



## Research Article

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### QUALITY CONTROL STUDIES OF *ARGYREIA SPECIOSA* SWEET LEAVES

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#### ABSTRACT

The plant *Argyrea speciosa* Sweet (Syn: *Argyrea nervosa* Burm. f.), is a woody climber which belongs to the family Convulvulaceae. The plant is commonly known as Elephant creeper in English and in Hindi, it is known as ‘Vryddhadaru’. It has been used as a ‘‘Rasayana’’ drug (rejuvenating drugs) in the traditional Ayurvedic system of medicines. Authentication and identification has been done taxonomically. The quality control studies of the leaves have been performed as per WHO guidelines-1998. The leaf has been heart-shaped up to 1 foot across, back with silvery hairs on the lower surface, glabrous above, white tomentose beneath and long stalked. The most distinct anatomical character in TS of petiole has been the occurrence of intra-xylary phloem, where the xylem is above and the phloem is below. Unicellular trichomes are numerous and are present in the dorsal side only. These palisade cells are rectangular in nature where the length is twice its breadth. Total ash (15.5% w/w), acid insoluble ash (3.6% w/w), water soluble ash (2.18% w/w), alcohol soluble extractive (1.66% w/w), water soluble extractive (3.56% w/w) and moisture content (14.07%) of the crude drug have been obtained. A fingerprint of fluorescence has been observed in fluorescence analysis. Chlorinated pesticide in first and second elute from column have been 0.034 and 0.081mg/kg of the crude drug respectively. Phosphated pesticides are found to be 0.012, 0.095 and 0.004mg/kg respectively from first, second and third elute. The heavy metals content have been negligible.

**Keywords:** *Argyrea speciosa*, Quality control, WHO guidelines

#### INTRODUCTION

It has estimated that 60% of the world’s inhabitants rely on traditional medicines as primary source of health care<sup>1</sup>. These traditional medicines are primarily plant-based. Even in the remaining population, natural products are important in health care. It is estimated that 25% of all prescriptions dispensed in the USA contained a plant extract or active ingredients derived from plants. It is also estimated that 74% of the 119 currently most important drugs contain active ingredients from plants used in traditional medicine<sup>2</sup>. The plant *Argyrea speciosa* Sweet (Syn: *Argyrea nervosa* Burm. f.), is a woody climber which belongs to the family Convulvulaceae. The plant is commonly known as Elephant creeper in English and in Hindi, it is known as ‘Vryddhadaru’. It has been used as a ‘‘rasayana’’ drug (rejuvenating drugs) in the traditional Ayurvedic system of medicines<sup>3</sup>. A large number of alkaloids have been reported in the seed of the plant which include agroclaine, chanoclavine I, chanoclavine II, racemic chanoclavine II, elymoclavine, festuclavine, lysergene, lysergol, iosolysrgol, penniclavine, setoclavine, ergine, isoergine, ergometrine, isoergometrine, lysergic acids  $\infty$ -hydroxyethylamide, isolysergic  $\infty$ -hydroxyethylamide acids and a few unknown alkaloids<sup>4</sup>. The plant contains 9-keto-octadec-15-enoic acid<sup>4</sup>, 6-methoxy coumarin-7-O- $\infty$ -glucopyranoside<sup>5</sup>, quercetin and kaemferol<sup>6</sup>.

#### MATERIALS AND METHODS

##### Collection and authentication of plant material

The plant twig of *Argyrea speciosa* Sweet has been collected in and around the Banaras Hindu University campus. The identity of the plant has been confirmed by Prof. N. K. Dubey, Reader, Department of Botany,

Banaras Hindu University, Varanasi, India. A herbarium (COG/H.No.-024) of the leaves has been prepared and deposited in the Pharmacognosy division of Department of Pharmaceutics, IIT (BHU) Varanasi. The leaves of the plant have been taken for quality control studies.

##### Morphological and microscopical studies

The morphology of the leaves was studied according to standard methods<sup>7,8</sup>.

##### Preparation of specimen for Microscopical studies

The specimen was preserved in glycerine: 50% alcohol: water (25:50:25) mixture for pharmacognostical studies. Free hand section was taken in the usual way. For studies on isolated tissues and cell, small piece of materials from different portion was macerated separately as per WHO’s guidelines 1998. Representative diagrams were sketched with the help of camera lucida<sup>9</sup>.

##### Disintegration of tissues

For disintegration of various tissues, a procedure as per the WHO’s guidelines was followed. The fresh leaf material is sliced into small pieces, about 2mm thick and 5mm long. These pieces were then taken in a test tube containing about 5ml of KOH (110gm/L), and then heated on a water bath for 15 to 30minutes until a portion breaks easily when pressure is applied with the help of a glass rod. The liquid was decanted and washed the soften material several times with fresh quantities of water. The materials were then transferred into a slide and tease it out with a needle. 1drop of glycerol is added and a cover glass is applied. The representative diagram was sketched with the help of camera lucida<sup>9</sup>.

**Table 1: Physicochemical parameters of *Argyreia speciosa* leaf**

Parameter	% w/w*
Total ash	15.5
Acid-insoluble ash	3.6
Water-soluble ash	2.18
Alcohol soluble extractive	1.66
Water soluble extractive	3.56
Moisture content	14.07

\*average of three readings

**Table 2: Fluorescence drug analysis of *Argyreia speciosa***

Powder + Reagent	Fluorescence in daylight	Color code	Fluorescence under UV	Color code
Powder as such	Golden rod	218,165,32 DAA520	NF	-
Powder + 1N NaOH in methanol	Brown	165,42,42 A52A2A	Antiquewhite	250,235,215 FAEBD7
Powder + 1N NaOH in water	Dark red	139,0,0 8B0000	Green yellow	173,255,47 ADFF2F
Powder + 1N HCl in methanol	Dark golden rod	184,134,11 B8860B	Lime green	50,205,50 32CD32
Powder + 1N HCl in water	Dark golden rod	184,134,11 B8860B	Green yellow	173,255,47 ADFF2F
Powder + 1N HNO <sub>3</sub> in methanol	Maroon	128,0,0 800000	Green yellow	173,255,47 ADFF2F
Powder + 1N HNO <sub>3</sub> in water	Light yellow	255,255,224 FFFFE0	Medium sea green	60,179,113 3CB371
Powder + Iodine (5%)	Golden rod	218,165,32 DAA520	Light green	144,238,144 90EE90
Powder + FeCl <sub>3</sub> (5%)	Brown	165,42,42 A52A2A	NF	-
Powder + KOH (50%)	Brown	165,42,42 A52A2A	Green yellow	173,255,47 ADFF2F
Powder + Ammonia (25%)	Dark golden rod	184,134,11 B8860B	Light green	144,238,144 90EE90
Powder + Picric acid (saturated)	Yellow	255,255,0 FFFF00	NF	-
Powder + Acetic acid	Golden rod	218,165,32 DAA520	Green yellow	173,255,47 ADFF2F

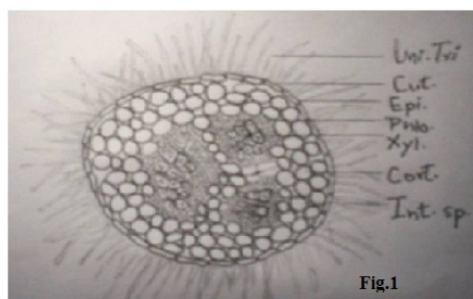


Fig.1

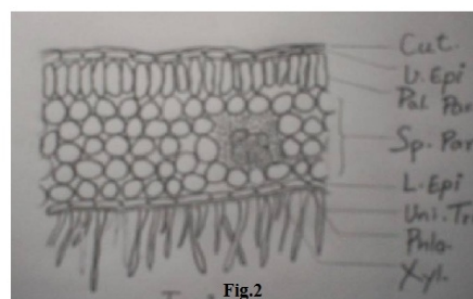


Fig.2

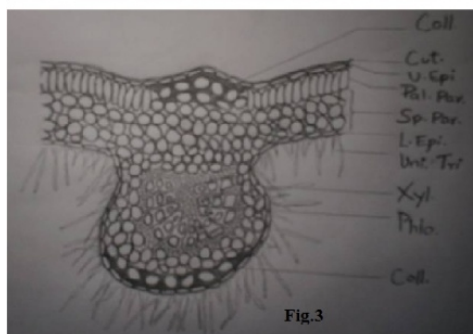


Fig.3

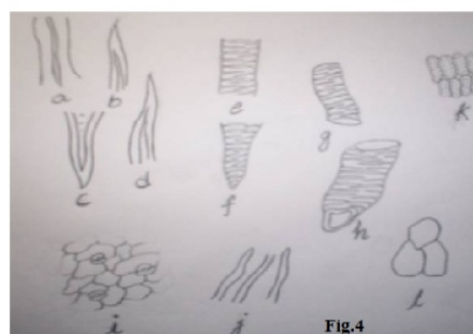


Fig.4

**Figure 1:** Transverse section of the Petiole of *Argyreia speciosa* Sweet. **Figure 2:** Transverse section of the Lamina of *Argyreia speciosa* Sweet. **Figure 3:** Transverse section of the Midrib of *Argyreia speciosa* Sweet. **Figure 4:** Isolated elements of *Argyreia speciosa* Sweet. Uni. Tri, Unicellular Trichomes; Cut., Cuticle; Epi., Epidermis, Phlo., Phloem; Xyl., Xylem; Cort., Cortex; Int. sp., Intercellular space; U. Epi, Upper Epidermis; Pal. Par., Palisade Parenchyma; Sp. Par., Spongy Parenchyma; L. Epi, Lower Epidermis; Coll, Collenchyma; a-d: xylem fibre; e-f: tracheids; g-h: vessels; i: anisocytic stomata; j: unicellular trichomes; k: palisade parenchyma cells; l: xylem parenchyma.

#### **Determination of physicochemical parameters**

Physico-chemical parameters i.e. percentage of moisture content, percentage of ash values and extractive values were performed as per the WHO guidelines on the quality control methods for medicinal plant materials<sup>9</sup>.

#### **Fluorescence analysis**

Fluorescence analysis was carried out following reported methods of Chase & Pratt<sup>10</sup>.

#### **Determination of pesticide residues**

Determination of chlorinated and phosphated pesticide residues in the crude drug was done according to WHO guidelines- 1998<sup>9</sup>.

#### **Determination of heavy metal content**

It was performed according to WHO guidelines- 1998<sup>9</sup>.

### **RESULTS**

#### **External morphology of the leaf**

The leaf was heart-shaped up to 1 foot across, back with silvery hairs on the lower surface. Leaves were ovate or cordate, up to 12 inch long, glabrous above, white tomentose beneath and long stalked.

#### **Microscopical characters**

##### **Section through the petiole (Figure 1)**

The transverse section of the petiole was somewhat circular in outline surrounded by numerous number of unicellular trichomes types. The epidermis was composed of cubical type of cells which were radially elongated and were covered externally by thick cuticle. Below the epidermis, a wide zone of cortex of three to four layers was seen. This was followed by 4-5 layers of parenchymatous cells. These parenchymatous cells were round to oval in shape and were having small intercellular spaces in between them. Central pith was absent. Four numbers of vascular bundles were seen where the two below were continuous in nature. The arrangement of xylem and phloem were clearly observed through close observation. One of the most distinct anatomical characters was the occurrence of intra-xylary phloem, where the xylem was above and the phloem was below. Xylem comprised of vessels, tracheids, xylem fibers and xylem parenchyma. Xylem vessels were mostly with spiral type of thickenings. The phloems consisted of sieve tube, companion cells and phloem parenchyma.

##### **Section through the lamina (Figure 2)**

Lamina showed a dorsiventral arrangement of the mesophyll with the palisade cells being present only in the ventral side of the section. The palisade cells were single layered and radially elongated in nature. The cells of the upper and the lower epidermis were of the same type being cubical to tangentially elongated and covered internally by a thick striated cuticle. The epidermal cells in the lower surface elongated to form unicellular trichomes. However, trichomes were completely absent in the upper surface. In surface view, the cells were polygonal in shape. Below the palisade cell layer laid a four to five layered of spongy parenchyma cells. These cells were round to oval in shape with small intercellular

spaces in between them. Veins were seen traversing here and there in the mesophyll tissue and the vascular bundles were surrounded by a layer of bundle sheath cells which were comparatively smaller in size than the cells of the spongy tissue.

##### **Section through the midrib (Figure 3)**

The midrib was bulged on both the ventral and dorsal sides but dorsal convexity was more prominent than the ventral one. The epidermis was mostly composed of cubical type of cells covered over by thick layer of cuticle. Similarly the lower epidermis was covered of the same. Most of the cuticular cells elongated to form trichomes in the lower epidermis. Unicellular trichomes were numerous and were present in the dorsal side only. Below the epidermis lied a single layer of palisade cells. These palisade cells were rectangular in nature where the length was twice its breadth. Following these palisade cells was a layer of spongy parenchyma cells. These spongy parenchyma cells were 4-5 layered and were round to oval in shape with small intercellular spaces in between them. The vascular bundle was collateral in nature. The phloem consisted of sieve tubes, companion cells and phloem parenchyma. Xylem comprised of vessels, tracheids, xylem fibers and xylem parenchyma. Xylem vessels were mostly with spiral type of thickenings. One of the distinct anatomical features of the section was the occurrence of intra-xylary phloem where the xylem lied above and the phloem lied below. Rest of the ground tissue was composed of parenchymatous cells, mostly isodiametric in nature with small intercellular spaces in between them. Simple starch grains were scattered here and there. This was evidenced when a thick section through the midrib was stained with a dilute solution of iodine, it stained blue in appearance.

##### **Disintegration of tissues (Figure 4)**

For disintegration of various tissues, a procedure as per the WHO's guidelines was followed. The various characteristics observed were Xylem Fibres, Vessels, Xylem parenchyma, Tracheids, Palisade parenchyma cells, Trichomes and Anisocytic stomata.

#### **Physicochemical parameters**

Physico-chemical constants like percentage of moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash, petroleum ether soluble extractive, chloroform soluble extractive, water soluble extractive and ethanol soluble extractive were determined and depicted in Table 1.

#### **Fluorescence analysis**

The results of fluorescence analysis of the drug powder are presented in Table 2.

#### **Determination of pesticide residues**

Chlorinated pesticide obtained in first and second elute from column were 0.034 and 0.081 mg/kg of the crude drug respectively. Phosphated pesticide obtained in first, second and third elute from column were 0.012, 0.095 and 0.004 mg/kg of the crude drug respectively.

### Determination of heavy metal content

The heavy metals content of *Argyrea speciosa* was found to be negligible. They were Cd- 0.000mg/L, Cr- 0.013mg/L, Ni- 0.021mg/L and Pb- 0.011mg/L.

### CONCLUSION

The various morphological, microscopical, physicochemical standards developed in this study will help for botanical identification, quality control and standardization of the drug in crude form. High ash value is the indication of either presence of high amount of inorganic salts or high amount of extraneous matter in the crude drug. Fluorescence analysis of the drug powder gives a fingerprint of color pattern that helps in identification. Since both pesticide residues and heavy metal contents are negligible, hence the crude drug may be considered safe for biological use. Further, the authentic plant material can be explored for its pharmacological and phytochemical potential.

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