Pharmacognostical And Phytochemical Studies Of Strychnos Potatorum Linn Seeds

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ABSTRACT:

Introduction: Strychnos potatorum Linn (Fam: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lanka and Burma. In traditional system of medicine the seeds are used for the treatment of various ailments like jaundice, bronchitis, diabetes, conjunctivitis, chronic diarrhoea, dysentery etc. They are also used to clear muddy water by its coagulant action. Although the seeds are useful in various treatments, its pharmacognostic features and phytochemical analysis were not studied.

Methods: The pharmacognosy of the seed was studied by evaluating the macroscopic and microscopic characters, whereas the phytochemistry was studied by fractionation and HPTLC fingerprinting.

Results: Microscopic evaluation of the seed revealed the presence of testa with tangentially elongated shrunken parenchyma in the outer zone and trichome zone in the inner layer. Calcium oxalate crystals were seen on the surface of the seed. Endosperm tissue consists of palisade like epidermal cells, thick and prominent cuticle on the surface of the epidermis. Physiochemical constants of the seed powder showed 7.65% moisture, 4.39% alcohol soluble extractive, 12.25% water soluble extractive, 1.43% total ash, and 0.09% acid insoluble ash. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, steroids/triterpenes, polyphenolics, reducing sugars, saponins and anthocyanins. HPTLC screening of the ether fraction of SPP showed seven peaks, unsaponifiable fraction showed five peaks and alkaloidal fraction showed eight peaks at 260 nm.

Conclusion: The pharmacognostical features and physiochemical constants of Strychnos potatorum reported here will be useful to identify the seeds from its adulterants.

Keywords: Strychnos potatorum; pharmacognostical studies, physiochemical constants, HPTLC.

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INTRODUCTION

Strychnos potatorum Linn (Fam: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lanka and Burma[1]. In traditional system of medicine, the seeds are used in the treatment of gonorrhoea, leucorrhoea, gastropathy, bronchitis, chronic diarrhoea, dysentery, renal and vesicle calculi, diabetes, conjunctivitis, scleritis, ulcers and other eye disease[2]. The ripe seeds are used for clearing muddy water. The clarification is due to the combined action of colloids and alkaloids in the seeds[3].

Phytochemical studies revealed the presence of diaboline (major alkaloid) and its acetate[4]; brucine, loganin, mannose, sucrose, arachidonic, lignoceric, linoleic, oleic, palmitic and stearic acids[5]; on saponification of the oil: β-sitosterol, stigmasterol (also in leaves and bark along with campesterol); oleanolic acid and its 3β acetate, saponins containing oleanic acid, galactose and mannose (seeds)[6]; triterpenes and sterol[7]mannogalactans[8,9,10].

The total alkaloid fraction isolated from the seeds of Strychnos potatorum when administered in mice and rats at the doses of 70–100 mg/kg, i.p. produced restlessness, irritability and tremors, followed by convulsions of tonic type all over the body[11] and hypotensive action[12]. The methanolic extract of the dried seeds was found to have antidiarrhoeal[13] and diuretic[14] activities. The seed powder was found to possess antidiabetic activity[15]. Mannogalactans isolated from the seeds of Strychnos potatorum showed antihypercholesterolemic activity in experimental rats[8].
MATERIALS AND METHODS

**Plant material**

The seed specimens for the study were collected from crude drug market, Chennai and the genuinity of the seed specimen was confirmed by Dr. S. Jayaraman, Botanist, Plant Anatomy Research Centre, Chennai, Tamilnadu. The following protocol was used for the pharmacognostic authentication.

**Macroscopic evaluation**

The seeds were evaluated and its macroscopy was photographed to view its special characteristics like shape, size and colour.

**Microscopic evaluation**

1) **Processing of the specimen**

The seed specimens were boiled in distilled water for about 30 min in order to soften the tissues and fixed in FAA (Formalin 5 ml + acetic acid + 70% ethyl alcohol 90 ml). After 24 hrs of fixing, the specimen was dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimen was carried by gradual addition of paraffin wax until, tertiary butyl alcohol solution attained super saturation\(^{[16]}\). The specimens were cast into paraffin blocks.

2) **Sectioning**

The paraffin embedded specimens were sectioned to a thickness of 10–12 μm and stained with toluidine blue\(^{[17]}\). For studying the epidermal trichomes, the seeds were immersed in hot water for few min and the trichomes were removed by scraping the surface of the seed with the scalpel. The scrapped material was mounted in a drop of glycerin and sealed with the cover slip.

3) **Photomicrographs**

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Calcium oxalate crystals and trichomes were studied under polarized light and photographed.

**Physiochemical constants**

Determination of moisture content, extractive values (Alcohol and water soluble extractives) and ash values (Total ash, acid insoluble ash and water soluble ash) were done according to the methods given in Ayurvedic Pharmacopoeia of India, 1999\(^{[18]}\).

**Phytochemical analysis**

**Fractionation**

The seed powder (SPP) was processed to obtain different fractions and used for preliminary qualitative chemical analysis. The TLC and HPTLC patterns of specific fractions were also developed.

The seed powder was processed as follows:

### Phase I

- Extracted with ether
- Ether extract (1) (Volatile oils/ terpenes)
- Saponification with methanolic KOH and extracted with ether
- Ether soln (2) (Unsaponifiables) (Steroids/triterpenes/saponins)
- Aqueous soln
- Acid extract made alkaline with ammonia soln
- Alkaline extract extracted with CHCl\(_3\), three times
- CHCl\(_3\) extract (Presence of alkaloids) (3)

### Phase II

Powdered *Strychnos potatorum* seeds was extracted with methanol for 5 min on a water bath at about 60°C and then filtered. The methanol extract (4) was tested for alkaloids, flavonoids and tannins. The marc obtained was extracted with hot water. The water extract (5) was tested for sugars, tannins etc.

Preliminary qualitative phytochemical screening was carried out according to the method of Kokate, 1997\(^{[19]}\) and Trease and Evans, 1983\(^{[20]}\).

**TLC analysis**

Thin layer chromatography was developed in precoated silica gel plates Merck 60 F\(_{254}\) of 0.2mm thickness.

**SPP fractions**

1) **Ether extract**

Solvent systems: Hexane: Ethyl acetate (9: 1).
Detection: Vanillin sulphuric acid
ii) Unsaponifiables in ether

Solvent system: Toluene: Ethyl acetate (4:1).
Detection: UV at 260 nm, Libermann burchardt reagent,

iii) CHCl₃ extract (Alkaloid fraction)

Detection: Under UV at 260 nm

**High-pressure thin layer chromatographic study (HPTLC)**

The phytoconstituents identified through qualitative chemical analysis and TLC was further processed for HPTLC in precoated silica gel plates (Merck 60F254) of 0.2 mm thickness and 10x10 cm plate size. About 3 μl of the samples were applied in the plates using Linomat IV Automatic Spotter. Then the plates were developed in CAMAG Twin Trough Chamber of dimension 20x10 cm. The developed plates were air-dried and detected by UV (under deuterium lamp, 260 nm) / spraying reagents (under tungsten lamp, 550 nm) and densitometric scanning was done using CAMAG TLC scanner to record the peaks. The Rf values and the percentage area of the separated phytoconstituents were determined.

**RESULTS AND DISCUSSION**

**Macroscopic features of the seed**

The gross morphology of the unground whole seed was circular in surface view and ellipsoidal in lateral view. The edge of the seed is marked by thin, smooth, even circular ridge. The hilum occurs at the center of the seed and appears as a minute scar. The micropyle lies on the median portion of the lateral ridge. The seeds are ash grey and measure 7 mm in diameter, and 5 mm in thickness. The seed surface is smooth and even. No specific odour or taste is evident. The seeds are hard and strong, become soft on prolonged boiling. The surface of the dry seed exhibited fine reticulate marking which are visible only to the hand-lens. (Fig.1)

**Microscopic features (Figs. 2, 3, 4 & 5)**

This is a valuable test for both powders and for unground drugs. The identity of many adulterants of unground drugs can be established or confirmed by an examination...
Spectrum 2: HPTLC fingerprinting of Unsaponifiable fraction of SPP.

Spectrum 3: HPTLC fingerprinting of alkaloidal fraction of SPP.
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**Figure 1** Macroscopic features of the seeds. H – Hilum; LV – Lateral View; M – Micropyle; SV – Side View of the seed.

**Figure 2** Microscopic features of the seeds. Sectional view of the seeds. C – Central cavity; En – Endosperm; T – Testa.

**Figure 3** TS of the seeds to show enlarged view of the Testa and endosperm. 1. A portion of the Testa and Endosperm. 2. Basal part of the trichomes with horizontal part and epidermis of the endosperm. Cu – Cuticle of the epidermis; En – Endosperm; EP – Epidermis; Pa – Parenchyma zone; T- Testa; Tr – Trichome.
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**Figure 4** Seed coat under polarized light. 1. TS of the Testa and endosperm showing horizontally oriented trichomes. 2. Crystal deposition along the cuticle of the epidermis. B- Basal part of the Trichome; Cr – Crystal; En- Endosperm; Tr- Trichome.

**Figure 5** Trichomes of the seed under polarized light. 1. Bunch of trichomes scrapped out of the seed. 2. A single trichome showing the basal part cell lumen. B – Basal part of the trichome; L – Lumen of the trichome.

of calcium oxalate crystals, by the details of structure of the trichomes and other features. In transverse-vertical section, the seeds are elliptical and consist of a central cavity, which is broader in the middle and become gradually tapering on the opposite ends. The seed is occupied by massive endosperm around the central narrow elliptical cavity. The testa of the seed is uniformly thick in all parts of the seed.

**Testa (seed coat)**

The seed coat or testa consists of two distinct zones: the outer zone is somewhat uneven and consists of tangentially elongated, thin walled very much shrunken parenchyma, inner to the parenchyma zone is the trichome zone where dense, thick walled trichomes occur very close to each other. These trichomes have dilated basal part with which
the hairs are attached on the surface. The major portion of the trichome has thin narrow longitudinally running lumen. The unique feature of the trichomes is that all of them are bent at the base right angles and lie prostrate over the seed. The hairs are highly thick walled and walls are lignified. The lignification of the walls is evident, as the hairs appear brightly glittering when seen under the polarized light. It was also observed that calcium oxalate prismatic crystals were frequently seen on the surface of the seed i.e. at the basal part of the trichomes and on the surface of the endosperm tissue.

**Endosperm**

The endosperm tissue consists of vertically elongated, palisade-like epidermal cells on the surface zone of the seed. A thick and prominent cuticle is seen on the surface of the epidermis where the crystals occur. The cells inner to the palisade-like epidermal cells become gradually polygonal in outline. These cells have very thick walls and narrow lumen where cell inclusions are seen. The cell wall consists of cellulose and no lignification of the thick walls is evident. The narrow lumen of the endosperm cells contains nucleus and storage food materials. Plasmodesmatic connections are frequently seen crossing the cell walls and connecting the cytoplasm of adjacent cells.

**Physiochemical constants**

The physiochemical constants were determined and shown in table 1. The determination of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter, it is more especially applicable to powdered drugs. The total ash value is useful to exclude drugs, which have been coated with chalk, lime, or calcium sulphate to improve their appearance. The total ash value vary within wide limits for different specimens, whereas acid insoluble ash i.e. the ash insoluble in dilute hydrochloric acid, is often of more value than the total ash. The water-soluble ash is used to detect the presence of material exhausted by water. The water soluble ash is subjected to much greater reduction than is the total ash and is therefore used as an important indication of the presence of exhausted material substituted for the genuine article[21]. Alcohol soluble extractive value is frequently employed to determine the approximate resin content of the drug.

Determination of moisture content indicates the percentage of active chemical constituents in crude drugs mentioned on air-dried basis. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination[19].

**Phytochemical screening**

On detailed phytochemical screening by qualitative analysis, the SPP fractions showed the presence of steroids, triterpenoids, saponins and alkaloids in Phase I whereas flavonoids, tannins, carbohydrates, reducing sugars in Phase II. (Table 2)

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>SPP fractions</th>
<th>Methanol fraction of SPE (6)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
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<tr>
<td>Carbohydrates (Molisch’s test)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Proteins and aminoacids (Millon’s and Biuret test)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Alkaloids (Mayer’s, Wagner’s and Dragendorff’s tests)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Steroids/Triterpenes (Libermann burchardt test)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Polyphenolics (Flavonoids/Tannins) Ferric chloride and Lead acetate test</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Reducing sugars (Benedict’s and Fehling’s test)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saponins (Foam test)</td>
<td>–</td>
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(+) Presence
(-) Absence.
**HPTLC analysis**

HPTLC profile of all the fractions showed well-resolved peaks on densitometric scanning at both UV light and after spraying specific reagents.

- **SPP (Ether fraction):** Scanning at 550 nm showed seven well resolved peaks, which were detected by spraying Vanillin sulphuric acid reagent. The Rf values were 0.09, 0.17, 0.26, 0.36, 0.45, 0.58 and 0.69 (Spectrum 1).
- **SPP (Unsaponifiable fraction):** At UV 260 nm, densitometric scanning gave five resolved peaks. The Rf values were 0.35, 0.43, 0.49, 0.60 and 0.71 (Spectrum 2).
- **SPP (Alkaloid fraction):** Scanning at UV 260 nm gave eight different peaks with Rf values 0.15, 0.21, 0.31, 0.39, 0.42, 0.54, 0.64 and 0.74 (Spectrum 3).

In conclusion, the pharmacognostic evaluation proves the authenticity of the seeds of *Strychnos potatorum* Linn. The preliminary chemical and chromatographic analysis of SPP and SPE showed the presence of phytochemicals like steroids, triterpenoids, saponins, polysaccharides and polyphenolics which may contribute various pharmacological activities of these drugs (SPP and SPE).

**REFERENCES**