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Original article

Pharmacognostic study of *Argyreia pilosa* stem

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Abstract

Background and objectives: *Argyreia pilosa* (Convolvulaceae) has been utilized for many aliments in the conventional system ethnomedicinally; most significantly against sexually transmitted diseases, skin troubles, diabetes, rheumatism, cough, and quinsy. The key challenge experienced in the standardization of herbal drugs is the correct identification of the plant source. Thus, setting up quality control parameters by means of pharmacognostic and phytochemical analysis which assures the purity, safety, and efficiency of *A. pilosa* is necessary. The current research was conducted to assess the pharmacognostic characteristics including macroscopic, microscopic, phytochemical and physicochemical parameters of the stems of *A. pilosa*. **Methods:** Micro as well as macroscopic characteristics were investigated. Physicochemical parameters had been done by implementing WHO suggested parameters; preliminary phytochemical and fluorescent evaluation of stem was executed for appropriate identification and standardization. **Results:** The color, shape, size, odor and surface characteristics were reported from the stem and powdered stem material of *A. pilosa.* Light microscope images of cross section and powdered stem revealed the presence of phloem fibers, multicellular uniseriate trichomes, sclerides, lignified xylem fibers, xylem vessels, parenchyma cells and medullary rays. Phytochemical testing confirmed the presence of flavonoids, alkaloids, tannins, phenols, steroids, fixed oils, fats, acid compounds, glycosides, amino acids, and proteins. Physicochemical parameters such as moisture content, ash value, extractive value and fluorescent behavior of stem powder have also been established. **Conclusion:** The current research would be useful in order to supplement the information regarding to standardization, identity and in performing additional explorations in Ayurvedic system of medicine.

Keywords: *Argyreia pilosa*, microscopic, pharmacognostic, phytochemical, standardization

Introduction

The native Indian system of medicine, mainly including Ayurveda, Siddha, and Unani, is the oldest alternative management system with meticulously documented therapies. Ayurveda, a part of ethical traditions of India, is extensively well known for its uniqueness and worldwide acceptance mainly because it provides natural approaches to treat disorders and promote

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healthcare [1]. In accordance with the World Health Organization (WHO) conventional or non-conventional medicines are employed by 70-95% of the worldwide population especially in developing nations for their healthcare [2]. Furthermore, the usage of herbal medicines has grown astonishingly in line with the worldwide trend of individuals returning to natural remedies [3]. The increasing usage of medicinal plants by the public is driving to evaluate the health claims of these agents and to establish standards of quality and manufacture. The most important crucial issue with regard to consumers about medications is purity, safety, efficiency, and effectiveness; therefore, standardization and quality control of herbal medicines and raw materials are always needed. The popularity of the natural medicines is rising globally in general and especially in the developed nations, however, one of the hurdles is the absence of standard quality control profiles [4].

Standardization of drug means confirmation of its identity, quality, and purity throughout all phases of its cycle. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, specified qualitative and quantitative values that carry an assurance of quality, efficacy, safety, and reproducibility. A herbal product is not considered scientifically valid when the medication examined has not been authenticated and described in order to assure reproducibility in the production of the product. Furthermore, several harmful and fatal side effects have recently been claimed, including direct toxic effects, hypersensitive reactions, effects from contaminants, and interactions with herbal medications [3]. One of the primary issues associated with phytomedicine studies is the complexness of the procedure to assess natural products because their quality and compounds characterization is difficult and variations might be caused in the pharmacokinetic, pharmacodynamic and safety profiles [5]. Additionally, the chemical constituents of medicinal plants may vary based on genetic aspects, weather, soil and other external factors [6]. Without appropriate quality control parameters for the production of medicinal plants, it is impossible to assure the reproducibility between various batches, or to assay for the lack of contaminants. Therefore, Standardization, as well as modern analytical methods, should be used to help define the features of natural drugs, in order to recognize and understand their possible variations [6,7].

In the current study, we have emphasizied our investigation on one of the commonly available plants in India *i.e*., *Argyreia pilosa* Wight & Arn. , belonging to Convolvulaceae family. The Convolvulaceae family contains nearly 1650 predominantly exotic species. The genus *Argyreia* Lour., with around 135 species includes some important species such as *A. aggregate, A.cuneata, A. cymosa, A. daltoni, A. elliptica, A. fulgens, A. kleiniana, A. malabarica, A. nervosa, A. pilosa, A. setosa, A. strigosa* and *A. speciosa* [8-10]. Ethnomedicinally, the plants belonging to genus *Argyreia* have long been used in various ailments in the Traditional system; most significantly against rheumatism, syphilis, gleet, gonorrhea, chronic ulcer, skin diseases and also as tonic. *Argyreia pilosa* is a twiner, branchlets reddish and hirsute plant; other characteristics are: leaves simple, alternate, broadly ovate, 7-10×7-9 cm, apex acute, base subcordate, margin entire, and nerves prominent up to 7-8 pairs. Flowers pink, in axillary, capitates heads, peduncle long 23 cm, bracts linear, bristly hair to 1 cm long, calyx 5 lobed, lobes unequal, nearly free to base, oblong - lanceolate to 0.8 cm long, corolla infundibular, to 4 cm, lobes spreading, stamens included. Fruits are berries [11,12].

Argyreia pilosa is also an ornamental, in addition to being a medicinal plant. All parts of this plant are widely used as a folklore medicine for the treatment of various ailments by the Indian traditional healers. Its root is utilized to cure various illnesses like sexually transmitted diseases *viz*., gonorrhea and syphilis and blood diseases. Traditionally, the paste of the leaves has been applied to the neck region for coughs,

quinsy and applied externally in case of itching, eczema and other skin troubles. It has also been used as antidiabetic, antiphlogistic, for rheumatism and to reduce burning sensation [11,12]. Young wines are mixed together with rhizome of ginger and spread all around the body to relieve from fever. The decoction of its root has been used to treat diarrhea and cathartic conditions [13,14]. A vast range of phytochemical constituents have been isolated from the genus *Argyreia i.e*., glycosides, alkaloids, amino acids, proteins, flavonoids, triterpene and steroids [15]. The genus *Argyreia* has been reported to have various biological activities including nootropic, aphrodisiac, antioxidant, antiulcer, immunomodulatory, hepatoprotective, anti-inflammatory, antihyperglycaemic, antidiarrheal, antimicrobial, antiviral, nematicidal, anticonvulsant, analgesic, anti-inflammatory, wound healing and central nervous depressant activities [15-19]. Even though the drug has many uses, it's pharmacological and phytochemistry has been poorly explored [20].

For this reason, we have carried out pharmacognostic study of *Argyreia pilosa* [21]. This kind of study will not only assist in authentication but also assures reproducibility of herbal products in marketing.

Experimental

Plant material

The plant material was obtained from Tirupati, Chittoor district of Andhra Pradesh, India during March 2016 and authenticated by Dr. K. Madhava chetty, Taxonomist, Sri Venkateswara University Tirupati, India. Voucher specimen No. 1922 was deposited at the Herbarium of V. V. Institute of Pharmaceutical Sciences, Gudlavalleru for future reference.

One portion of the stem was preserved for 48 h in formalin (5 mL): acetic acid (5ml): 70% alcohol (90 mL) mixture to improve the staining property of cell components for histological studies and the remaining portion was shade dried, powdered and sieved through mesh No. 20 and kept in an airtight container for future use [22,23].

Chemicals

All analytical grade chemicals were procured from Merck, Germany. Absolute alcohol, phloroglucinol, acetic acid, chloral hydrate, H_2SO_4, NaOH, HNO_3, $FeCl_3$, distilled water, Conc. HCl, Chloroform, Ethyl acetate, and Methanol.

Pharmacognostic evaluation

Organoleptic evaluation

Organoleptic evaluation of stem of *A. pilosa* has been carried out for the color, size, odor, shape, taste, surface and fracture as per WHO quality control methods of herbal medicine [24].

Microscopic evaluation

Preparation of sections

Microscopic studies had been done by preparing a thin section of the stem by hand using a sharp cutting edge of the blade, then cleared with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid (1:1) and mounted in glycerin. The model of microscope used for the study of different characters was Olympus CX-21i trinocular microscope, illumination halogen.

Powdered microscopy

The powder microscopy was carried out in accordance with the procedure described by Khandelwal [25].

Preparation of extracts and preliminary phytochemical analysis

The powdered material had been extracted with various solvents according to its polarity *i.e*., petroleum ether, chloroform, ethyl acetate, and methanol. One hundred g stem powder was extracted with 500 mL of the respective solvent by maceration at room temperature for 24 h. Then, filtered through Whatman filter paper and the filtrate was collected with rotary evaporator. The extract was then subjected to preliminary

phytochemical screening according to standard methods [25,26].

Physicochemical analysis

Physicochemical analyses were carried out in accordance with the references cited in the text [4,24-27].

Fluorescence analysis

Various reagents were utilized to check the fluorescence activity. Thus, 0.1 g of plant powder was blended with 1.5 mL of respective reagent. The mixture was placed on a slide for a minute and observed under visible light, short ultra-violet light (254 nm) and long ultraviolet light (365 nm) [22].

Results and Discussion

Stem powder was buff in color with no characteristic odor, and taste (figure 1).

Figure 1. Macroscopic characteristics of the stem of *Argyreia pilosa*

Transverse (TS) section of stem showed barrel shaped cells constituting the epidermis, which was arranged compactly without intercellular spaces with multicellular uniseriate covering trichomes. The hypodermis was composed of collenchyma cells, which were arranged in 4-5 layered, provided additional protection and support. It was followed by sclerenchymatous cells arranged in 2-3 layers. Vascular bundles were conjoint, collateral and open. Xylem was present in the continuous ring consisting of vessels, fibers, and xylem parenchyma. Phloem consisted of phloem fibers, sieve cells, companion cells and phloem parenchyma. Uniseriate medullary rays are present. The central portion is occupied by parenchymatous cells as shown in figure 2.

The powdered stem was pale in color, which revealed the presence of sclerides, xylem fibers, xylem vessels, parenchyma cells, phloem fibers and medullary rays as shown in figure 3.

The results of qualitative phytochemical analysis of crude powder of *A. pilosa* stem were tabulated in table 1 and the results attained from various determinations of physicochemical analysis have been tabulated in table 2.

Fluorescence analysis of stem powder was performed after treating with different solvents. Fluorescence was observed at 254 and 365 nm comparing its change of color in visible light. The observations were tabulated in table 3 showing the variation in color. Indian native systems of medicine utilize the crude drugs of plant origin. It is crucial that standards be set up to regulate and check the identity of the plants and ensure their quality before usage. Consequently, a detailed pharmacognostic assessment is incredibly an important prerequisite. In accordance with the World Health Organization (WHO), the organoleptic and histological criteria of a medicinal plant could be the first step in the direction of developing its identity and purity which needs to be accomplished in advance of any assessments [28].

Argyreia pilosa, substantially employed in conventional medicines provides remarkable therapeutical prospective for diverse pharmacological activities. The notable diagnostic features of stem revealed the existence of sclerides, xylem fibers, xylem vessels, parenchyma cells, phloem fibers and medullary rays.

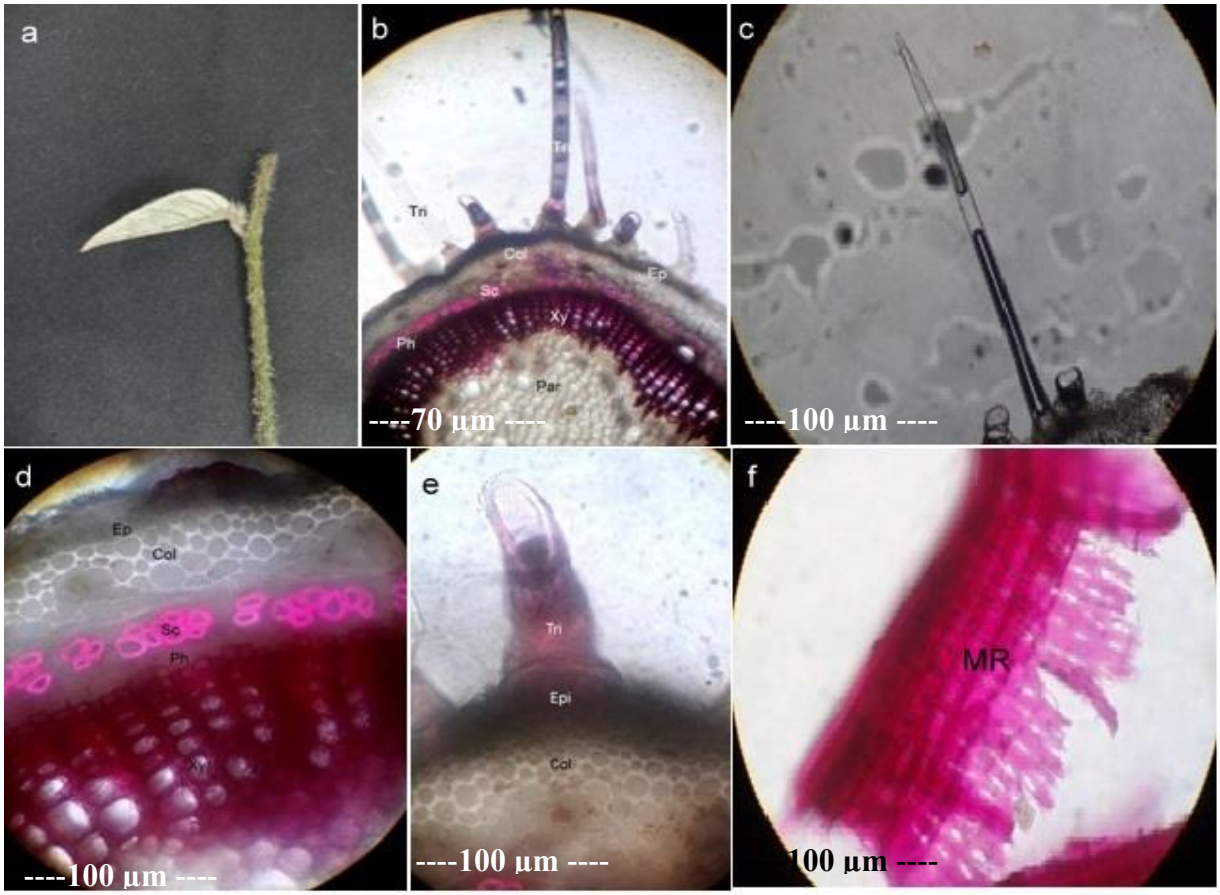

Figure 2. T. S of Stem of *Argyreia pilosa*; a: morphology of stem; b: TS of stem entire view; c: unicellular covering trichome; d: TS of the stem at a magnification of 40X; e: TS of stem showing trichome, epidermis, and collenchyma; f: TS of stem showed uniseriate medullary rays. Abbreviations: Tri: trichome, Ep: epidermis, Col: collenchyma, Sc: scleroidal cells, Xy: xylem, Ph: phloem, MR: medullary rays

These characteristics work extremely well for standardization of drugs and also for preparation of monographs as well as minimizing the chances of adulteration when the drug comes in the powdered form. The study of physicochemical parameters is a significant source to gauge the purity and quality of crude drugs. Ash value is used to ascertain the quality and purity of the crude drug. It signifies the presence of several impurities like carbonate, oxalate, and silicate. The water soluble ash is water dissolvable portion of total ash, exercised to compute numerous inorganic substances within the drugs. The acid insoluble ash includes mainly silica and implies contamination with earthy matter. The moisture content of drugs could be the minimal level to be able to control the growth of microorganisms like bacteria, yeast or fungi while in storage.

The extractive values are helpful to gauge the chemical constituents found in the crude drug and even aid in the assessment of specific constituents soluble in a particular solvent. [29-31].

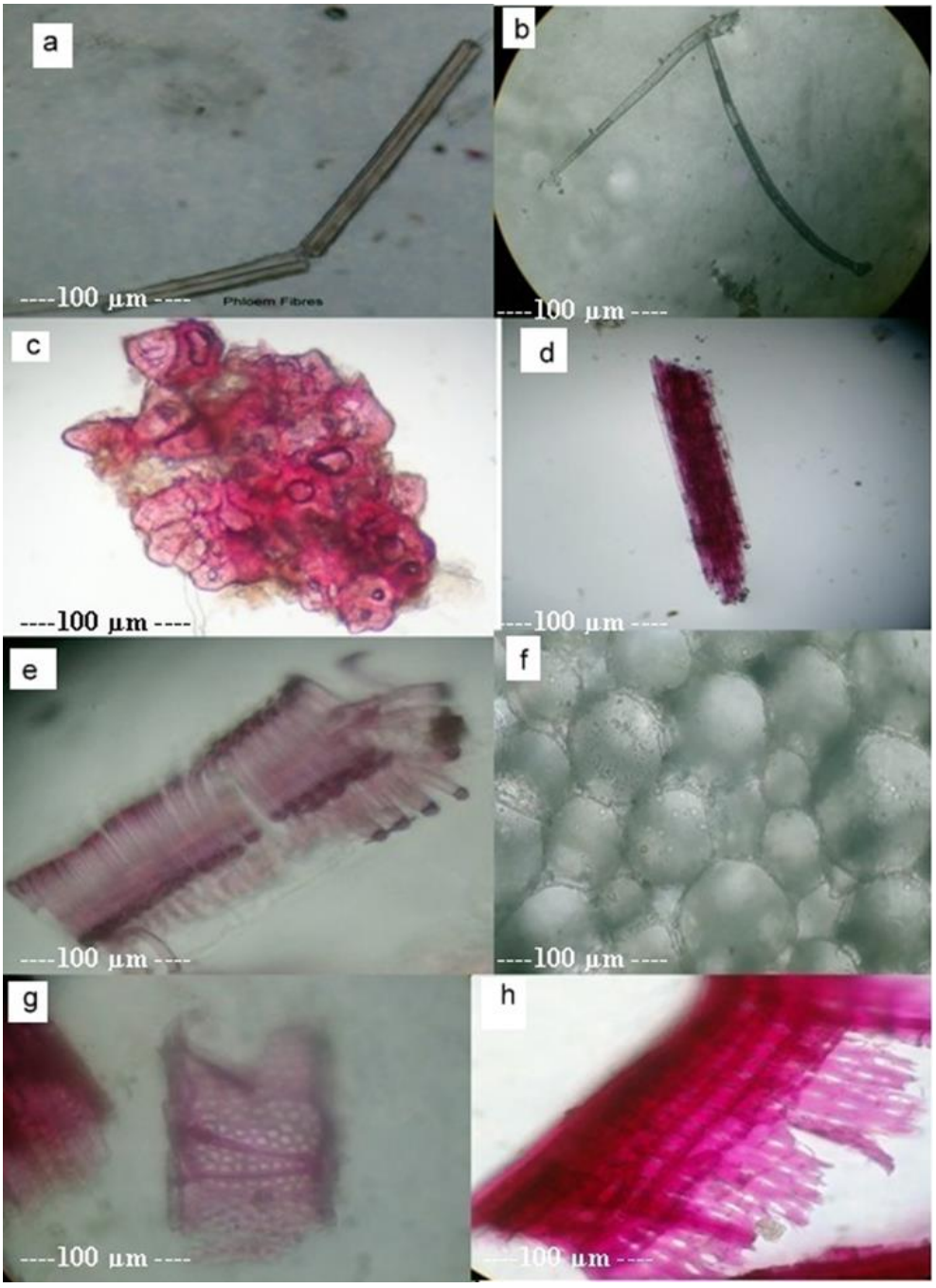

Figure 3. Powder microscopy of stem of *Argyreia pilosa*; a: phloem fibers; b: multicellular uniseriate trichomes; c: sclerides; d: lignified xylem fibers; e: xylem vessels; f: parenchyma cells; g: xylem vessels in tangential longitudinal view; h: Xylem vessels with medullary rays

Table 1. Phytochemical analysis of various extracts of stem of *Argyreia pilosa*

Phytoconstituents	Method	Pet. ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract
Flavonoids	Shinoda Test	-	+	-	+
	Zn. Hydrochloride test	-	+	-	+
	Lead acetate Test	-	+	-	+
Volatile oil	Stain test	-	-	-	-
Alkaloids	Wagner Test	-	-	+	+
	Hager's Test	-	-	+	+
Tannins & phenols	$FeCl_3$ Test	-	+	-	+
	Potassium dichromate test	-	-	-	+
Saponins	Foaming Test	-	-	-	-
Steroids	Salkowski test	+	-	+	+
Fixed oils and fats	Spot test	+	-	-	-
Carbohydrates	Molish test	-	-	-	+
Acid compounds	Litmus test	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	+
Proteins	Biuret	-	-	-	+

Table 2. Physicochemical parameters of stem powder of *Argyreia pilosa*.

Parameters	Values %w/w
Moisture content (Loss on drying)	8.25 ± 0.31
Total ash	6.33 ± 0.22
Acid insoluble ash	2.48 ± 0.10
Water soluble ash	1.86 ± 0.12
Petroleum ether soluble extractive value	0.67 ± 0.05
Chloroform soluble extractive value	2.06 ± 0.04
Ethyl acetate soluble extractive value	3.10 ± 0.07
Methanol soluble extractive value	7.54 ± 0.06
Water soluble extractive value	11.24 ± 0.03

Table 3. Fluorescence analysis of powdered stem of *Argyreia pilosa*

Solvent	Visible light	UV light	
		254 nm	366 nm
Distilled water	Pale Buff	Brown	Dark Brown
1 N NaOH	Brownish black	Black	Green
1N HCl	Buff	Black	Black
50% HNO_3	Buff	Black	Black
$FeCl_3$	Buff	Bluish yellow	Bluish green
Ethyl acetate	Buff	Black	Light green
Picric acid	Brownish yellow	Black	Black
Methanol	Buff	Black	Light green

The phytochemical analysis of different solvent extracts *viz.*, petroleum ether, chloroform, ethyl acetate, and methanol was carried out and indicated the presence of tannins, flavonoids, steroids, glycosides, amino acids, proteins, and alkaloids.

Standardization of herbal drugs is very much crucial because these drugs produced from heterogeneous sources which could result in variations. These kinds of variations can cause spurious results in various pharmacological and phytochemical studies.

The whole plant of *Argyreia pilosa* has been used for many therapeutical properties, therefore, the current study might be beneficial to supplement the information in respect to its identification, authentication, and standardization; no such information is available for the same till date.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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