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Pharmacognostic and phytochemical evaluation of the *Solanun sisymbriifolium* leaf

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Solanum sisymbriifolium **Lam. (Solanaceae) is an important medicinal herb in Ayurvedic medicine. In the present investigation, the detailed pharmacognostic study of *S. sisymbriifolium* leaf was carried out to lay down the standards which could be useful in future experimental studies. The study included macroscopy, microscopy, preliminary phytochemical screening and physicochemical evaluation. These observations will help in the Pharmacognostical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulteration.**

Key words: *Solanum sisymbriifolium*, pharmacognosy, microscopy.

INTRODUCTION

Herbal drugs play an important role in health care programs especially in developing countries. Such herbal drugs are promising choice over modern synthetic drugs, as they show minimum/ no side effects and are considered to be safe (Gokhale, 1979; Shankar and Ved, 2003). Generally, herbal formulations involve use of fresh or dried plant parts. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances (Mukherjee, 2002). However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines (Dahanukar et al., 2000). With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies (Ozarkar, 2005). These studies help in identification and authentication of the plant material.

Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Anonymous, 1998). *Solanum sisymbriifolium* Lam. (Solanaceae) is commonly known as sticky nightshade, the fire-and-ice plant, Litchi tomato or Morelle de Balbis. It is a perennial herb that has been used as a traditional medicine possessing diuretic and antihypertensive properties in Paraguay. In Argentina, the roots of the plant were traditionally used as diuretic, analgesic, contraceptive, antisyphilitic and hepatoprotective, while the aerial parts of the plant were used in treatment of diarrhea, infections of respiratory and urinary tracts. The flowers are used in India as analgesic and the leaves are used as febrifuge in Peru (Ibarrola et al., 1996; Ferro et al., 2005). The solasodine, the steroidal saponin isolated from the plant was found very potent for the treatment of neurological disorders (Chauhan et al., 2011). Literature details of morphology, phytoconstituents, medicinal properties and uses of *S. sisymbriifolium* are very scarce;

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therefore, in the present study, pharmacognostic and phytochemical standards of the leaves of *S. sisymbriifolium* WEre studied.

These standards are of utmost importance not only in finding out genuity, but also in the detection of adulterants in marketed drug (Johanson, 1940).

MATERIALS AND METHODS

Collection of the plant material

Fresh leaves of *S. sisymbriifolium* were collected from Saurashtra University Campus, Rajkot, Gujarat (India). The sample was authenticated for its botanical identity by Botanist Botanical Survey of India, Rajasthan and voucher specimen deposited in herbarium of the institute. Dried leaves were made into powder.

Morphological evaluation

The macroscopic characters such as size, shape, surface, margin, colour, odour, taste etc. were studied for morphological investigation (Kokoski et al., 1958; Brain, 1975; Khandelwal, 1998; Kokate et al., 1999; Anonymous, 2001).

Microscopic evaluation

For microscopical studies, free hand section of leaf were cut, cleared and stained with saffranine according to the prescribed method (Kokoski et al., 1958; Brain, 1975; Khandelwal, 1998; Kokate et al., 1999; Anonymous, 2001). The results were registered by botanical illustration and photos taken by means of the digital light microscope fitted with 1/3" CCD camera imaging accessory with Scoptek image 2000 image analysis software.

Histochemical colour reactions

The different histochemical colour reactions were performed on the leaf transverse sections to differentiate the different cell compositions and identification (Trease and Evans, 1986).

Physicochemical evaluation

The foreign matter, ash values, extractive values and foaming index were performed according to the officinal methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials (Anonymous, 1996).

Phytochemical screening

The preliminary phytochemical tests for dried leaves powder were also carried out according to the standard procedures described by Kokate (1986) and Horborne (1998). All the reagents used were of analytical grade obtained from Fine Chemicals Ltd., Mumbai, India.

Thin layer chromatography (TLC)

Ethanolic extract of *S. sisymbriifolium* leaves was prepared by using Soxhlet extraction method. The extract was filtered and concentrated on rotary evaporator. The concentrated extract was spotted on a normal phase plate previously activated at 110°C for 2 h, using a capillary tube. The plate was developed using mobile phase of toluene:ethylacetate (9:1). Further, the TLC was sprayed with 10% methanolic sulphuric acid and Dragendorff's reagent. The Retardation factor (R_f) was determined using this formula:

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

RESULTS

Macroscopic features

Phyllotaxy: Alternate.
Shape: Ovate-oblong, deeply pinnatisect or pinnatifid many prickles.
Size: Leaf blade – 10 to 15 cm in length and 6 to 10 cm in width.
Petioles: 1.5 to 5 cm, spiny.
Surface: sparsely stellate-hairy above.
Margin: Sinuate, lobes rounded.
Venation: Reticulate (Figures 1 and 2).

Microscopic features

Epidermis

In transection (Figure 3), the blade epidermis was single-layered (Figure 5A). The epidermis shows presence of anisocytic stomata (Figure 5B), sharp pointed unicellular type (Figure 5C) and multicellular collapsed type (Figure 5D) trichomes occur predominantly on leaf epidermal cell surface.

Mesophyll

The mesophyll was dorsiventral, consisting of one layer of palisade parenchyma (Figure 5A) and four strata of spongy parenchyma (Figure 5F). The midrib region (Figures 3 and 4), in transverse section, is biconvex. The epidermis is uniseriate and has multicellular trichomes similar to the blade. They are seldom unicellular too. Adjacent to the epidermis, angular collenchymas (Figure 3) occur, comprising approximately four to six rows on the dorsal side and six to eight on the ventral one. The calcium oxalate prisms are found in some of the spongy parenchymatous cells (Idioblasts).

Conducting tissue

Compactly arranged endarch and collateral type vascular bundles (Figure 5E) are found, which are embedded in the ground parenchyma region (Figure 5F).

Histochemical color reactions

The results of histochemical color reactions performed

Figure 1. Entire plant of *S. sisymbriifolium.*

Figure 2. Compound leaf of *S. sisymbriifolium.*

on transverse section and dry powder of leaf were given in Table 1.

Physicochemical evaluation

The moisture content, ash values likes (total ash, acid insoluble ash, water soluble ash), water soluble extractive, methanol soluble extractive and foaming index of stem powder were evaluated. The data obtained are shown in Table 2.

Qualitative phytochemical screening

The extracts and powder drug were subjected to preliminary phytochemical screening for the presence of type of phytoconstituets. The extracts and powder were found to contain alkaloids, terpenoids, saponins, sterols, carbohydrates and amino acids as shown in Table 3.

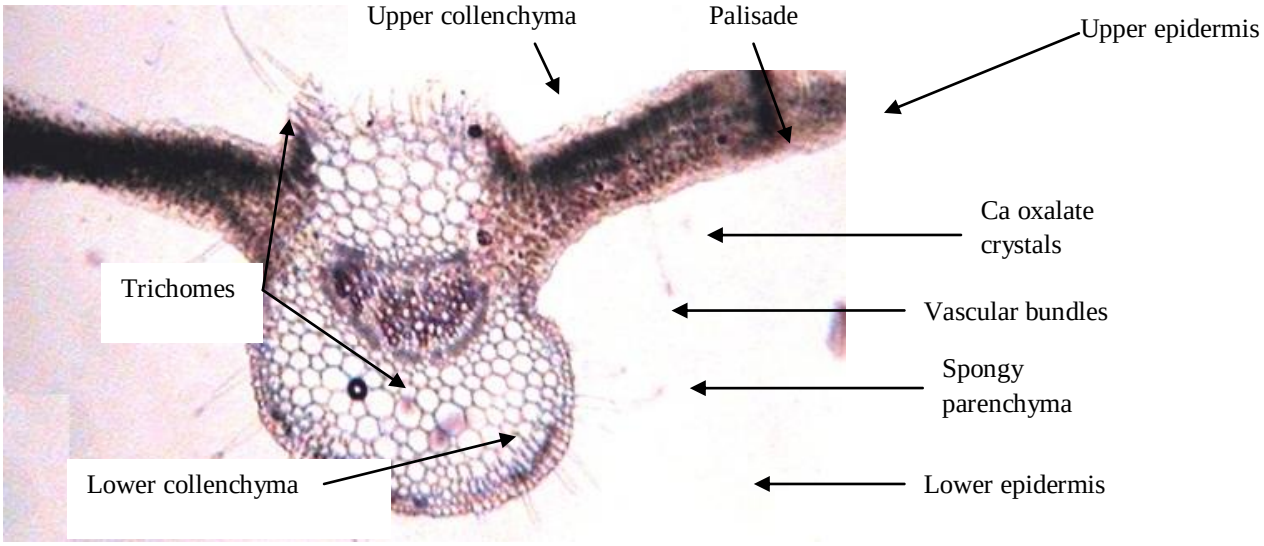

Figure 3. Transverse section of *S. sisymbriifolium* leaf (4X).

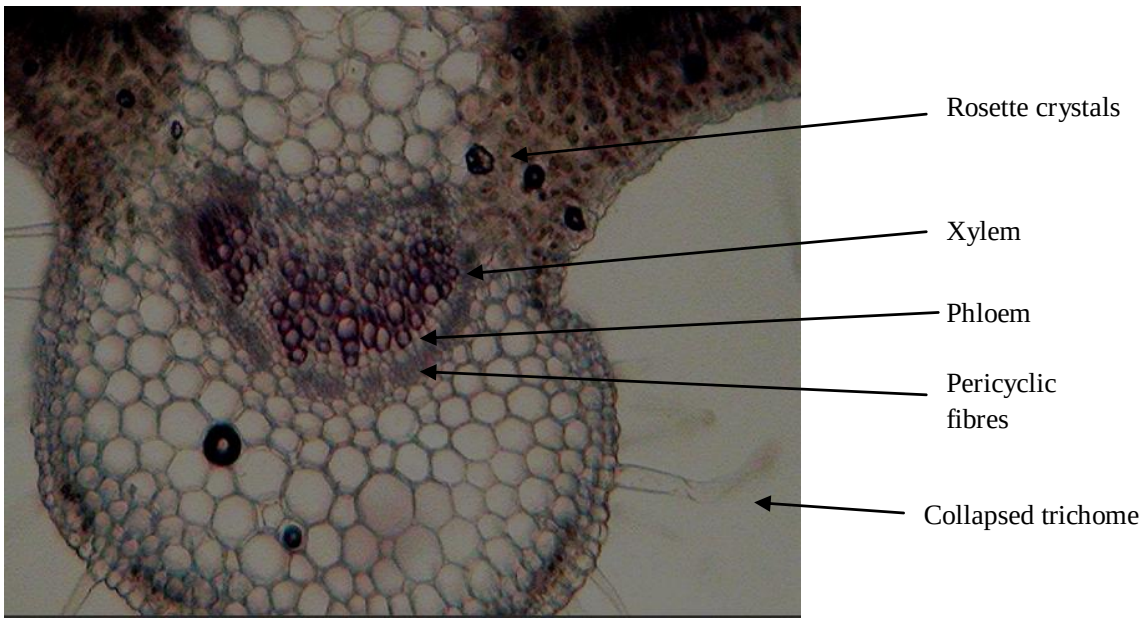

Figure 4. Transverse section of *S. sisymbriifolium* leaf showing leaf blade region (10X).

Thin layer chromatography profile of ethanolic extract of *S. siymbriifolium* leaves

Six different compounds were separated when sprayed with 10% methanolic H_2SO_4 (Figure 6A, Table 4); whereas, when sprayed with Dragendorff's reagent, two different spots were examined (Figure 6B, Table 5).

DISCUSSION

In the present study, the pharmacognostic and phytochemical standards for the leaves of *S. sisymbriifolium* were shown for the first time. Morphological and anatomical studies of the leaf will enable the identification of the crude drug. Various diagnostic characters were identified in the leaf like single layered palisade tissue; anisocytic type of stomata; uni- and multicellular collapsed trichomes; endarch and collateral type vascular bundles. These characters might be a very important tool to identify or to authenticate the drug for future reference. The information obtained from physicochemical evaluation will be useful in finding out the genuity of the drug. In that respect, various physicochemical parameters like moisture content, loss on drying, ash values, and extractive values

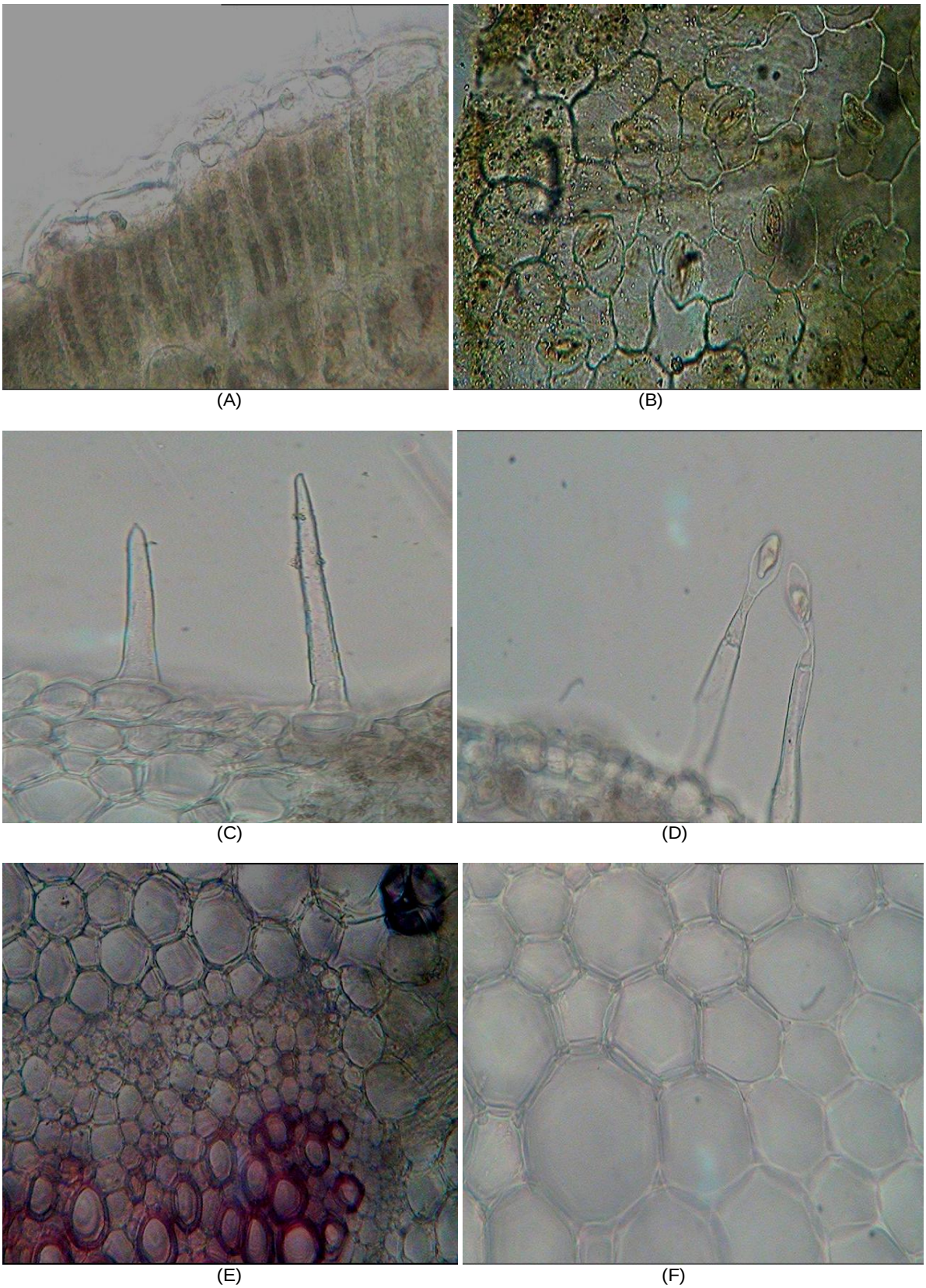

Figure 5. Magnified view of transection of *S*. *sisymbriifolium* leaf showing various characters (40X). (A). Palisade layer (40X), (B) anisocytic stomata in surface preparation (40X), (C) pointed unicellular trichomes (40X) , (D) collapsed glandular trichomes (40X), (E) vascular bundles, (F) spongy parenchyma.

Table 1. Histochemical color reactions of *S. sisymbriifolium* leaf powder.

Reagent	Constituent	Color	Histological zone	Degree of intensity
Phloroglucinol + HCl	Lignin	Pink	Xylem, sclerenchyma	+++
Aniline SO_4 + H_2SO4	Lignin	yellow	Xylem	++
Weak Iodine solution	Starch	-	-	-
H_2SO_4	Calcium oxalate	Needles	Mesophyll and midrib parenchyma	+
$SbCl_3$	Steroids/triterpenoids	Reddish pink	Mesophyll	++
Dragendroff's reagent	Alkaloids	Cream	Spongy paranchyma	+++

Table 2. Physicochemical parameters of *S. sisymbriifolium* leaf powder.

Parameter	Values
Ash values	
Total ash	6.00% w/w
Acid insoluble ash	1.00% w/w
Water soluble ash	0.5% w/w
Extractive values	
Ethanol soluble extractive	34 .00% w/w
Water soluble extractive	15.00% w/w
Moisture content or water content	
Loss on drying at 110°C	9.5% w/w
Foaming index	
Foaming index of entire powder	<100

Table 3. Qualitative phytochemical screening of successive extracts of *S. sisymbriifolium* leaves.

Test	P. E.	Benzene	Chloroform	E. A.	Methanol	Water	95% ethanol
Carbohydrates	–	–	+++	+++	+++	+++	+++
Alkaloids	-	-	++	++	++	++	++
Protein	–	–	+	+	+	+	+
Fats and oils	+	+	+	+	+	+	+
Terpenoid/steroid	–	++	++	++	++	++	++
Tannins	–	–	–	–	–	–	–
Glycosides	–	–	–	+	+	++	++
Flavonoids	–	–	–	–	–	–	–

P. E., Petroleum ether; E. A., ethyl acetate; + present; - absent.

were also determined; those can be used as reliable aid for detecting adulteration in the drugs. Also, for identification of allied species as well as adulterants, such parameters are useful. Plants show their biological activity through their secondary metabolites, those are actually the biologically active constituents of the plant. Therefore, the quality as well as quantity of such phytoconstituents must be evaluated during standardization of the plant drug.

In the present study, preliminary screening was performed

Table 4. R_f values for thin layer chromatography sprayed with methanolic sulphuric acid.

R_f	Colour
0.23	Brown
0.29	Yellowish pink
0.34	Pink
0.40	Light pink
0.71	Yellow
0.85	Reddish yellow

Table 5. R_f values for thin layer chromatography sprayed with Dragendorff's reagent.

R_f	Colour
0.58	Yellowish orange
0.85	Yellowish orange

by conducting qualitative chemical tests and the results of screening showed the presence of alkaloids, terpenoids (steroids) and glycosides, as the major class of phyto-constituents. TLC analysis was carried out with the leaf extract that showed the presence of seven components in the extract. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also, the manufacturers can utilize them for identification and selection of the raw material for drug production.

Conclusion

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy, physiochemical and phytochemical parameters. As there is no record on pharmacognostical and phytochemical work on *S. sisymbriifolium* leaf, the present work is undertaken to produce some pharmacognostical and phytochemical standards.

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