

Phytochemical Investigation And Standardization Of Some Medicinal Plants

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Abstract

To evaluate pharmacognostic features including macroscopic, microscopic and physicochemical parameters of the five plants *Argyrea speciosa*, *Balanitesa egyptiaca*, *Gloriosa superba*, *Tagetes erecta* & *Cyathocline purpurea*. Micro and Macroscopic features of fresh and dried root samples were explored. Physicochemical variables were done by implementing WHO urged variables, preliminary phytochemical and fluorescent evaluation of root sample had been performed intended for correct recognition and standardization of All five plants *Argyrea speciosa*, *Balanitesa egyptiaca*, *Gloriosa superba*, *Tagetes erecta* & *Cyathocline purpurea*. The colour, shape, size, odor, and surface features had been observed from the root and then powder root material of all five plants. Light electron microscope i.e. Olympus CX-21i trinocular Microscope images of cross section of root and powdered root revealed that the existence of cork cells, Xylem fibers with tapered ends, lignified xylem vessels, phloem fibers, medullary rays, sclerides and parenchymatous cells. Phytochemical testing revealed the existence of flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides, amino acids, and proteins. Physicochemical parameters including moisture content, ash value, extractive value and fluorescent behavior of root powder had been identified. These variables are useful which will distinguish the powdered drug material. The present studies useful in an attempt to augment the data with regards to its standardization, identity and performing additional exploration on Ayurveda approach to medicine.

Key words: Standardization; Phytochemicals: Microscopy; Morphology; Extractive value

Introduction

India being a botanical garden of the world and a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. WHO encourages the traditional drugs because of its fewer side effects and most of the European countries expanding towards Ayurvedic medicines? Since ancient times, people have been exploring the nature particularly plants in search of new drugs [1]. Plant materials have been used for the treatment of various diseases throughout the world before the advent of modern clinical drugs. The use of medicinal plants still play an important role to cover the basic health needs in the developing countries and the industrialized societies has been traced to the extraction and development of several drugs from these plants as well as from traditionally used folk medicine [2]. Various medicinal properties have been attributed to natural herbs; medicinal plants constitute the main source of new pharmaceuticals and health care products. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc; [3]. Previously the crude drugs were identified by comparison only with the standard descriptions available, but recently due to advancement in the field of pharmacognosy various techniques have been following for the standardization of crude drugs [4]. Medicinal herbs have been used in one form or another under indigenous systems of medicine. Dubey et al. [5] mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. Number of plants were screened for secondary

metabolites for their medicinal values [6] *Artemisia annua*, [7] *Svensonia hyderabadensis*, [8] *Thymus vulgaris*, [9] *Allium giganteum*, [10] *Cephalotaxus koreana*, [4] *Boswellia ovalifoliolata*, [11] *Memecylan umbellatum* and [12] *Dalbergia sisso*. Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds, etc [13] i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers [14, 1, 15]. In the present work, qualitative phytochemical analysis was carried out in 5 medicinal plants. Phytochemicals are natural and nonnutritive plant bioactive chemical compounds that have protective or disease preventive properties against external stress and pathogenic attack [4]. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. The plant-derived phytochemicals with therapeutic properties could be used as a single therapeutic agent or as combined formulations in drug development [5]. The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated. The phytochemical investigation of a plant may involve extraction of plant materials, phytochemical screening, separation and isolation of the constituents, characterization of the isolated compounds [6]. Liver has a pivotal role in the maintenance of normal physiological process through its multiple and diverse functions, such as metabolism, secretion, storage and detoxification of a variety of drugs. It is exposed to a wide variety of xenobiotic, hepatotoxins and chemotherapeutic agents that lead to damage and subsequent impairment of its function. Therefore herbal and other indigenous sources have been adequately explored for the safe and effective hepatoprotective action. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine [7]. There are numerous plants and traditional formulations available for the treatment of liver diseases. Treating liver diseases with botanical drugs has a long tradition, but evidence for efficacy is sparse. Moreover, synthetic drugs available in the market may cause serious side effects. To pursue scientific proof, the present work is designed and screened with the seven medicinal plants, which were used traditionally for treating liver disorders.

Material & Methods Selection and collection of Plant material

Plants were searched in Madhya Pradesh particularly areas near to Indore. All plants were collected from area near Indore. Plants were collected as whole and herbarium was also Prepared and submitted. Plant was authenticated by Dr. Zia Ul Hasan, Botanist, Department of Botany, Safia College of Science, Bhopa (M.P.) [Voucher No. *Argyreia speciosa*, *Balanitesa egyptiaca*, *Gloriosa superb*, *Tagetes erecta* & *Cyathocline purpurea* 318/BOT/SAFIA/18, 319/BOT/SAFIA/18, 320/BOT/SAFIA/18, 321/BOT/SAFIA/18, 322/BOT/SAFIA/18]. Plant materials was washed and dried under shade. Dried plant material was grinded using electric grinder at Department of Pharmacy, Dr. A.P.J. Abdul Kalam University, Indore, M.P. Dried plant material was kept in closed air tight container till any further use.

Morphological studies Organoleptic evaluation

Organoleptic evaluation comprises the color, odor and texture

Microscopic examination

Microscopic examination comprises the Epidermal layer, Trichomes, Vascular bundle and Stomata **Physiochemical examination**

Foreign matter

The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious foreign matter when examined as given below. Took a representative portion from a large container, or remove the entire contents of the packing if 100 g or less, and spread in a thin layer in a suitable dish or tray.

Examine in daylight with unaided eye. Transferred suspected particles, if any, to a petri dish, and examine with 10x lens in daylight.

Ash value Total ash

Place about 2-4g of the ground air-dried material, accurately weighed, in a previously ignited and tarred crucible (usually of platinum or silica). Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cool in a desiccators and weigh. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 ml of water or a saturated solution of ammonium nitrate. Dry on a water-bath, then on a hot-plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, then weigh without delay. Calculate the content of total ash in mg per g of air-dried material.

Acid insoluble ash

To the crucible containing the total ash, add 25 ml of hydrochloric acid (~70g/l) TS, cover with a watch-glass and boil gently for 5 minutes. Rinse the watch-glass with 5 ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash less filter-paper and wash with hot water until the filtrate is neutral. Transferred the filter-paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes, then weigh without delay. Calculate the content of acid-insoluble ash in mg per g of air-dried material.

Water soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ash less filter-paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material

Sulphated ash

Heat a silica or platinum crucible to redness for 10 minutes; allow cooling in a desiccators and weighing. Put 1 to 2 g of the substance, accurately weighed, into the crucible; ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of sulphuric acid, heat gently until white fumes are no longer evolved and ignite at 800 ± 250 until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before, allow cooling and weighing. Repeat the operation until two successive weighing do not differ by more than 0.5 mg.

Loss on drying

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tarred evaporating dish. For example, for ungrounded or unpowered drug, prepared about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness. Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tarred evaporating dish, dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.01 g difference.

Extractive value Ethanol soluble extractive value

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 1050, to constant weight and weigh. Calculate the percentage of alcohol soluble extractive with reference to the air-dried drug. For determination of methanol soluble extractive use methanol on place of alcohol.

Water soluble extractive value

Proceed as directed for the determination of alcohol-soluble extractive, using *chloroform-water* instead of ethanol.

Crude extract preparation

In present study, plant materials was extracted by continuous hot percolation method using Soxhlet apparatus (Kokate, 1994). Powdered material of *Argyrea speciosa*, *Balanitesa egyptiaca*, *Gloriosa superb*, *Tagetes erecta* & *Cyathocline purpurea* was placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethyl acetate and methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchi type) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts were observed for organoleptic characters (percentage yield, colour and odour) and were packed in air tight container and labeled till further use (The Ayurvedic Pharmacopoeia of India).

Screening of phytochemical constituents

Qualitative phytochemical testing of extracts was done to study the presence or absence of various phytochemical constituents using standard tests (Kokate CK et al., 1993).

Tests for Carbohydrates

- Molish Test: 2 ml of aqueous extract was treated with 2 drops of alcoholic α -naphthol solution in a test tube and then 1 ml of conc. H_2SO_4 was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.
- Fehling's Test: To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicated the presence of reducing sugar.
- Benedict's test: Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Tests for Protein and Amino acids

- Biuret's Test: The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink color indicated the presence of proteins.
- Million's Test: 3 ml of extract was mixed with 5 ml of Million's reagent. White precipitate formed which on heating turned to brick red, indicated the presence of proteins.
- Ninhydrin Test: 3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue color indicated the presence of amino acids.

Tests for Glycosides:

- Borntrager's Test: To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and mixed well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammoniacal layer indicated presence of anthraquinone glycosides.
- Legal's Test: 1 ml of test solution was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicated the presence of cardiac glycosides.
- Keller-Killiani Test: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicated the presence of cardiac glycosides.

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added; further the solution was mixed well and filtered. With the filtrate, the following tests were performed:

- Mayer's Test: To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicated the presence of alkaloids.
- Dragendorff's Test: To 1-2 ml of filtrate, few drops of Dragendorff's reagent were added in a test tube. Formation of red precipitate indicated the presence of alkaloids.
- Hager's Test: To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicated the presence of alkaloids.
- Wagner's Test: To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicated the presence of alkaloids.

Tests for Saponins

- Froth Test: The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicated the presence of saponins.

Tests for Flavonoids

- Lead Acetate Test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.
- Alkaline Reagent Test: The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicated presence of flavonoids.

- Shinoda test: To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicated presence of flavonoids.

Tests for Triterpenoids and Steroids

- Salkowski's Test: The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicated the presence of triterpenes.
- Libermann-Burchard's Test: The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the side of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicated the presence of steroids and formation of deep red color indicated the presence of triterpenoids.

Tests for Tannin and Phenolic compounds

- Ferric Chloride Test: Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicated the presence of phenolic compounds.
- Lead Acetate Test: Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicated the presence of phenolic compounds.
- Dilute Iodine Solution test: To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicated the presence of phenolic compounds.
- Gelatin Test: Some quantity of extract dissolved in distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate indicated the presence of phenolic compounds. **Tests for Fats and Oils**
- Solubility test: To 2-3 ml of the alcoholic solution of extract, few ml of chloroform was added and solubility was observed, or to 2-3 ml of the alcoholic solution of extract, few ml of 90% ethanol was added and solubility was observed.

Result & Discussion Morphological studies Organoleptic evaluation of powder of sample

S. No.	Analysis Parameters	Test Sample				
		<i>Argyreia speciosa</i>	<i>Balanitesa egyptiaca</i>	<i>Gloriosa superb</i>	<i>Tagetes erecta</i>	<i>Cyathocline purpurea</i>
1.	Colour	Light brown	Brown	Greenish	Yellowish Brown	Brown
2.	Odour	Aromatic	Aromatic	Not specific	Aromatic	Not specific
3.	Taste	Bitter	Sour	Astringent	Bitter	No taste
4.	Texture	Fibrous	Fibrous	Coarse powder	Coarse powder	Coarse powder

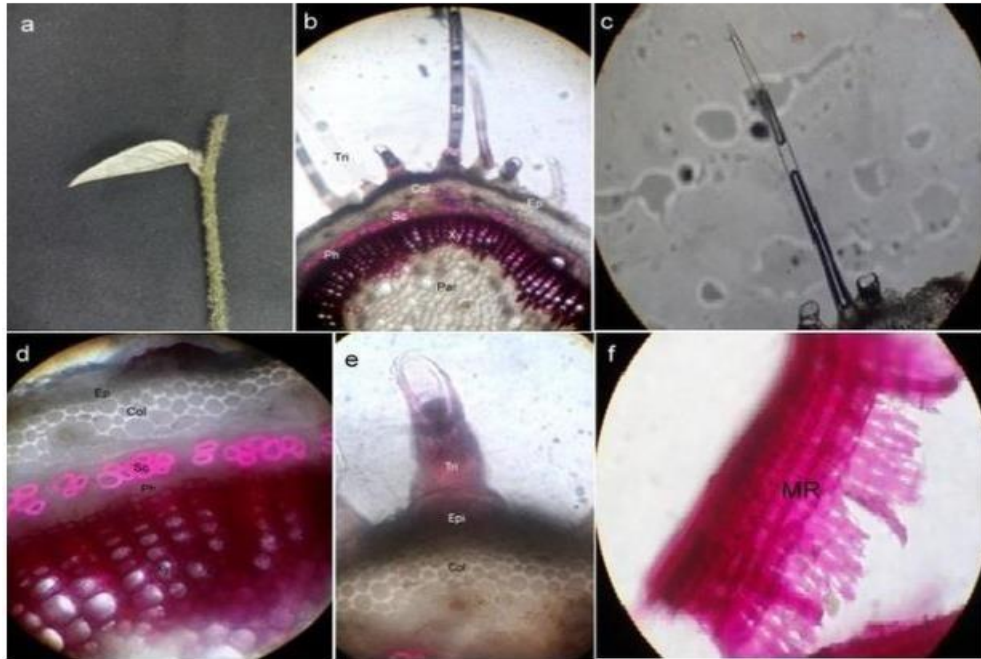


Fig. 1: T. S of Stem of *Argyreia pilosa*; a: morphology of stem; b: TS of stem entire view; c: unicellular covering trichome; d: TS of the stem at a magnification of 40X; e: TS of stem showing trichome, epidermis, and collenchyma; f: TS of stem showed uniseriate medullary rays. Abbreviations: Tri: trichome, Ep: epidermis, Col: collenchyma, Sc: scleroid cells, Xy: xylem, Ph: phloem, MR: medullary rays

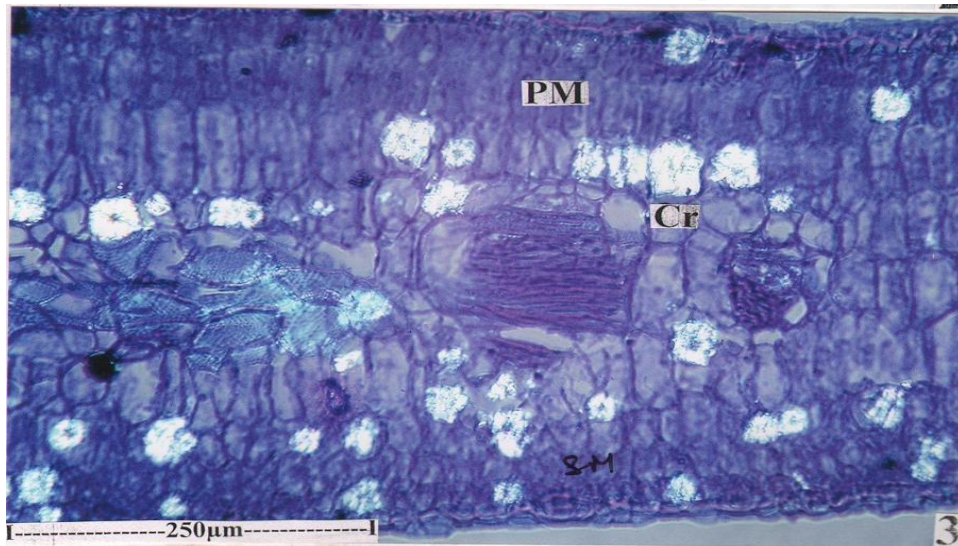
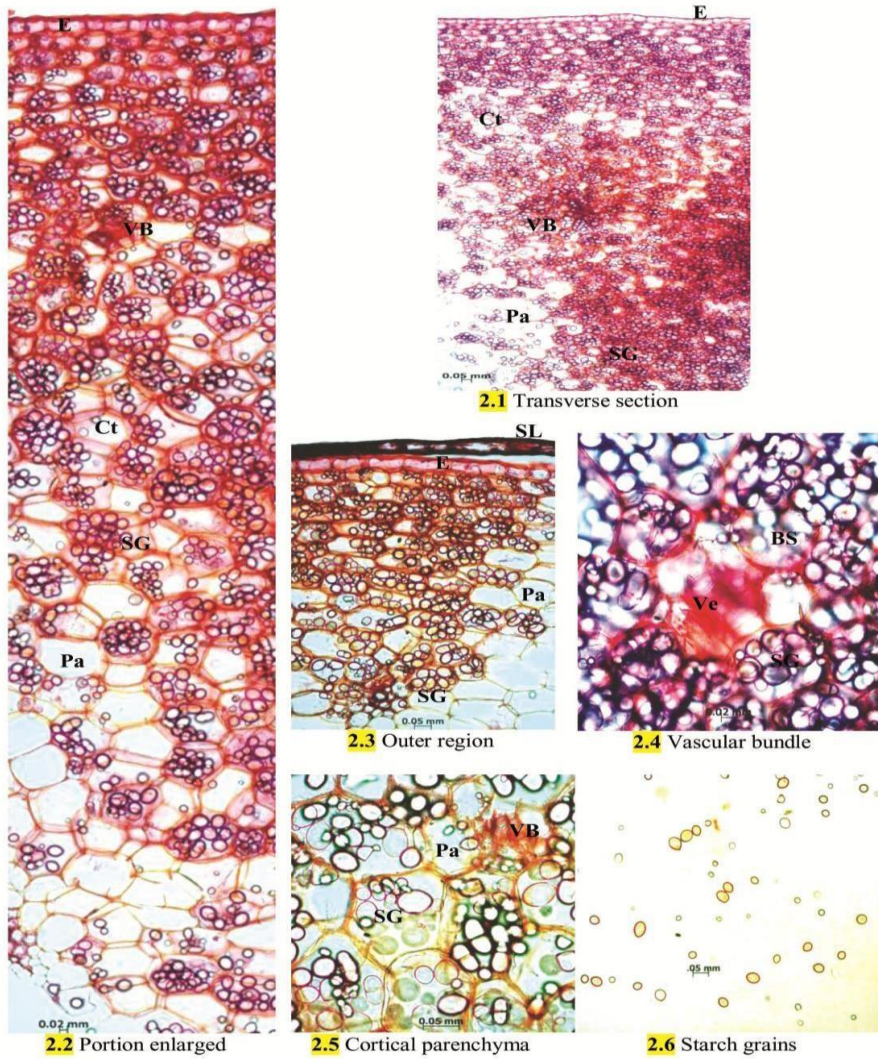


Fig. 2: T.S. of leaf showing cristal distribution (Under polarized light.)

(AdE- Adaxial Epidermis, BS- Bundle Sheath, Cr- Crystals, Cu- Cuticle, Ep- Epidermis, Gc- Guard cells, LM- Leaf margin, Ph- Phloem, PM- Palisade Mesophyll, Sc- Subsidiary cell, St- Stomata, X- Xylem.)



BS, bundle sheath; Ct, cortex; E, epidermis; Pa, parenchyma; SG, starch grains; SL, scaly leaf; St, stem; TS; transverse section; VB, vascular bundle; Ve, vessel.

Fig. 3: Microscopical Examination of *Balanitesa egyptiaca*

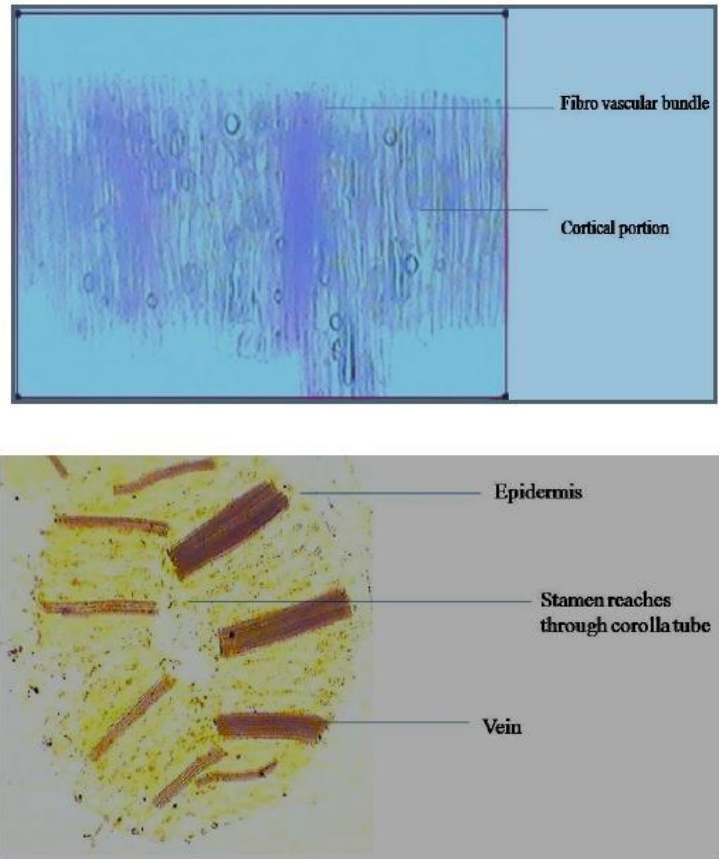


Fig. 4: Transverse section of sepal (calyx) of *Tagetes erecta* Linn flower

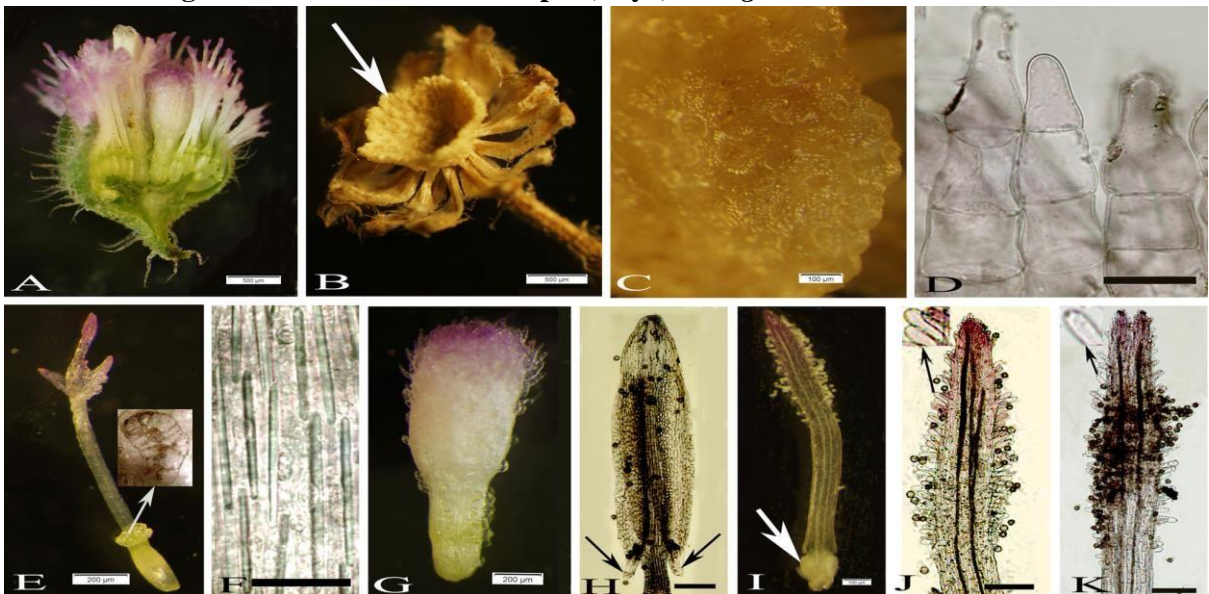


Fig. 5: Some morphological and micromorphological features of *Cyathocline purpurea* (a to i) Physiochemical examination Foreign organic matter Table 1: Foreign organic matter of different plant species

S. No.	Test sample	% w/w
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1.	<i>Argyreia speciosa</i>	0.67±0.04
2.	<i>Balanitesa egyptiaca</i>	0.88±0.12
3.	<i>Gloriosa superb</i>	0.46±0.14
4.	<i>Tagetes erecta</i>	0.52±0.18
5.	<i>Cyathocline purpurea</i>	0.61±0.14

Ash value**Table 2: Ash value of different plant species**

S. No.	Analysis Parameters	Test Sample				
		<i>Argyreia speciosa</i>	<i>Balanitesa egyptiaca</i>	<i>Gloriosa superb</i>	<i>Tagetes erecta</i>	<i>Cyathocline purpurea</i>
1	Total Ash	8.4	12.64	3.45	4.21	3.85
2	Acid Insoluble	1.48	2.47	0.28	0.56	1.26
1.	Water soluble	6.72	9.23	1.27	2.64	2.56
2.	Sulphated ash	2.35	1.34	2.12	3.54	1.02

Loss on drying Table 3: Loss on drying of different plant species

S. No.	Test sample	% w/w
1.	<i>Argyreia speciosa</i>	5.63±0.63
2.	<i>Balanitesa egyptiaca</i>	4.28±0.08
3.	<i>Gloriosa superb</i>	6.44±0.02
4.	<i>Tagetes erecta</i>	7.32±0.12
5.	<i>Cyathocline purpurea</i>	6.24±0.34

Swelling index Table 4: Swelling index of different plant species

S. No.	Test sample	Index
1.	<i>Argyreia speciosa</i>	3.46±0.05
2.	<i>Balanitesa egyptiaca</i>	3.12±0.26
3.	<i>Gloriosa superb</i>	2.85±0.18
4.	<i>Tagetes erecta</i>	2.14±0.12
5.	<i>Cyathocline purpurea</i>	3.42±0.35

Crude fiber percentage yield**Table 5: Crude fiber percentage yield of different plant species**

S. No.	Test sample	% w/w
1.	<i>Argyreia speciosa</i>	26.65±1.24
2.	<i>Balanitesa egyptiaca</i>	31.28±0.86
3.	<i>Gloriosa superb</i>	08.44±0.54

4.	<i>Tagetes erecta</i>	12.35±0.37
5.	<i>Cyathocline purpurea</i>	11.65±0.71

Extractive value Table 6: Extractive values of different plant species

S. No.	Solvent	Test Sample				
		<i>Argyreia speciosa</i>	<i>Balanitesa egyptiaca</i>	<i>Gloriosa superb</i>	<i>Tagetes erecta</i>	<i>Cyathocline purpurea</i>
1.	Ethanol	14.56±1.24	11.34±0.63	12.66±0.22	13.72±1.22	04.56±0.79
2.	Water	16.28±0.54	14.38±1.05	17.45±0.63	12.41±1.46	07.73±0.49

Physical evaluation of extracts**Table 7: Physical evaluation of different plant species**

S. No.	Evaluation parameter	Sample (hydroalcoholic extract of)				
		<i>Argyreia speciosa</i>	<i>Balanitesa egyptiaca</i>	<i>Gloriosa superb</i>	<i>Tagetes erecta</i>	<i>Cyathocline purpurea</i>
1	Colour	Brown	Brownish Black	Brown	Brown	Brown
2	Odour	Aromatic	Odourless	Aromatic	Pungent	Aromatic Characteristic
3	% Yield	18.58	16.05	20.24	11.54	18.12

Screening of phytochemical constituents in *Argyrea speciosa* extracts

S. No.	Test Methanol	Result				PEBE	EABE	CHBE	MEBE	PEGS	EAGS	CHGS	MEGS
		PEAS	EAAS	CHAS	MEAS								
Test for carbohydrates													
1	Molish test	-	-	-	+	-	-	-	+	-	-	-	+
Test for reducing sugars													
2	Fehling's test	-	-	-	-	-	-	-	+	-	-	-	+
Test for monosaccharides													
3	Barfoed's test	-	-	-	+	-	-	-	+	-	-	-	+
Test for proteins													
4	Biuret test	+	+	-	+	-	-	-	+	-	-	-	+
Test for steroids													
5	Salkowski's test	+	-	+	+	-	-	-	+	-	-	-	+
Test for cardiac glycosides													
6	Keller-killiani test	-	-	-	+	-	-	-	+	-	-	-	+
Anthroquinone glycosides													
7	Borntrager's test	-	-	+	+	-	-	-	+	-	-	-	+
Test for saponin glycosides													
8	Foam test	-	-	-	-	-	-	-	+	-	-	-	+
Test for flavonoids													
9	Shinoda's test	-	+	-	+	-	-	-	+	-	-	-	+
Test for fats and oils													
10	Chloroform	+	+	+	+	-	-	-	+	-	-	-	+
Phenolic compounds													
10	Ferric chloride test:	-	+	-	-	-	-	-	+	-	-	-	+
Test for alkaloids													

11	Dragendorff's test	+	-	+	+	-	-	-	+	-	-	-	+
12	Mayer's test	-	+	-	+	-	-	-	+	-	-	-	+
Test for amino acids													

13	Ninhydrin test	-	-	-	+	-	-	-	+	-	-	-	+
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Screening of phytochemical constituents in *Argyrea speciosa* extracts

S. No.	Test Methanol	Result							
		PETE	EATE	CHTE	METE	PECP	EACP	CHCP	MECP
Test for carbohydrates									
1	Molish test	-	-	-	+	-	-	-	+
Test for reducing sugars									
2	Fehling's test	-	-	-	-	+	-	+	+
Test for monosaccharides									
3	Barfoed's test	-	-	-	+	-	-	-	+
Test for proteins									
4	Biuret test	+	+	-	+	-	-	-	+
Test for steroids									
5	Salkowski's test	+	-	+	+	-	-	-	+
Test for cardiac glycosides									
6	Keller-killiani test	-	-	-	+	-	-	-	+
Anthroquinone glycosides									
7	Borntrager's test	-	-	+	+	-	-	-	+
Test for saponin glycosides									
8	Foam test	-	-	-	-	+	-	+	+

Test for flavonoids									
9	Shinoda's test	-	+	-	+	+	-	+	+
Test for fats and oils									
10	Chloroform	+	+	+	+	+	-	+	+
Phenolic compounds									
11	Ferric chloride test:	-	+	-	-	+	-	+	+
Test for alkaloids									
12	Dragendorff's test	+	-	+	+	-	-	-	+
13	Mayer's test	-	+	-	+	-	-	-	+
Test for amino acids									
14	Ninhydrin test	-	-	-	+	+	-	+	+

In transactional view, the leaf exhibits dorsiventral midrib and smooth, even and thick lamina. The midrib is more or less flat on the adaxial side and slightly conical on the abaxial side. The midrib is 500 µm thick. It consists of thin epidermal layers of small thick walled cells. The cuticles were prominent and uneven. The vascular bundle of the midrib is single, more or less circular, prominent and collateral. The vascular bundle consists of wide cluster of narrow thick walled xylem elements and thin is of phloem located on the lower end of the xylem strand. The vascular strand is surrounded by a thin layer of sclerenchyma sheath. There are also one or two layers of large hyaline parenchyma cells around the vascular strand. The vascular bundle is 200 µm in transverse plane. The vascular bundles of the lateral veins are circular and they do not project beyond the level of the surface of the lamina.

Lamina

The lamina is 350 µm thick. It is isolateral, ie; The adaxial and abaxial sides are not well differentiated. The epidermal cells on both sides are large and thick walled with thick cuticle. The mesophyll tissue is consists of adaxial zone of two to three layers of vertically elongated compact palisade cells. Similar type of palisade cells are also seen on the abaxial part, the abaxial palisade cells are in two layers. The median part of the lamina consists of two or three layers of spherical, less compact spongy parenchyma cells. The stomata occur at the level of epidermis, the guard cells have short cubicular ledges. The epidermis including cutical is 20 µm thick.

Powder Microscopy

Fragments of lamina were observed under the microscope.

Epidermal trichomes

Druse non glandular epidermal trichomes are seen the abaxial surface of the lamina. The trichomes are unicellular, unbranched and thick walled. The trichomes are pointed at the cell inclusions. The trichomes are up to

250 µm long and 15 µm thick.

Veins and terminal sclereids

Veins with vein islets and Veins terminations are also seen on the leaf fragments. The vein-islets are many sided, and have thick, straight vein boundaries. The vein terminations have characteristic cluster of squarish sclereids with wide lumen and fairly thick walls.

Crystal bodies are wide spread in the powder. The crystals are exclusively druses, which are spherical bodies comprising many pointed triangular small crystal units.

Langali consists of dried tuberous roots of *Gloriosa superba* Linn., classified as one among upavisha dravya. Garbha patani, garbhantaka, and garbhaghna are some special synonyms suggestive of its abortifacient activity. Colchicine an alkaloid and its glycoside colchicoside are the two compounds which of its roots and having increased demand by the pharmaceutical companies within and outside India. As *Langali* forms an ingredient of many

Popular Ayurvedic formulations it has become an endangered species. Rhizomes of *Costus speciosus* are said to be sold in the market under the name of Langali. Authentication should be the primary criteria of any research using plants, which will help to ensure the quality of any medicinal product. A characteristic macro-microscopic fingerprint of a drug is diagnostic in identifying crude drugs of plant origin.

This study is an attempt to establish, the diagnostic characteristics of *Argyreia speciosa*, *Balanitesa egyptiaca*, *Gloriosa superba*, *Tagetes erecta* & *Cyathocline purpurea*. These results can be employed as suitable quality control measures to ensure the quality, safety and efficacy of this herbal drug material. The parameters studied here are useful to identify and authenticate the traditionally important medicinal plants *Argyreia speciosa*, *Balanitesa egyptiaca*, *Gloriosa superba*, *Tagetes erecta* & *Cyathocline purpurea* and this will prove helpful in the preparation of herbal monographs and pharmacopoeial standards as emphasized by WHO.

Because the safety and efficacy are the ultimate goals, to ensure the reproducible quality of the herbal drugs, the exact identification and quality assurance of the raw material is essential [15]. Mostly the herbal materials are

supplied to the market is shrunken, twisted, rolled and deformed and without trade name and proper identification. So, such drugs can easily adulterated or substituted. The application of pharmacognostic protocols such as macromorphology, micromorphology, organoleptic tests, ash value, extractive values, foreign matters, swelling index and histochemical studies will help in identifying genuine drugs because these tests result in specific results for a particular drug.

Microscopy also plays an important role in drug identification. The importance of epidermal characters, in general, are widely recognized in taxonomic considerations and in many cases these are successfully used in the identification of taxa at genus as well as species levels [16]. Similarly, studies in stomata have a great taxonomic as well as pharmacognostic value in proper identification of medicinal plants [17].

The physical parameters are almost constant for a plant therefore these are helpful in setting standards for a crude drug. Various physicochemical parameters were evaluated for the leaf, stem bark and fruit parts as mentioned in WHO guidelines. These parameters are important for detection of drug adulteration or improper handling of raw materials [1]. One such parameter is ash value, which gives an idea of inorganic composition and other impurities in a plant drug. The total ash value is also important for detection of metal, salts, and silica [18].

There are always chances of microbial growth when the crude drug is stored for a longer period of time and the moisture content of crude drug is directly related to its stability and consequently with the shelf life of crude drug. The lower the moisture content, the higher will be the stability of that drug and chance of microbial growth will be less and vice versa [19]. Identification of the different classes of phytochemical constituents of the plant is an important parameter, which gives an indication of the pharmacological active metabolites present in the plant [20].

Conclusion

Phytochemical studies on *Argyreia speciosa*, *Balanitesa egyptiaca*, *Gloriosa superb*, *Tagetes erecta* & *Cyathocline purpurea* plants indicate it to be a useful plant to investigate for phytochemical and biological assays. HPLC analysis may serve as a useful data for the standardization of the drug. The data generated from this study would help in the authentication of various parts of *Argyreia speciosa*, *Balanitesa egyptiaca*, *Gloriosa superb*, *Tagetes erecta* & *Cyathocline purpurea*, a very important constituent of various herbal drug formulations. This may lead to easier authentication of herbal drugs procured from markets for the correct identification of the medicinal plant ingredients. **References**

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