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Pharmacognostic and Phytochemical Investigations in *Strychnos potatorum* Linn. F.

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ABSTRACT

Objective: To investigate the pharmacognostic and preliminary phytochemical characteristics of leaves of an endangered medicinally important forest tree species *Strychnos potatorum*. **Methods:** Pharmacognostic and phytochemical investigations were conducted in terms of macroscopic, microscopic, physicochemical and preliminary phytochemical parameters. **Results:** The macro and microscopic study revealed that leaves are simple, petiolated, glabrous, dorsiventrally differentiated, hypostomatic, without trichomes and with conjoint, bi-collateral, exarch vascular bundles which are surrounded by sclereids on all sides. These leaves showed the presence of fibers, parenchyma, xylem vessels and amphibrachyparacytic stomata. This crude drug (leaf powder) showed the characteristic physicochemical values like 3.5% (total ash), 2.8% (water soluble ash), 0.5 % (acid insoluble ash), and 3.4% (moisture content). Leaf powder showed the characteristic fluorescence when treated with different reagents which supported results of phytochemical studies. Preliminary phytochemical investigations in leaf powder showed the presence of alkaloids, glycosides, tannins, flavonoids, sterols, triterpenoids, phenols, quinones, saponins and absence of fats and oils. **Conclusions:** Various pharmacognostic and preliminary phytochemical characters observed in this may help in standardization, identification and carrying out further research in *S. potatorum* leaf based drugs used in Ayurveda and also in modern pharmacopoeia.

Keywords: *Strychnos potatorum*, Pharmacognostic studies, Phytochemical studies

1. Introduction

According to World Health Organization (WHO), it was estimated that 80% of the population in developing countries rely mostly on traditional medicine like plant drugs, for their primary health care needs^[1]. Medicinal plants being natural, non-narcotic, having no side effects, cost effective, preventive and curative therapies which could be useful in achieving the goal of "Health for all" in a cost effective manner. Due to this fact, medicinal plants occupied an important position in the socio-cultural, spiritual and medicinal arena of people. These medicinal plants are always busy in synthesizing new compounds called secondary metabolites while interacting with their environment which help them to cope up with continuous changes in environment. Very less fraction of these secondary metabolites were isolated and characterized, which play an important role in human health care.

The Indian Systems of Medicine, viz Ayurveda, Siddha, Unani and Homeopathic system is predominantly rely on plant based raw materials and their preparations and formulations. Modern pharmacopoeia also contains at least 25% drugs derived from plants and many other are synthetic analogues of prototypic plant compounds. Due to increase in demand for plant based crude drugs supplied to pharmaceutical, phytochemical and perfumery industries in both developing and developed countries, are frequently adulterated by foreign organic matters resembling the standard drugs or substituted by inferior quality of crude drugs. Hence, this has necessitated developing a systematic approach to their study in modern pharmacognosy.

Strychnos potatorum Linn. F. (Family- Loganiaceae) is one such medicinally important forest tree species which popularly known as nirmali or clearing nut tree.

In our traditional medicinal systems like Ayurveda, Siddha and Unani, the plant parts are used for treating urinary tract and eye infections; gonorrhoea and kidney troubles, leucorrhoea, tuberculosis, diabetes, venereal diseases and acute diarrhea [2, 3]. Though, very little phytochemical and pharmacognostic studies were carried out in this plant on stem, roots and seeds [2] there is no report on leaves. Hence, in the present study, pharmacognostic and preliminary phytochemical investigations have been carried out in the leaves of elite medicinally important forest tree *S. potatorum*.

2. Materials and Methods

2.1. Plant Material

S. potatorum leaves were collected from the Govt. Timber Depot (GTD) premises, Mahadevpur, Karimnagar, Andhra Pradesh, India during October 2012 and authenticated by Prof. N. Rama Swamy, Department of Biotechnology, Kakatiya University, Warangal. These leaves were washed under running tap water followed by Bavistin (Fungicide) (15% W/V) solution for 5 min. Later, these were washed with sterile distilled water for three times. Fresh leaves were used to study the macroscopic and microscopic parameters; whereas shade dried powdered leaf material was used for the physico-chemical, pharmacognostic and preliminary phytochemical investigations.

2.2. Macroscopic and Organoleptic studies

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug. The fresh leaves were subjected to macroscopic studies which comprised of organoleptic characters viz., color, odour, appearance, taste, texture etc.

2.3. Microscopic studies

2.3.1. Leaf Microscopy

For qualitative microscopic evaluation, the collected fresh leaves were fixed in FAA solution (Formalin-Aceto-Alcohol: Formalin, Acetic acid each 5 ml, in 90 ml of 70% ethanol) for 24 hrs then dehydrated with graded series of tertiary-butyl alcohol [4] and castled in paraffin blocks. Later, the paraffin embedded specimens were subjected for sectioning with the help of rotary microtome with a thickness of 10-12 μ m and de-waxed the sections. These sections were stained with safranin and observed under a compound microscope at projection 10X and 40X.

2.3.2. Quantitative Microscopy

In transverse sections it is not possible to study nature of epidermal cells, trichomes and stomata; stomatal index, vein islet number and vein termination number which play an important role in identifying characteristics of crude drugs and adulterants [5, 6]. But, these can be determined by quantitative microscopy. These quantitative microscopic values are comparatively constant for a particular species and can be used to make difference in closely related species.

For the study of epidermal cells and stomatal index, leaf segments were boiled in chloral hydrate solution and the epidermis was peeled with forceps and then mounted on slide with 5% (v/v) glycerin in water and observed under microscope. Stomatal index was calculated as Number of stomata/Total number of epidermal

cells multiplied by 100. Microscopic descriptions of tissue are supplemented with micrographs wherever necessary.

For determination of vein islet number and vein termination number a 1 mm square leaf piece was cleared by boiling in chloral Hydrate solution and then observed under microscope for determining vein islet and terminations [7].

2.3.3. Powder Microscopy

To study the presence or absence of various types of tissues or structures, the shade dried leaves are powdered using electric grinder, passed through sieve No. 60 and then subjected for microscopic studies [8, 9].

2.4. Physicochemical parameters

The various physicochemical parameters were studied as per standard protocols [10] which include determination of ash contents (total ash, water soluble and acid insoluble), extractive values, moisture content and fluorescence analysis.

2.4.1. Determination of ash values

For determining ash content of drug, about 3 g of powder was spread in a pre-ignited and weighed silica crucible. Then the crucible was incinerated gradually to make the crucible free from carbon. After cooling, crucible was weighed to get the total ash content and then the ash was subjected for determining the acid insoluble and water soluble ash. The percentage of total ash was calculated by taking the air dried sample as standard [11].

2.4.2. Determination of extractive values

Considering the diversity and chemical nature of drug, five different solvents viz. water, ethanol, chloroform, acetone and ethyl acetate were used for determination of extractive values. About 5 g of powdered leaf material was subjected for cold maceration extraction with 100 ml of above solvents. Determination of extractive values of a crude drug is beneficial in its evaluation process wherever evaluation of chemical components in drugs is not possible by any other means [12]. After extraction, the extracts are concentrated in rota vaporizer and dried in vacuum desiccator. Then the extractive values are calculated as percentage w/w of solvent soluble extractive with reference to the air dried drug.

2.4.3. Determination of moisture content

Moisture content was determined by loss of weight on drying (LOD) method [12]. For this 5 gm of drug (powdered leaf material) was taken and kept in an oven at 105 °C till a constant weight was obtained. Amount of moisture present in the sample was calculated as reference to the air dried leaf material.

2.4.4. Fluorescence analysis

Crude drugs show their own characteristic fluorescence when exposed to ultra violet radiation and is dependent on its chemical constituents. This analysis is useful to identify adulterants during crude drug evaluation. In the present study, one gram of crude drug was taken in watch glass and subjected for fluorescent analysis as such and after treatment with different reagents. [13, 14, 15]

2.5. Preliminary phytochemical screening

Plants are considered as bioreactors or biosynthetic laboratories as they synthesize wide range of characteristic therapeutically

important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. For preliminary phytochemical screening 50 g of shade dried leaf powder was extracted using cold maceration technique with ethanol, chloroform, acetone, water and ethyl acetate. Later the extract was filtered and concentrated using rota evaporator and vacuum desiccator. Then the extracts were subjected for preliminary screening using standard procedure for identifying various phytoconstituents^[16].

3. Results

A systematic approach is necessary in pharmacognostic study which helps in confirmation and determination of identity, purity and quality of a crude drug. This detailed and systematic pharmacognostic study will give valuable information for future research work.

3.1 Macroscopic and Organoleptic Studies

Macroscopic study helps in rapid identification of plant material and is the primary step in characterization of crude drug. In *S. potatorum* the leaves are simple, entire and are oppositely arranged. Stipules are completely absent. Petiole is small ranging from 1 to 7 mm long and is glabrous in nature. Leaf blade is elliptical to ovate (with a size of 6 to 15 cm X 3 to 9 cm), cuneate to acuminate, glabrous. Leaves are five veined from the base and show pinnate type of venation.

The organoleptic studies revealed that the leaves are dark green in color on dorsal surface, light green in color on ventral surface with characteristic pungent odour. The powdered leaf material is light green in color, coarse in texture, slightly pungent in odour with bitter taste.

3.2. Microscopic Studies

3.2.1. Leaf Microscopy

The microscopic study of a plant material (leaf, stem, bark or root) mainly serves as an important diagnostic method in differentiation and identification of a particular plant species. The transfer section

of leaf showed the presence of dorsiventral nature. The upper and lower surface is covered by single layer of epidermis. The epidermal cells are undulating. The upper epidermis showed the presence of well-developed cuticle, while stomata appeared only on the lower epidermis (hypostomatic) without any trichomes. The mesophyll showed the presence of distinct palisade and spongy parenchyma. The midrib showed the presence of 3-5 vascular bundles arranged in the form of arc. Vascular bundles are conjoint, bi-collateral and exarch. Each vascular bundle is surrounded by sclereides on all sides. (Figure 1. a & b).

3.2.2. Quantitative Microscopy

The peeled epidermal layers under microscopy revealed that the upper epidermis is made up of polygonal paranchymatous cells without stomata, whereas the lower epidermis showed the presence of number of amphibrachyparacytic stomata (Fig. 1 c). In both the epidermal layers trichomes are absent. The stomatal index in upper epidermis is zero and in the lower epidermis 20.1. The number of vein islets and vein terminations was found to be 12 and 16 respectively.

3.2.3. Powder Microscopy

Powder microscopy mainly helps in detection of adulterated substances and also in confirmation of purity of crude drug. The powder microscopy showed the presence of phloem fibres (Fig 2 d & e), parenchyma cells (Fig 2 a & b), spiral xylem vessels (Fig 1 d; 2 c) and amphibrachyparacytic stomata.

3.3. Physicochemical parameters

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physicochemical parameters like ash values, extractive values, moisture content and fluorescence on reaction with various chemical reagents were investigated and the results are presented (Tables.1-3).

Table 1: Physicochemical parameters of *S. potatorum* leaf powder.

| <i>Physicochemical parameter values (% w/w)</i> | |
|---|-------|
| Total ash | 3.5 % |
| Water soluble | 2.8% |
| Acid insoluble | 0.5% |
| Moisture content | 3.4% |

Table 2: Solvent extractive values (%w/w) of *S. potatorum* leaf powder

| Name of the extract | color | extractive value |
|-----------------------|-----------------|------------------|
| Ethanol extract | Green | 2% |
| Chloroform extract | Dark green | 0.95% |
| Acetone extract | Green | 2.5% |
| Aqueous Extract | Green | 3% |
| Ethyl acetate Extract | Yellowish brown | 1.5% |

Table 3: Fluorescence analysis of *S. potatorum* leaf powder with various reagents

| Reaction mixture | Day light | UV light |
|---|---------------|-------------|
| Powder as such | Green | Dark green |
| Powder + conc. HCl | Dark green | Dark green |
| Powder + conc. H ₂ SO ₄ | Brown | Dark brown |
| Powder + conc. HNO ₃ | Reddish brown | Black |
| Powder + 5% I ₂ Solution | Brown | Dark green |
| Powder + 1N NaOH in water | Dark green | Dark green |
| Powder + 1N NaOH in Methanol | Light green | Light green |
| Powder + 5% FeCl ₃ | Dark green | Dark green |
| Powder + Glacial acetic acid | Green | Dark green |
| Powder + 1% Picric acid in water | Brown | Dark green |

3.4. Preliminary Phytochemical Analysis

The extracts obtained after successive solvent extraction by cold maceration technique with ethanol, chloroform, acetone, water and ethyl acetate were subjected for standard qualitative phytochemical tests to identify the presence of chemical constituents (*viz.*, alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. This type of phytochemical screening of extracts mainly helps in determining the proper polar

solvent for extraction. Results of these tests are presented in Table 4. The preliminary screening in *S. potatorum* leaf powder revealed that the acetone, water and chloroform are the most suitable solvents for extraction of most of the phytochemicals. According to our investigations, the leaves showed rich glycosides, alkaloids and sterols.

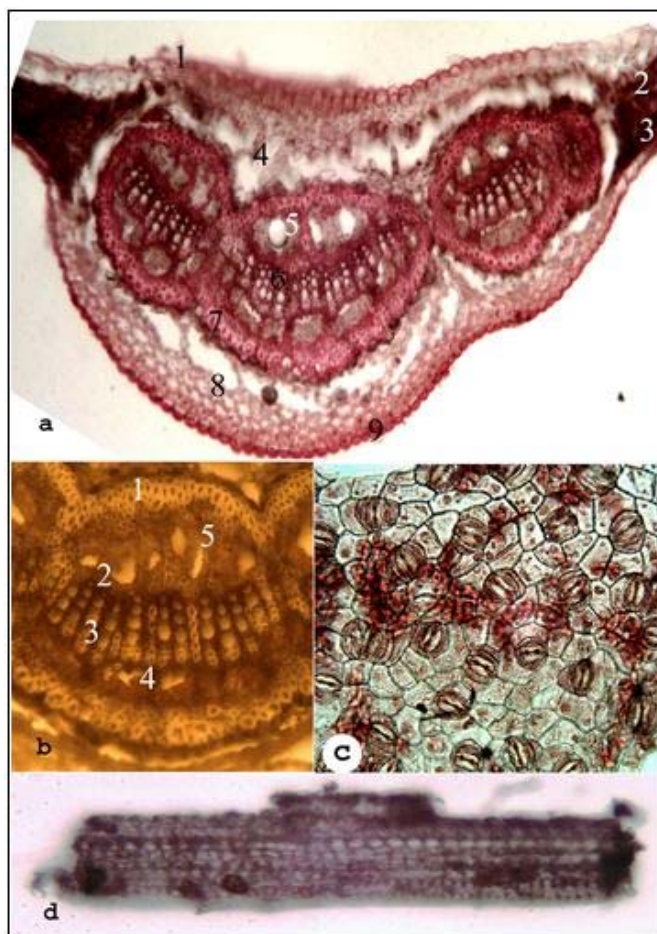


Fig 1. Microscopic study of *S. potatorum* leaf: (a) Transverse section of *S. potatorum* leaf; 1) Upper epidermis; 2) Palisade cells; 3) Spongy cells in lamina; 4) Spongy cells in midrib region; 5) Phloem; 6) Xylem; 7) Vascular bundle; 8) Collenchyma; 9) Lower epidermis; (b) Vascular bundle; 1) Sclerenchyma; 2) Protoxylem; 3) Metaxylem; 4) Abaxial phloem; 5) Adaxial phloem; (c) Lower epidermis showing amphibrachyparacytic stomata; (d) Xylem vessel.

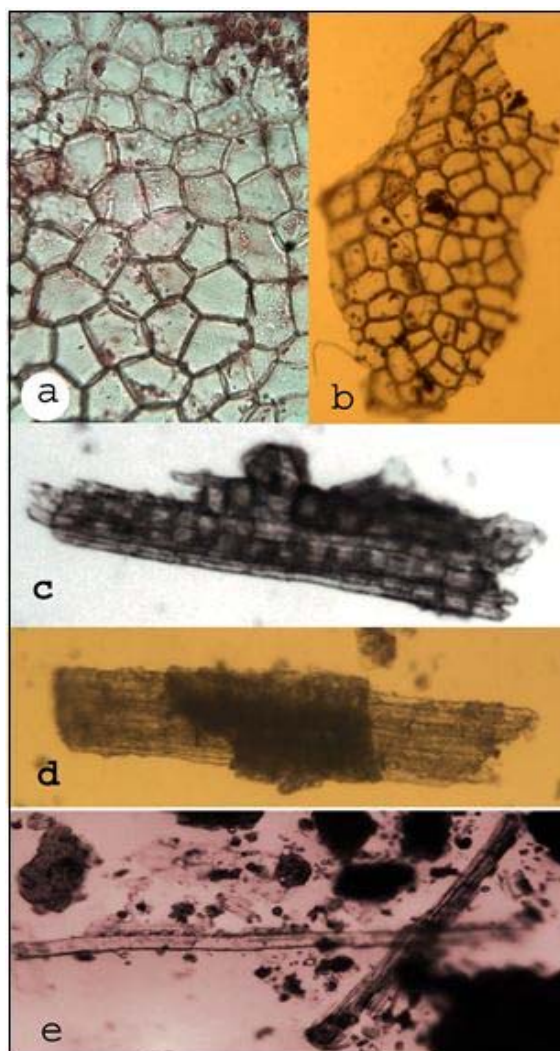


Fig 2: Powder microscopy of *S. potatorum* leaf: (a) Upper epidermis showing paranchymatous cells; (b) Parenchymatous tissue; (c) xylem vessels; (d & e) Fibers.

Table 4: Phytochemical analysis of leaf extracts of *S. potatorum*.

| Compound name | Ethanol extract | Chloroform extract | Acetone extract | Aqueous extract | <i>Ethyl acetate extract</i> |
|-----------------------|-----------------|--------------------|-----------------|-----------------|------------------------------|
| Alkaloids | +++ | +++ | ++ | +++ | + |
| Glycosides | +++ | +++ | +++ | +++ | +++ |
| Tannins | + | ++ | + | ++ | - |
| Flavonoids | - | ++ | - | +++ | - |
| Sterols/Triterpenoids | + | + | ++ | ++ | + |
| Fats & Oils | - | - | - | - | - |
| Phenols | - | + | - | + | - |
| Quinones | - | - | - | + | - |
| <i>Saponins</i> | - | - | + | + | - |

+: Weak positive test; ++: Low positive test; +++: Strong positive test; -: negative test.

4. Discussion

Physicochemical parameters like ash values, moisture content and fluorescence analysis are helpful in determining the physiological and non-physiological ash, possibility of microbial growth or contamination and presence of impurities respectively. The relative low acid insoluble ash value (0.5%) and a high ratio of water soluble ash content (2.8%) of *S. potatorum* indicates that the crude drug contains more physiological ash or contents (i.e. related to plant tissue) than the non-physiological content. The relative low moisture content (3.5%) indicates that the drugs give less possibility for microbial growth and contamination.

The preliminary phytochemical investigations in *S. potatorum* leaves indicated the presence of alkaloids, glycosides, tannins, flavonoids, sterols and triterpenoids in more quantity where as the phenolics, quinones and saponins in less quantity, but there are no fats and oils. Similar findings were also found in root, stem bark and seed extract of *S. potatorum* [2]. Presence of these phytoconstituents was also reported in *S. potatorum* seeds, roots, stem and bark which are pharmaceutically important [17,3]. Presence of such phytochemical constituents may be responsible for various pharmacological activities of this elite medicinal plant.

Medicinal plants are considered as living factories as they produce various phytochemicals viz., alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc in the form of secondary metabolites. These serve as life saving drugs. Due to this, day by day demand for crude drugs is increasing and also adulteration of crude drugs. Hence, standardization of a crude drug is essential to avoid and identify adulteration [15]. In standardization of a crude drug, macroscopic and microscopic evaluation is the primary step. According to WHO, botanical standard investigations like epidermal cells, stomatal index, vein islet and termination values etc are mandatory for the diagnosis of the herbal crude drug [18,19].

5. Conclusion

In the present investigation, a set of pharmacognostical standardization parameter studies were conducted on *S. potatorum* leaves as per pharmacopoeia and WHO guidelines. These studies revealed the presence of various important bioactive compounds and proved that the plant leaves are also medicinally important. These results may help in standardization, identification and in carrying out further research in *S. potatorum* leaf based drugs which are used in Ayurveda and modern pharmacopoeia.

6. Conflicts of interest statement

We here with declare that we have no conflict of interest.

7. Acknowledgements

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