1). The vein terminations are branched repeatedly to form dendroid outline (Fig. 4.2). The petiolar tissues comprise epidermis outer ground tissue, vascular cylinder and inner ground tissue (Fig. 5.1). The outer ground tissue is differentiated into about five layers of collenchyma followed by five layers of fairly large thin walled and compact parenchyma cells (Fig. 5.2). The epidermis bear dense epidermal trichomes. The vascular cylinder is circular and closed. It is 30 m thick (Fig. 6.1, 2). Secondary xylem is a circular wide dense cylinder of vessels and fibres (Fig. 7.1, 2). The ground tissue of the xylem is fibres which posses thick lignified walls and narrow lumen (Fig. 7.3). Periderm is superficial in position and it is 150 m thick. Shallow fissures are frequently seen on the periderm (Fig. 8 & 9). Cortex is wide, homocellular and parenchymatous. It is 800 m wide. Some of the cells contains mucilage (Fig. 9). The vessels are narrow in the centre and become gradually wider towards the periphery. They are angular to circular; solitary or in multiples of two or three (Fig. 10).

POWDER MICROSCOPIC OBSERVATIONS

The powder and macerated preparations of the plant show the following elements when examined under the microscope. Clavate type of trichome is one of the trichomes found in the powder (Fig. 11.1, 2). The trichome is 250 m in height and 100 m thick. The peltate type of glandular trichome have 2 or 3 celled stalk. The trichome is 280 m long; the secretary body is 40-80 m wide (Fig. 12.1, 2, 3).

1. Xylem elements (Fig. 13, 14, 15)

The xylem elements include mostly fibres and vessel elements (Fig. 13.1, 2). The fibres are of two types:

a) Wide fibres
b) Narrow fibres

a)Wide fibres (Fig. 14.2): The wide fibres are more or less spindle shaped; they are thick in the middle and abruptly tapering at the ends. This walls are thin and the cell lumen is wide.
b)Narrow fibres (Fig. 14.1, 2): The narrow fibres are uniformly narrow and gradually tapering. Their walls are thick and the cell lumen is narrow. They are 700-750 m long and 8-10 m wide.

2) Vessel elements (Fig. 13.1, 2; 15.1, 2): Vessel elements are less abundant than the fibres. The vessel elements are narrow, cylindrical and mostly tailed. They have wide circular multiseriate bordered pits an the lateral walls. The perforation plate is wide and oblique or horizontal. The vessel elements are 250-400 m long.

Table-1: Comparative studies on measurements of tissues and cells in root, stem & leaf

Organ	Measurements ( )	
Root	***Asclepias curassavica***	***Martynia annua***
Thickness of Root	2.6 mm	2-2.5 mm
Secondary xylem	1.9 mm	1.8 mm
Vessels	30-70 m	50-100 m
Stem		
Vascular cylinder	700 m	300 m
Cortex	350 m	500 m
Leaf		
Thickness leaf margin	200 m	180 m
Thickness of lamina	250 m	220 m

ORGANOLEPTIC AND POWDER MICROSCOPY COMPARISON (Table 2-9)

Comparative organoleptic characters revealed that in both the plants have similar properties since. There is a possibility of adulterations. Table 2 gives the stated character, powder analysis differed when the whole plant powder is treated with 5% aq. NaOH in colouration (Table-3).

Table-2: Powder characteristics of the drug

Name of the plant	Colour	Appearance	Odour	Taste
Asclepias curassavica	Green	Fine powder	No characteristic	Bitter
Martynia annua	Light brown	Fine powder	No characteristic	Very bitter

Ash, solubility values, extractive values showed differences (Table 4, 5, 6). Fluorescence analysis of various extracts of the drug eluted various colours for both the plants which are described in Table 7. In Table 8 & 9 fluorescence analysis of the drug powder of Asclepias curassavica and Martynia annua plant powders are tabulated. These two plants showed variation under the fluorescence microscopy.

Table-3: Powder analysis of the drug

Treatment	Observation	
	Asclepias curassavica	***Martynia annua***
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-4: 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
Martynia annua	8.45	23.66	0.4	56.33

Table-5: 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
Martynia annua	74.23	32.43	82.43

Table-6: 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
Martynia annua	65.23	24.84	7.46	62.56

Table-7: Fluorescence analysis of various extracts of the drug

Extract	Treatment	Observation	
		Asclepias curassavica	*Martynia annua*
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-8: 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-9: Fluorescence analysis of the drug powder of Martynia annua

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

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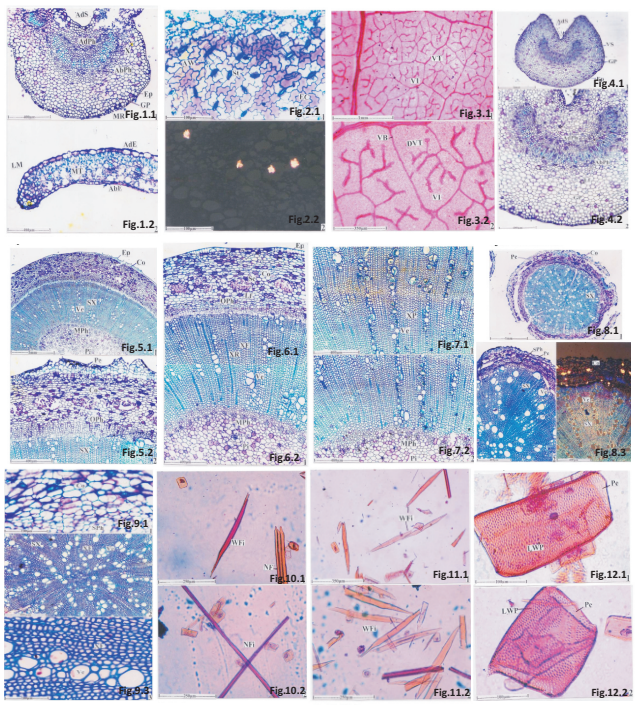

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Fig. 1.1: T.S. of midrib; Fig. 1.2: T.S. of leaf margin; Abph: Abaxial phloem; AdE: Adaxial epidermis; AdS: Adaxial side; Ep: Epidermis; Gp: Ground plan; LM: Leaf margin; MR: Midrib; MT: Mesophyll tissue; X : Xylem; Adph: Adaxial Phloem; AbE: Abaxial epidermis; Fig. 2.1: Paradermal view of epidermal cells and stomata; Fig. 2.2: Calcium oxalate crystals in the mesophyll tissue as seen under polarized light; Aw: Anticlinal wall; EC: Epidermal cell; St: Stomata; Fig. 3.1: Venation pattern; Fig. 3.2: Vein-islet and vein terminations enlarged; DvT: Dendroid vein termination; VB: Vein boundary; VI: Vein-islet; VT: Vein-termination; Fig. 4.1: T.S. of petiole entire view; Fig. 4.2: A sector enlarged; Lf: Laticifer; Vs: Vascular strand; Fig. 5.1: T.S. of stem half sector; Fig. 5.2: T.S. of stem periderm, cortex and outer phloem enlarged; Co: Cortex; Mph: Medullary phloem; Oph: Outer phloem; Pe: Periderm; Pi: Pith; Sx: Secondary xylem; Ve : Vessel; Fig. 6.1: T.S. of stem showing cortex, outer of inner phloem and secondary xylem; Lf: Laticifer; Sc: Sclerides; XF: Xylem fibre; XR: Xylem ray; Fig. 7.1: T.S. of old stem showing radial lines of vessel of fibres with circular masses of medullary phloem; Fig. 8.1: T.S. of root entire view; Fig. 8.2: A sector enlarged; Fig. 8.3: Crystals in the cortex (polarized light); Sph: Secondary phloem; Fig. 9.1: T.S. of root phloem zone; Fig. 9.2: Secondary xylem along the middle point; Fig. 9.3: Secondary xylem showing vessels and fibres; Fig. 10.1: Wide fibres; Fig. 10.2: Narrow fibres; NFi: Narrow fibre; WFi: Wide fibre; Fig. 11.1, 2: Wide fibres; Fig. 12.1,2: Vessel elements with wide perforation and dense lateral wall pits; LWP: Lateral wall pits; Pe: Perforation.

LEGEND FOR THE FIGURES

Fig. 1.1: T.S. of leaf through midrib; Fig. 1.2: T.S. of leaf through later view; Fig. 1.3: T.S. of leaf margin; Abp: Abaxial phloem; AdC: Adaxial cone; Ep: Epidermis; GT: Ground tissue; Vs: Vascular strand; AdS: Adaxial side; La: Lamina; LM: Leaf margin; AbE: Abaxial epidermis; AdE: Adaxial epidermis; Ph: Phloem; Pm: Palisade mesophyll; SM: Spongy mesophyll; Lv: Lateral view; Gtr: Glandular epidermal trichome; Fig. 2.1: T.S. of midrib; Col: Collenchyma; X: Xylem; Fig. 3.1: Paradermal section of the lamina; Fig. 3.2: Stomata and epidermal cells enlarged; EC: Epidermal cell; St: Stomata; Fig. 4.1: Venation pattern; Fig. 4.2: Vein islet of vein termination; VI: Vein islet; VT: Vein termination; Fig. 5.1: T.S. of petiole; Fig. 5.2: T.S. of petiole-A sector; Col: Collenchyma; IGT: Inner ground tissue; OGT: Outer ground tissue; Pa: Parenchyma; VC: Vascular cylinder; Ph: Phloem; Fig. 6.1: T.S. of stem; Fig. 6.2: T.S. of stem – enlarged; Pa: Parenchyma; Pi: Pith; Fig. 7.1: T.S. of old root; Pe: Periderm; Sph: Secondary phloem; XFi: Xylem fibre; XR: Xylem ray; Fig. 8.1: Old root showing periderm, cortex of secondary phloem; Fig. 9.1: Clavate and long stalked trichome; Fig. 9.2: Clavate trichome enlarged; BC: Body cell; GTr: Glandular epidermal trichome; NC: Neck cell; SC: Stalk cell; Fig. 10.1,2: Xylem fibres of vessel elements; Fi: Fibre; Ve: Vessel element; Fig. 11.1: Narrow fibre; Fig. 11.2: Wide fibres of narrow fibres; Fig. 12.1: A tailed vessel element; Fig. 12.2: A vessel element with tail at one end; Pe: Perforation; Pi: Pits; Ta: Tail