ORIGINAL ARTICLE

Pharmacognostic Investigations and Phytochemical Screening of *Argyreia speciosa* Linn.

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Abstract

Context: *Argyreia speciosa* has multifarious biological activities and used traditionally to treat various diseases. *A. speciosa* regarded as a "Rasayan" drug in the Ayurvedic system of medicine to cure diseases of nervous system. Due to its high demand, there are chances of adulteration of this important drug. Therefore, this study was designed to investigate its pharmacognostic characters for its standardization. **Materials and Methods:** Macroscopic, microscopic, and physiochemical evaluation of *A. speciosa* was done using standardized procedures mentioned in the WHO guidelines. Thin-layer chromatography was done to check the purity and identification of drug. **Results:** Morphology of *A. speciosa* showed that the outer surface of stem was light brown and inner surface was cream white in color, having sweet taste and odorless. The presence of octahedral calcium oxalate crystal, parquetry layer, thin long fibers, cylindrical trichome, and hexagonal cork cells was observed in powder microscopy. Scanning electron microscopy showed the arrangement of vascular tissue in transverse section of stem. Physicochemical parameters showed that the total ash value was 3.95%, acid-insoluble ash was 1.25%, water-soluble ash was 2.33%, ether-soluble extractive was 0.24%, chloroform-soluble extractive was 0.88%, alcohol-soluble extractive was 2.12%, water-soluble extractive was 5.8%, and loss on drying was 10.3%. **Discussion:** Phytochemical analysis of crude extracts indicated the presence of lipids and steroids in hexane extract and alkaloids in chloroform extract. Methanol extract gave positive tests for alkaloids, glycosides, saponins, and flavonoids. Aqueous extract was reported to contain carbohydrates and proteins. Thin-layer chromatography of hexane extract, chloroform extract, and methanol extracts showed 5, 8, and 6 spots. **Conclusion:** All the above pharmacognostic parameters could be useful for the authentication and preparation of monograph for *A. speciosa*.

Key words: *Argyreia speciosa*, pharmacognostic evaluation, scanning electron microscopy, standardization

INTRODUCTION

India has a huge source of medicinal plants that are used in traditional system of medicine.[1] The use of herbal drugs becomes popular due to their less toxicity and side effects as compared to synthetic drugs. This led to sudden intensification in number of herbal drug manufactures.[2]

Standardization refers to the confirmation of identity, determination of quality, and purity and to detect the adulterant if any.[3] Methods of standardization have to include all aspects that contribute to quality of the herbal drugs, correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation such as ash values and extractive values, phytochemical evaluation, microbial load testing, toxicity testing, and biological activity.[4]

Argyreia speciosa regarded as a "Rasayan" drug in the Ayurvedic system of medicine to cure diseases of nervous system. Almost all parts of this plant are used in medicine.[5]

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It is commonly known as elephant creeper and distributed throughout India up to an altitude of 300 m.[6]

A. speciosa invites attention of the researchers worldwide for its pharmacological activities. In the traditional system of medicines, *A. speciosa* is used in gleets, gonorrhea, and chronic ulcers and in some skin disease such as ringworm, eczema, and itching. It is also used as analgesic and anti-inflammatory.[7] As this plant has multifarious biological activities and used traditionally to treat various ailments, therefore, it must be authenticated and standardized to avoid any kind of adulteration. Taking in view its importance, this study was designed to evaluate pharmacognostic parameters and develop its monograph.

MATERIALS AND METHODS

Plant material

The dried stems of *A. speciosa* were purchased from a registered dealer, and the taxonomic identity was confirmed by the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar. A voucher specimen No. 457/Bot. and Env. Sc. has been deposited in the same department's herbarium.

Pharmacognostic evaluation

Macroscopic characters

Morphological characteristics such as color, odor, and taste of the dried stems of *A. speciosa* were observed with naked eye or with the aid of a magnifying lens.

Microscopic characters

Transverse sections of stems of *A. speciosa* were cut and placed on stubs of scanning electron microscope (Zeiss Company EVO/LS10) and visualized.

Powder microscopic characters

Prepared glass slides were observed under the microscope (Magnus Ltd.). The powdered plant material was also treated with various reagents for the evaluation of microscopic features of the powdered plant material.

Physicochemical evaluation

Ash values

Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ash values of vegetable drugs is to determine the inorganic matter present in the drug. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates, sulfates, and silicates of sodium, potassium, calcium, and magnesium.[8]

Extractive values

The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Varieties of chemical compounds are available in crude drugs having variable properties. Various solvents are used for the extraction of various chemical compounds in a particular amount.[9]

Loss on drying

The moisture content of a crude drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. Hence, the moisture content of a drug should be determined and controlled. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.[10]

Fluorescence analysis

Dried and finely powdered stems of *A. speciosa* were subjected to fluorescence analysis in daylight and in the ultraviolet (UV) light (254 nm and 365 nm). Change in color was observed by application of different reagents.[10]

Extraction

The dried stems of *A. speciosa* were coarsely powdered, and 600 g of this powdered material was subjected to successive Soxhlet extraction using different solvents in an increasing order of their polarity, namely hexane, chloroform, and methanol (S.D Fine Chemicals Ltd., Mumbai) for not <48 h and then finally digested with distilled water.[11]

Phytochemical screening of extracts

The four extracts prepared from the powdered stems of *A. speciosa* were subjected to preliminary phytochemical screening using standard methods for different classes of phytoconstituents using specific standard reagents.[12,13] The phytochemical screening helps in identifying the chemical constituents belonging to a particular class as each class has its own pharmacological importance.

Thin-layer chromatography (TLC) of extracts

Thin-layer chromatography is a method for analyzing mixtures and determining the number of components present in it. It is also used for the identification and to check the purity of a compound. The identification of separated compounds can be achieved on the basis of their retention factor (R_f) values.[14]

RESULTS

Pharmacognostic evaluation

Macroscopic evaluation

Macroscopy of stems of *A. speciosa* was carried out and the characteristics observed were briefed in Table 1.

Table 1: Organoleptic features of stems of ***A. speciosa***

Characters	Dried stems
Color	Outer surface: Light brown Inner surface: Creamish white
Odor	Odorless
Taste	Sweet

A. speciosa: Argyreia speciosa

Table 2: Physicochemical parameters of dried stems of ***A. speciosa***

Physical parameter	% age yield w/w (with reference to air-dried drug)
Ether-soluble extractive	0.24
Chloroform-soluble extractive	0.88
Alcohol-soluble extractive	2.12
Water-soluble extractive	5.8
Total ash	3.95
Acid-insoluble ash	1.25
Water-soluble ash	2.33
Loss on drying	10.3

A. speciosa: Argyreia speciosa

Microscopic evaluation

Transverse section of the stem was cut, placed on the stubs, and observed under scanning electron microscope. The characters observed were shown in Figure 1. Powder characteristics of drug were also studied shown in Figure 2.

Physicochemical parameters

The dried powdered stems of *A. speciosa* were subjected to physicochemical evaluation, and the results obtained were shown in Table 2.

Fluorescence analysis

Fluorescence analysis of the dried powdered stems of *A. speciosa* was carried out by observing it under the daylight, short UV (254 nm), and long UV (365 nm) in an ultraviolet chamber. The colors observed in different solvents under different ultraviolet radiations are summarized in Table 3 as follows:

Preparation of extracts

The extracts were prepared using successive extraction using Soxhlet apparatus. The solvents used are hexane, chloroform, methanol, and water. The percentage yield of each of the four extracts, i.e., hexane, chloroform, methanol, and aqueous is calculated and the results obtained are reported in Table 4.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical screening, and the results obtained are shown in Table 5.

Table 3: Results of fluorescence analysis of dried powdered stems of *A. speciosa*

Experiment	Visible/Daylight	UV light (254 nm)	UV light (365 nm)
Powder as such	Brown	Dark brown	Light brown
Powder+50% HNO_3	Brown	Dark brown	Green
Powder+1 N HCl	Brown	Dark brown	Light brown
Powder+conc. H_2SO_4	Brown	Dark brown	Blackish-brown
Powder+NH_3	Brown	Dark brown	Green
Powder+I_2 solution	Brown	Dark brown	Light brown
Powder+10% $BaCl_2$	Brown	Dark brown	Light brown
Powder+40% NaOH	Brown	Dark brown	Light brown
Powder+10% $AgNO_3$	Brown	Dark brown	Light brown
Powder+5% $FeCl_3$	Brown	Dark brown	Green
Powder+10% $MgSO_4$	Brown	Dark green	Light green
Powder+10% $FeSO_4$	Brown	Dark brown	Light brown
Powder+5% $CaCl_2$	Brown	Dark brown	Light brown
Powder+5% $CuSO_4$	Brown	Dark brown	Green

A. speciosa: Argyreia speciosa. UV: Ultraviolet

TLC fingerprinting

The different extracts, namely hexane, chloroform, and methanol of the stem of *A. speciosa* were subjected to thin-layer chromatography to identify the various chemical constituents present in each of them. The chromatogram developed for hexane extract, chloroform extract, and methanol extract is shown in Figure 3 and R_f values are calculated for each spot as shown in Table 6.

DISCUSSION

For centuries, medicinal plants are used to treat various diseases. Due to these advantages, the medicinal plants have been widely used by the traditional medical practitioners.[15] However, the adulteration is the major problem arised nowadays. The standardization of plant material was done to set the basic protocol for the evaluation of quality, safety, and efficacy of herbal products.

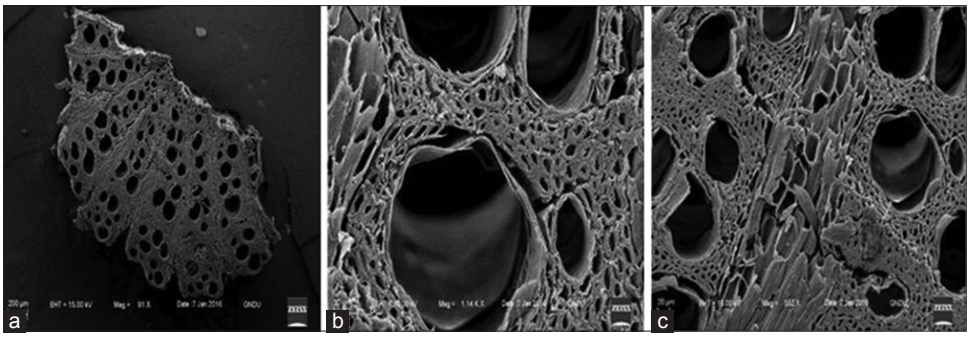

Figure 1: Scanning electron microscopy of the stem portion of *Argyreia speciosa* showing (a) transverse section of stem, (b) medullary rays, and (c) xylem vessels

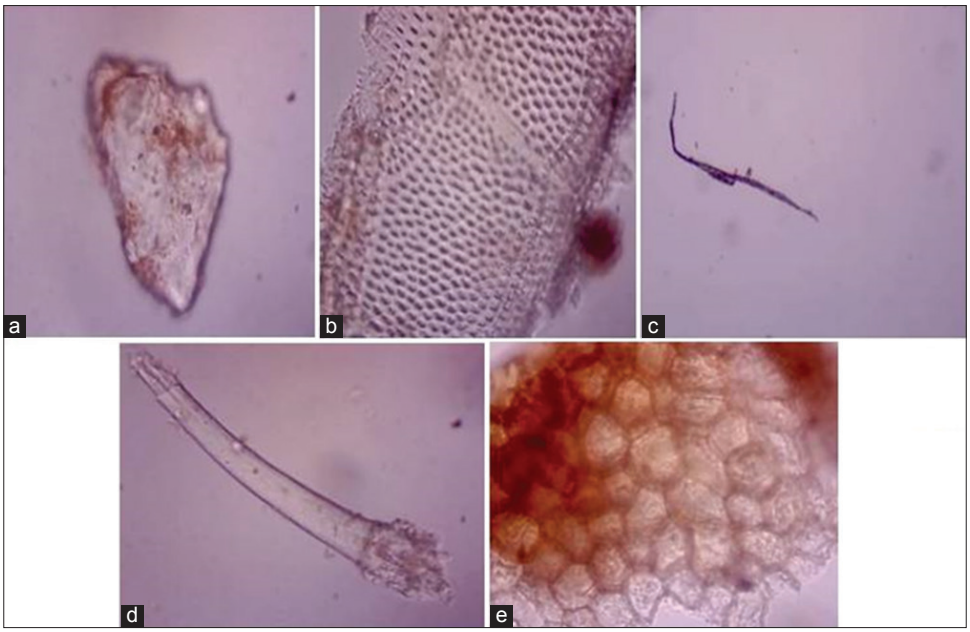

Figure 2: Powder microscopy of stems of *Argyreia speciosa* showing (a) octahedral calcium oxalate crystal, (b) parquetry layer, (c) thin long fiber, (d) unicellular pointed trichome, and (e) hexagonal cork cells

Table 4: Physical appearance and percentage yield of extracts

Extract	Color of extract	Odor	Physical appearance	Sense of touch	Amount of extract (gm)	% yield (w/w)
Hexane	Yellow	Characteristic	Semisolid	Sticky	2.73	0.55%
Chloroform	Brown	Characteristic	Semisolid	Sticky	3.58	0.59%
Methanol	Brown	Characteristic	Semisolid	Sticky	19.44	3.24%
Aqueous	Brown	Characteristic	Semisolid	Sticky	8.90	1.48%

Table 5: Results of preliminary phytochemical screening of extracts

Phytochemical constituents	Hexane extract	Chloroform extract	Methanol extract	Aqueous extract
Carbohydrates				
Molisch's test	-	-	-	+
Fehling's test	-	-	-	+
Benedict's test	-	-	-	+
Phloroglucinol test	-	-	-	-
Proteins				
Biuret test	-	-	-	+
Millon's test	-	-	-	+
Ninhydrin test	-	-	-	-
Xanthoprotein test	-	-	-	+
Steroids and Triterpenes				
Liebermann–Burchard test	+	-	-	-
Salkowski test	-	-	+	-
Antimony trichloride test	-	-	-	-
Trichloroacetic acid test	-	-	-	-
Saponins				
Foam test	-	-	+	-
Hemolysis test	-	-	+	-
Alkaloids				
Dragendorff's test	-	+	+	-
Mayer's test	-	+	+	-
Wagner's test	-	+	+	-
Hager's test	-	+	+	-
Tannic acid test	-	+	+	-
Anthraquinone glycoside				
Borntrager's test	-	-	+	-
Modified Borntrager's test	-	-	-	-
Cardiac glycosides				
Keller-Killani test	-	-	-	-
Legal test	-	-	-	-
Flavonoids				
Lead acetate test	-	-	+	-
Ammonia test	-	-	+	-
Shinoda test	-	-	+	-
Vanillin HCl test	-	-	-	-
Lipids				
Solubility test	+	-	-	-
Sudan IV test	+	-	-	-
Grease spot test	+	-	-	-
Emulsification test	+	-	-	-

+: present, -: absent

Authentication of plant material is precondition before using it as a research material. Therefore, it was planned to establish pharmacognostic standards of *A. speciosa*.

In the present study, *A. speciosa* was subjected to macroscopic and microscopic studies. Morphology of *A. speciosa* showed that the outer surface of stem was light brown and inner

Table 6: Results of TLC fingerprinting of extracts

Extract	Mobile phase	Number of spots	R_f value
Hexane extract	Benzene: methanol: ethyl acetate 6:2:2	5	0.1, 0.25, 0.4, 0.7, 0.8
Chloroform extract	Chloroform: methanol8:2	8	0.25, 0.3, 0.4, 0.5, 0.75, 0.8, 0.85, 0.9
Methanol extract	Chloroform: methanol6:4	6	0.12, 0.24, 0.44, 0.52, 0.68, 0.70

TLC: Thin-layer chromatography

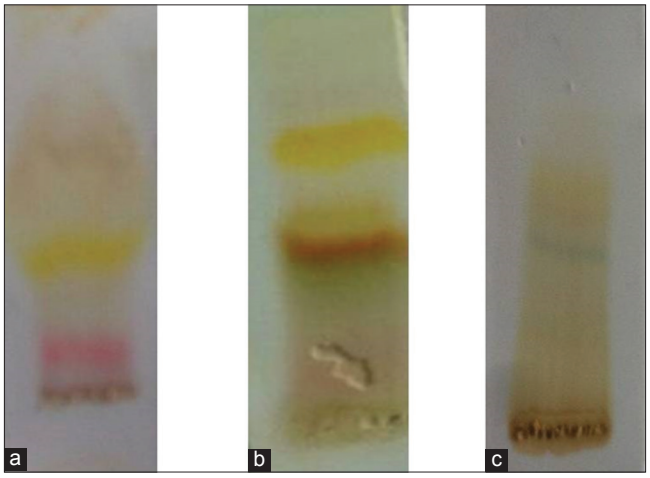

Figure 3: Thin-layer chromatogram of *Argyreia speciosa* (a) hexane extract, (b) chloroform extract, and (c) methanol extract

surface was cream white in color, having sweet taste and odorless. Microscopy showed the presence of octahedral calcium oxalate crystal, parquetry layer, thin long fibers, unicellular pointed trichome, and hexagonal cork cells. Scanning electron microscopy of the transverse section of stem showed the arrangement of vascular tissue.

Physicochemical study was done for the standardization of plant material. The powdered stems of *A. speciosa* showed that total ash value was 3.95%, acid-insoluble ash was 1.25%, water-soluble ash was 2.33%, ether-soluble extractive was 0.24%, chloroform-soluble extractive was 0.88%, alcohol-soluble extractive was 2.12%, water-soluble extractive was 5.8%, and loss on drying was 10.3%.

The dried plant material was coarsely powdered and subjected to successive Soxhlet extraction using different solvents in increasing order of their polarity, namely hexane, chloroform, and methanol for not <48 h and then finally digested with distilled water. Preliminary phytochemical screening study showed the presence of lipids and steroids in hexane extract and alkaloids in chloroform extract. Methanol extract gave positive tests for alkaloids, glycosides, saponins, and flavonoids. Aqueous extract was reported to contain carbohydrates and proteins.

To identify the different compounds from the extract, thin-layer chromatographic fingerprinting was carried out which gave an idea about the number of compounds present in extracts.

Therefore, the standardization process involves all the quality control parameters which are helpful in the authentication of plant material. All these parameters become necessary to ensure the quality and purity of a plant material and to supply medicinal plants of good quality having uniform efficacy.

CONCLUSION

All the above diagnostic macroscopic and microscopic features and pharmacognostic parameters could be a useful tool for the identification, authentication, and preparation of suitable monograph of *A. speciosa*. This study would also helpful to check the adulteration of this important medicinal plant and is of much importance for further research on this plant.

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