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Anatomical, physico-chemical, and phytochemical investigations of *Ceropegia bulbosa* var. *lushii*

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Ceropegia bulbosa Roxb. belonging to Asclepiadaceae family is found distributed throughout India. The root is extensively used in treatment of diarrhoea, dysentery, and kidney stone. It also exhibits analgesic and diuretic activities. The sweet sour leaves are edible and are considered to be tonic and digestive. Authenticity of the crude drug should be ensured when used for remedial purposes. The present study was thus undertaken for systematic pharmacognostical evaluation of the leaf, stem and root of the plant with respect to macroscopy, microscopy, and phyisco-chemical parameters. Preliminary phytochemical investigation indicated the presence of alkaloids, carbohydrates, sterols, glycosides among others. These parameters would be useful in identification and authentication of the crude drug.

Keywords: *Ceropegia bulbosa* Roxb., Macroscopy, Microscopy, Physico-chemical parameters.

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Introduction

Ceropegia bulbosa Roxb. (Family Asclepiadaceae) is classified as a 'Threatened Plant' distributed in the tropical and subtropical Asia[1]. There are two varieties of *C. bulbosa* Roxb.- One is *C. bulbosa* var. *lushii* and second one is *C. bulbosa* var. *esculenta*[2]. *C. bulbosa* var. *lushii* is a small, perennial, twining herb having a spherical or oblong, turnip-like root-tuber (3 X 2 cm), with 3-5 roots from its base. It has a very slender, annual terete and glabrous stems; leaves 2-4 x 1.5-2.5 cm, elliptic-oblong or obovate, elliptic-lanceolate, lowest almost orbicular, largest towards the middle of the stem, apiculate, rounded or turnate to subcordate at base; lateral nerves 3-5 pairs; petioles 3-10 mm, glabrous, channelled above; flowers on pedunculate, umbellate cymes; the peduncles 2-3 cm long, arising from between the petioles; pedicels 3-5 mm long, slender; bracts 2 mm long, linear, lanceolate, acute, and glabrous[3]. It is locally known as *Khappar-kaddu*, *Bhuu-tumbi*, *Paataal-tumbi*, *Gilothi*, *Galot* (Punjab)[4] and *Khadulo* (Rajasthan)[5]. Parts of the plant like leaves, roots, and seeds are used for the treatment of diseases like kidney stone, urinary bladder stone, stomach pain, diarrhoea, dysentery, deafness and promoting fertility and vitality[6-8]. The plant is a rich source of various phytochemicals and the tuber is reported to contains an active alkaloid namely cerpegin[9]. Roots also contain starch, sugars, gum, albuminoids, fats and crude fiber[4,10]. The aqueous extract of edible *Ceropegia* species contains steriods, polyphenols, sugars and potassium[4]. Currently, *C. bulbosa* is used in many herbal formulations. Since, there is lack of systematic pharmacognostical and phytochemical studies; the current work was designed to study the detailed macrosocpical, microscopical, physico-chemical characteristics of leaf, stem, and root of *C. bulbosa* var. *Lushii*, which may serve as a standard reference for identification and authentication.

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Materials and Methods

Plant material

The fresh plant was collected in September, 2010 from the forests of the Pali District, Rajasthan, India. The plant was authenticated from Botanical survey of India, Jodhpur, Rajasthan and a voucher specimen (No. JNU/PH/2010/$Cb_{LE}C_2$) was deposited in the herbarium of the Department of Pharmacognosy, Jodhpur Pharmacy College, Rajasthan.

Anatomical study

Macroscopy and microscopy

Fresh leaves, stem, and root were taken for macroscopical and histological studies. Shade dried

leaves, stem, and root were crushed and sieved through 60 no. mesh. The coarse powder was used to study microscopical characters, physico-chemical parameters, and phytochemical investigation. For the microscopical studies, transverse sections of leaves, stem, and root were prepared and stained as per standard procedure[11,12]. The powder microscopy was performed according to the method described by Khandelwal[13].

Physico-chemical investigation

The dried powder material was used for the determination of ash values, loss on drying, foreign matter, and extractive values[14].

Preliminary phytochemical investigation

For preliminary phytochemical investigation, individually powdered leaves, stem, and root were extracted successively with n-hexane, ethanol, and distilled water by cold maceration method[10]. The different extracts obtained by successive solvent extraction were tested separately for the presence of various phytoconstituents, viz. alkaloids, carbohydrates, phytosterols, glycosides, saponins, tannins and phenolic, flavonoids, fixed oils and fats, volatile oil, protein, and Mucilage[13].

Fluorescence analysis

The fluorescence examination and behavior of powder with chemical reagents were also studied[15], which may help to confirm purity of the drug.

Results and Discussion

Macroscopy

Leaves 2-4 x 1.5-2.5 cm, elliptic-oblong or obovate, elliptic-lanceolate, lowest almost orbicular, largest towards the middle of the stem, apiculate, rounded or turnate to subcordate at base; lateral nerves 3-5 pairs; petioles 3-10 mm, glabrous, channelled above (Plate 1a-b). Stems are annual, very slender, greenish, smooth, terete, profusely branched, branches polygonal, glabrous, ending in tuberous root (Plate 1a). It does not have a characteristic odour and taste. It is a small, perennial, twining herb having a spherical or oblong, turnip-like root-tuber (3 x 2 cm), with 3-5 roots from its base. Fresh root show creamy white color that turns brown after drying, having a characteristic odour and a mild sweetish taste (Plate 1c).

Microscopy

Leaf

Leaf surface shows the presence of veins, vein islets, vein terminations and palisade cells. Leaf constants such as stomatal index of lower and upper epidermis is 11.11 and 7.69, respectively, palisade ratio (lower epidermis) is 1:13.5, vein-islet number is 4.69 and veinlet termination number of 6.25 (Plate 2). Transverse section of the leaf consists of lamina and a midrib region as shown in Plate 3a-c. Lamina exhibits upper and lower epidermis; lower epidermal cells are smaller, measuring 4-5-6 µ, upper epidermal cells measure 8-9-10 µ; epidermal cells are rectangular and filled with chloroplast. Stomata anisocytic and anomocytic, found on upper and lower epidermis, many, sunken, each surrounded by 4 to 6 epidermal cells, mesophyll undifferentiated, mostly spongy parenchyma of varied shape and sizes, leaving large intercellular spaces, cells filled with chloroplast and starch grains. Midrib exhibits vascular bundle, which is cojoint, collateral, and closed consisting of xylem and phloem, next to the bundle sheath lies parenchymatous ground tissue; some peripheral cells are collenchymatous.

Petiole

Transverse section of the petiole is circular in outline. Epidermis is covered by cutical, cells are thick walled, single layered, and rectangular. Cortex made up of thin walled parenchymatous cells with intercellular spaces, cells contain abundant chloroplast and starch grains.

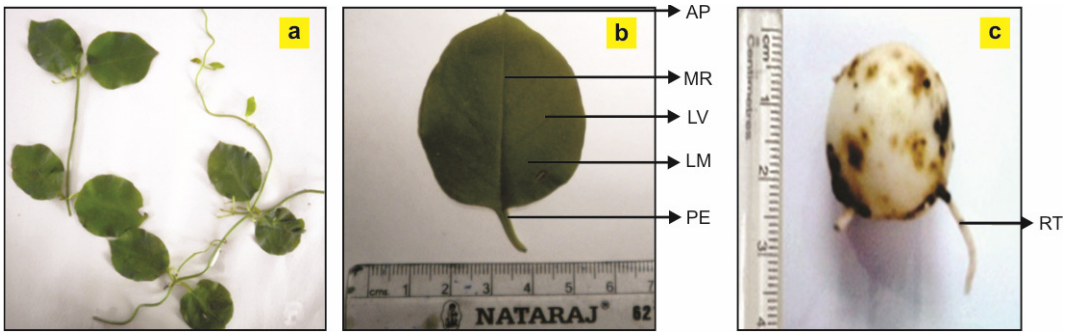

Plate 1—Macroscopical characters of the whole plant, leaves, and root of *C. bulbosa* var. *lushii*. a) Fresh twig, b) Fresh leaf, and c) Fresh root, where, AP-Apex, MR-Mid rib, LV-Lateral vein, LM-Lamina, PE-Petiole, and RT-Root.

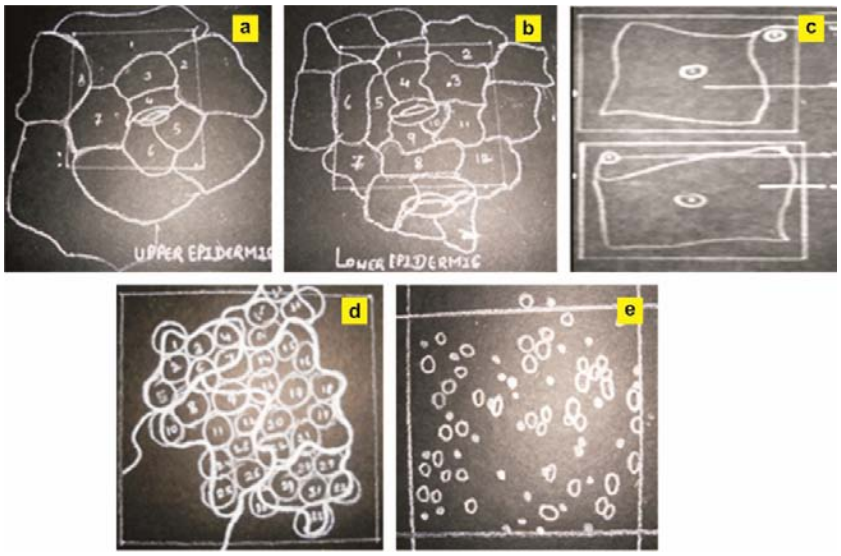

Plate 2—Leaf constants of leaf of *C. bulbosa*. a-d) Stomatal index of upper epidermis and lower epidermis, veinislet and vein termination number, palisade cells of lower epidermis (40X) and e) Lycopodium spore method.

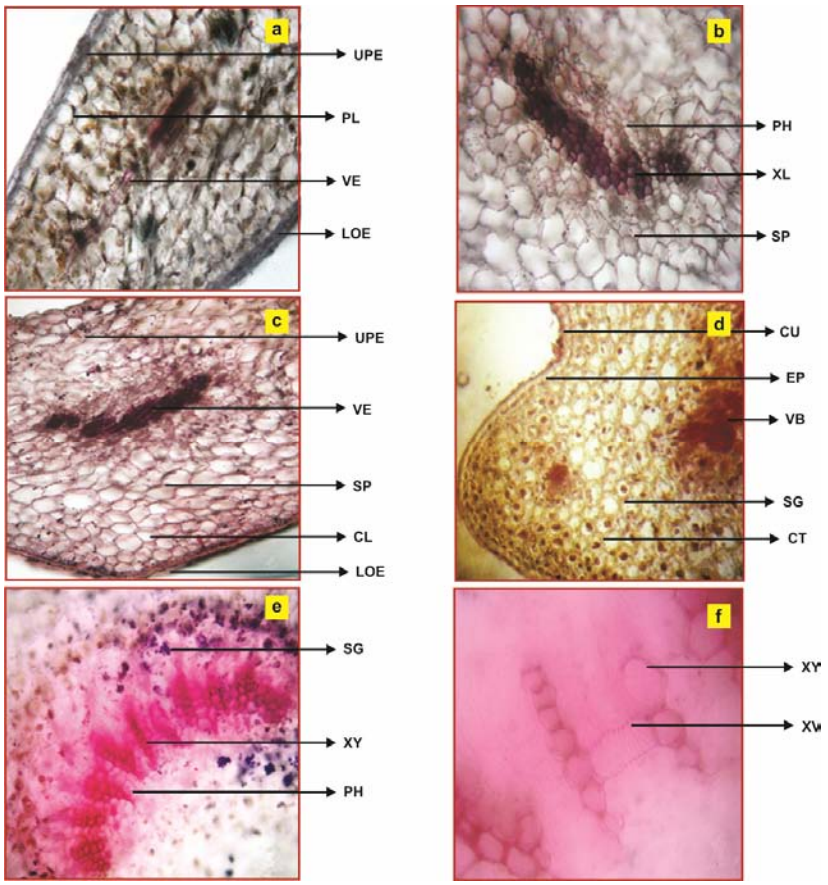

Plate 3—Microscopical characters of the leaves and petiole of *C. bulbosa* var. *lushii*. a-c) TS of lamina and midrib, where a) -Outline of lamina, b) -Vascular bundle in midrib region, and c) -Midrib enlarged, showing at 10X and 40X where, CU-Cutical, EP-Epidermis, VB-Vascular bundle, SG-Strach grain, CT- Cortex, XY-Xylem, XV-Xylem vessel PH- Phloem, UPE-upper epidermis, LOE-Lower epidermis, PL-Palisade, SP-Spongy parenchyma, VE- Veins, and CL-Collenchyma and d-f) TS of petiole, where d)- Outline of tissues, e) -Region enlarged, and f) -Vascular bundle enlarged.

Vascular bundle consists of phloem and xylem; phloem consists of companion cells and sieve tubes, whereas xylem consists of vessels, tracheids, fibers, and xylem parenchyma (Plate 3d-f).

Stem

Transverse section of the stem is oval in outline. Epidermis single layered thick walled, 5-9-16 µ, cells rectangular with cell contents, outer 4 to 5 layers of ground tissue cells 24-42-50 µ, isodiametric, with small intercellular spaces, some pitted, made up thickened cells. Vascular bundles in a ring, cojoint, collateral, open, 44-96-169 µ. Phloem consists of companion cells and sieve tubes, 12-24-26 µ while xylem consists of vessels, tracheids, fibers, and xylem parenchyma (Plate 4).

Root

The outer most layer is light brown, which is the piliferous layer found at the distal end consisting of rectangular cells, many extending as root hairs and measuring 30-85-110 µ. Next to this layer lies 6-8-layered exodermis consisting of slightly radially elongated and thick walled cells measuring 7-9-17 µ, which is followed by a large cortex made up of thin walled parenchymatous cells with intercellular spaces. Endodermis is single layered, consisting of narrow rectangular thick walled cells. Vascular bundles

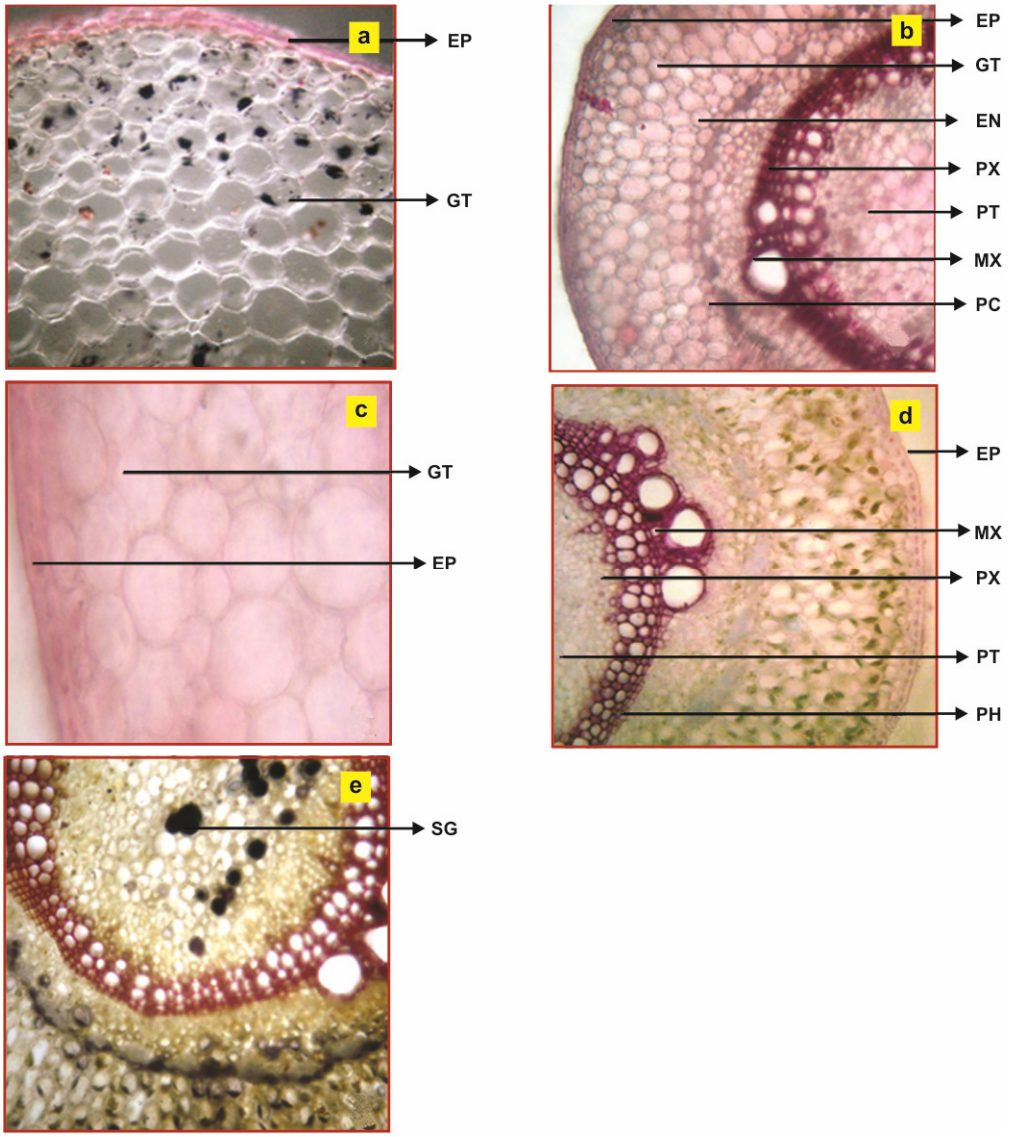

Plate 4—Microscopical characters of the stem of *C. bulbosa* var. *lushii*, a-d) TS of stem showing at 10X and 40X where, EP-Epidermis, GT-Ground tissue, EN-Endodermis, PC, Pericycle, VE- Vessel element MX, Metaxylem, PX, Protoxylem, PH, Phloem, PT, Pith and e) Stain with iodine showing blue color starch grain (SG).

consisting of 20-25 xylem groups; xylem exarch, measure 2-5-9 µ; phloem groups alternate with xylem and measure 4-8-10 µ; large parenchymatous pith was present (Plate 5).

Powder characteristic

Macerate of leaf exhibited stomata covered by epidermal cells measuring 30-45-60 µ; fibres with tapering ends, narrow lumen, measuring 310-535-705

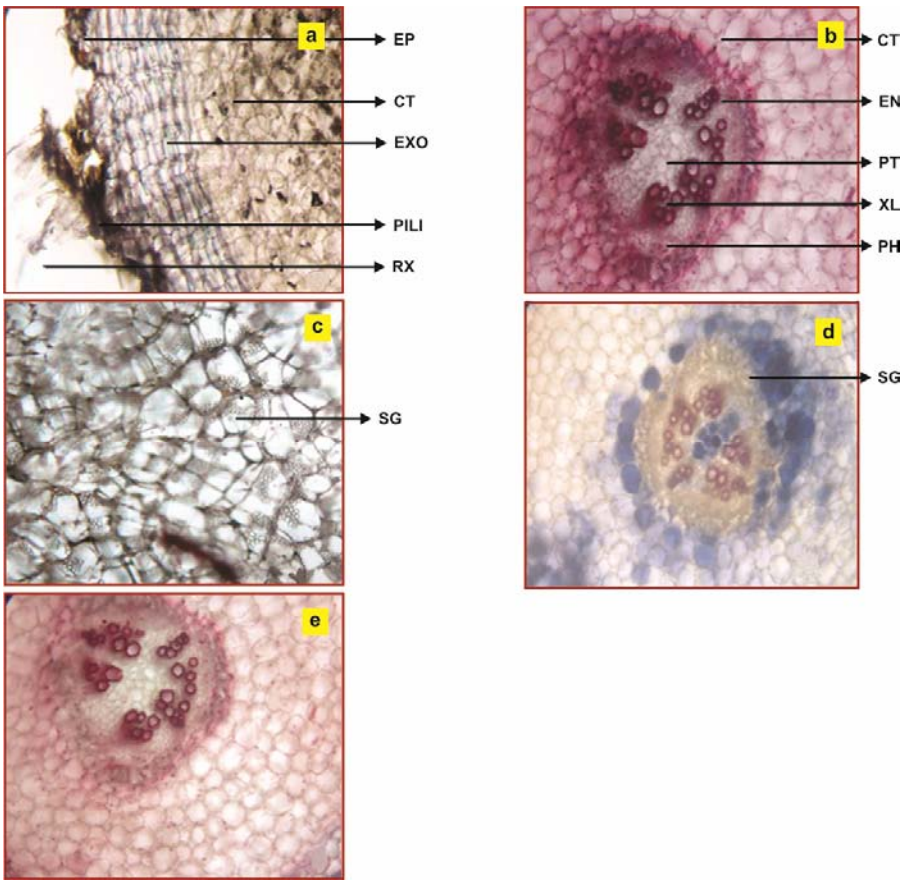

Plate 5—Microscopical characters of the root of *C. bulbosa* var. *lushii*. a-e) TS of root showing at 10X and 40X, where EP-Epidermis, EXO-Exodermis, CT-Cortex, EN-Endodermis, RH-Root hair, PILI-Piliferous layer, MX-Metaxylem, XY-Protoxylem (VB), PT-Pith, c) Starch grain showing without staining, and d) Stain with iodine showing blue color Starch grain (SG).

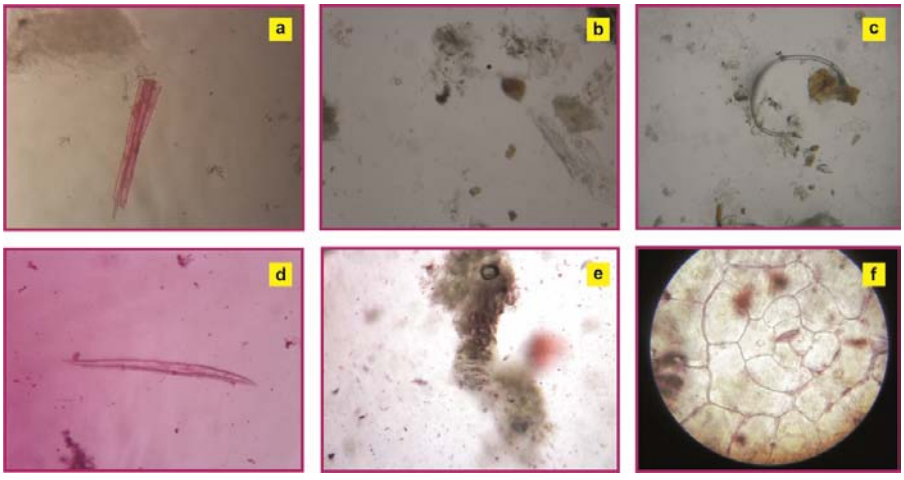

Plate 6—Powder microscopy of the leaf of *C. bulbosa* var. *lushii*. a) Composed of lignified fibers vessels, b) Stain with iodine showing blue color starch grain, c) Trichome, d) Fibers, e) Vascular bundle, and f) Stomata covered by epidermal cells.

µ; with phloroglucinol turned pink showing the presence of lignin; lignified vessels of different size and shape (cylindrical or barrel) measuring 90-250-257 µ; simple starch grain which appeared blue after staining with iodine 3-7-13 µ (diam.). Unicellular trichomes measuring 120-196-318 µ is seen in Plate 6 and Table 1. Macerate of root exhibit parenchyma cells of different size and shape with oil globules measuring 84-100-130 µ; fibers with tapering ends, narrow lumen measuring 310-435-505 µ; vessels of different size and shape (cylindrical or barrel) with reticulate and pitted thickening measured 240-250-254 µ; simple starch grain 3-7-13 µ (diam.) (Plate 7). Macerate of stem exhibited parenchymatous cells measuring 82-101-137 µ, vessels, tracheids, fibers, and xylem parenchyma (Plate 8).

Physico-chemical analysis

Standardization is an evaluation to ensure the quality control of herbal drugs[16]. Physico-chemical analysis of the whole and powdered drugs indicates identity, purity, and quality of herbal drugs[16]. Purity depends on the

Table 1—Histochemical test of leaf, stem, and root of *C. bulbosa* var. *lushii*

S. No.	Material	Reagent	Test for	Colour change	Result		
					Leaf	Stem	Root
1.	Section	Iodine solution	Starch	Blue	+	-	+
2.	Section	Ferric chloride	Tannin	Black	+	+	+
3.	Section	Ruthenium red	Mucilage	Pink	+	-	-
4.	Section	Conc. HCL	Crystals	Effervescence	+	-	-
5.	Section	Sudan III	Oil	Red color droplets	+	-	+
6.	Section	Pinch of phloroglucinol + dilute HCL	Lignin	Magenta color	+	+	+

+ = Present, - = Absent

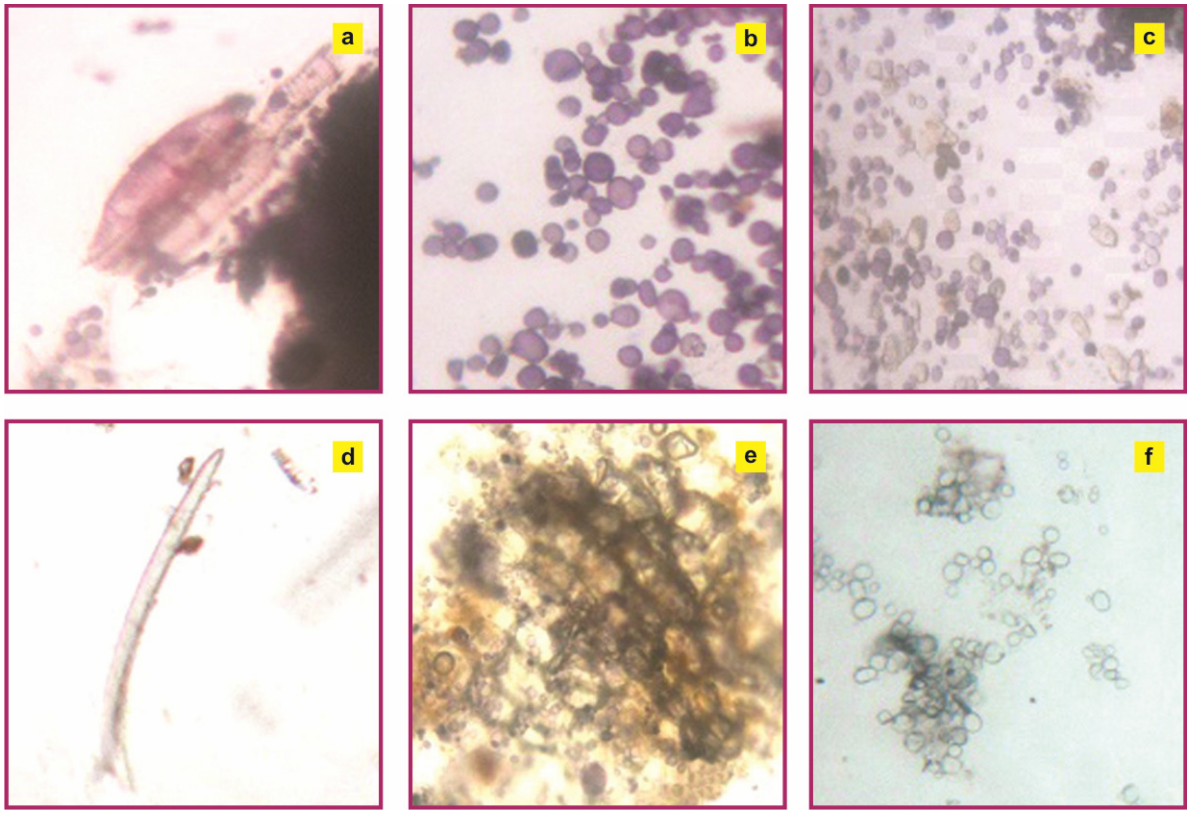

Plate 7—Powder microscopy of the root of *C. bulbosa* var. *lushii*. a) Composed of lignified fibers vessels, b) Stain with iodine showing blue color starch grain, c) Starch grain with aleurone grain, d) Fibers, e) Ground tissue with oil globules and starch grain, and f) Strach grain without staining.

absence of foreign matter, while quality explains the percentage of active constituents in the drugs that make it valuable as medicine[17]. Different ash values were determined to find the inorganic content in the sample. Table 2 reveal the values of different parameters for the leaf as foreign matter 0.81 %, LOD 10.0 %, total ash 11.75 %, acid insoluble ash 1.24 %, water soluble ash 3.85 %, water soluble extractive value 33.72 %, alcohol soluble extractive value 16.44 %, respectively; for stem as foreign matter 0.23 %, LOD 8.0 %, total ash value 5.25 %, acid insoluble ash value 0.28 %, water soluble ash value 2.95 %, water soluble extractive value 15.25 %, alcohol soluble extractive value 6.25 %, respectively; and for root as foreign matter 0.52 %, LOD 11.66 %, total ash value 6.25 %, acid-insoluble ash 0.52 %, water-soluble ash 3.03 %, water soluble extractive value 20 %, and alcohol soluble extractive value 14 %, respectively. .

Preliminary phytochemical analysis

Results of the different phytochemical tests are presented in Table 3. Presence of the volatile oil in leaves and root was confirmed by the red color obtained by globules in Sudan–III Test[13].

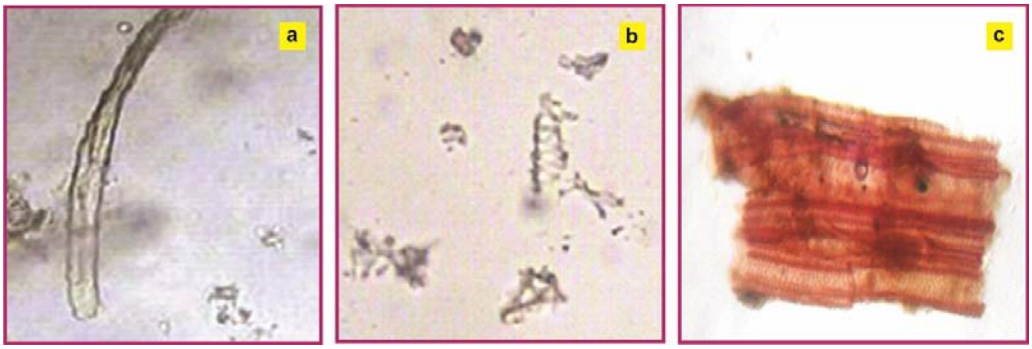

Plate 8—Powder microscopy of the stem of *C. bulbosa* var. *lushii*. a) Composed of fibers, b) Tracheids, and c) Pits vessel.

Table 2—Quantitative parameters of leaf, stem, and root of *C. bulbosa* var. *lushii*

S . No.	Parameters (% w/w)	Leaf	Stem	Root
1	Foreign matter	0.81±0.11	0.23±0.2	0.52±0.01
2	LOD	10.0 ± 0.29	8.0±0.2	11.66 ± 0.4
3	Total ash	11.75±0.26	5.25±0.19	6.25±0.1
4	Acid insoluble ash value	1.24±0.19	0.28±0.16	0.52±0.01
5	Water soluble ash value	3.85±0.12	2.95±0.4	3.033±0.03
6	Water soluble extractive value	33.72±0.2	15.25±0.21	20.0±0.16
7	Alcohol soluble extractive value	16.44±0.14	6.25±0.59	14.0±0.27

All results are represented in Mean±SEM (n=3)

Table 3—Preliminary phytochemical analysis of leaf, stem, and root extract of *C. bulbosa* var. *lushii*

S. No.	Test	Leaf extract			Stem extract			Root extract		
		HE	EE	WE	HE	EE	WE	HE	EE	WE
1.	Alkaloids	-	+	+	-	+	+	+	+	+
2.	Carbohydrates	-	+	+	-	+	+	-	+	+
3.	Phytosterols	-	+	-	-	+	-	-	+	+
4.	Glycosides	-	+	-	-	-	-	-	-	-
5.	Saponins	-	-	-	-	-	+	-	-	+
6.	Tannin and phenolic	-	+	+	-	+	+	-	+	+
7.	Flavonoids	-	+	-	-	+	-	-	+	-
8.	Fixed oils and fats	+	-	-	-	-	-	-	-	-
9.	Volatile oil	+	-	-	-	-	-	+	-	-
10.	Protein	-	+	+	-	+	+	-	-	-
11.	Mucilage	-	-	+	-	-	+	-	-	-

+- Present, -- Absent, HE- Hexane extract, EE- Ethanolic extract, WE- Water extract

Table 4—Fluorescence analysis of powdered leaf and root of *C. bulbosa* var. *lushii*

Plant part		Leaf			Root		
S. No.	Treatment	Under daylight	UV light		Under daylight	UV light	
			S.W. (254 nm)	L.W. (366 nm)		S.W. (254 nm)	L.W. (366 nm)
1.	Powder drug	Dark green	Pastel green	NF	Cream	cascade green	NF
2.	Ethanol	Venetian green	Pista T	NF	Cream	cascade green	NF
3.	Methanol	Pastel green	Pista T	NF	Light green	Dark green	NF
4.	HCl	Black	Pastel green	Venetian green	Pale cream	Cascade green	NF
5.	H_2SO_4 (50 %)	Black	Pastel green	Gypsum	Pale cream	Cascade green	NF
6.	HNO_3	Black	Dew drop	Venetian green	Pale cream	Green	NF
7.	5 % KOH	Venetian green	Pastel green	NF	Pale cream	Mint green	NF
8.	Ethanolic NaOH	Raw silk	Venetian green	NF	Light buff	Dark green	NF
9.	Methanolic NaOH	NF	Pista T	Venetian green	Green	Light green	NF

S.W.- Short wavelength, L.W. - Long wavelength, NF- No fluorescence

Fluorescence analysis

The characteristic fluorescent properties or colours recorded through this study could be used as a standard in the identification and authentication of the leaves and root of *Ceropegia bulbosa* var. *lushii* in its crude form. Further, the contents of this study can also be used as an aid to check adulteration, where the samples does not show more variation or difference in the emission of colours when compared with the genuine samples[15]. The powdered drug was treated with different reagents and examined under day light and UV light (Short, 254 nm). These values are summarized in Table 4.

Conclusion

Standardization of herbal drugs should be ensured to provide sound scientific footing to enhance consumer confidence and to improve business prospects for herbal medicines. The present work was thus planned to establish pharmacognostic standards for *C. bulbosa* var. *lushii* so as to have reliable parameters to authenticate the plant. Macroscopical and microscopical study will prove the authenticity of the drugs in their powdered form. Preliminary phytochemical analysis indicated the presence of carbohydrates, fixed oil, glycosides, alkaloid, tannins, flavonoids, saponins, steroids, and triterpenoids. The ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present. Extractive values are primarily useful for determination of exhausted or adulterated drug. Thus, phytochemical analysis, ash value, extractive value, and fluorescence analysis will be helpful in rapid identification of the drug.

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