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PHYTOCHEMICAL ANALYSIS AND HISTOLOGY OF *Strychnos potatorum* L. SEEDS

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ABSTRACT

Plan: *To evaluate the Pharmacognostic properties including physicochemical characters and HPTLC profile of seeds of Strychnos potatorum L.*
Methodology: *Micro and macroscopic characters of fresh and dried seed samples were analyzed. Physicochemical studies, fluorescent behavior of seeds and estimation of Quercetin by HPTLC were performing by using standard procedure.*
Outcome: *Microscopic studies revealed that testa comprises two different region: the outer region consists shrunken parenchyma and inner is trichome zone with dense trichomes occur very close to each other. Calcium oxalate prismatic crystals are frequently seen. Physicochemical parameters such as foreign matters, moisture content, extractive values, ash content, and fluorescent behavior of seed powder were also determined. This report on the pharmacognostic studies of S. potatorum may help investigators, in the characterization of the crude drug and to screen pharmacological activities of this species.*

1. INTRODUCTION

The genus Strychnos, the largest genus of the family Loganiaceae, was first described by Linnaeus, comprises about 200 species, which may be subdivided into three geographically separated groups widely distributed around the world's tropics: one in Africa with 75 species; one in America with 73 species; and one in Asia (including Australia) with 44 species. Only exception is *S. potatorum* which is found both in Africa and Asia. *Strychnos potatorum* L. commonly known as “clearing nut tree” or Nirmali is a medium sized glabrous deciduous tree having a height of 6-18 meters.

Seeds of *Strychnos potatorum*

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It is indigenous to India. The ripe seeds are used for clearing muddy water. This cleaning property is due to the communal action of alkaloids and colloids present in the seeds. The seeds are said to posses number of biological activities like diuretic activity, antidiarrheal activity, hepatoprotective and antioxidant, antiulcer, anti-inflammatory activity and antiarthritic activity[1-7] because of various constituents present in the drug like triterpenes Isomotiol and many alkaloids[8]. Traditionally, the seeds are used in diabetes and gonorrhea and have a bitter bad taste, astringent to the bowels, aphrodisiac, tonic, diuretic, good for liver, in kidney complaints, improve the eyesight and a good remedy for snake bite[9]. Pharmacognostic and phytochemical investigation reveals the comprehensive study on *Strychnos potatorum* Linn in order to establish standards to differentiate it from other species of drugs.

2. MATERIALS AND METHODS

2.1. Collection & authentication of plant material

Seeds of *S. potatorum* were procured from local herbal drug market of Allahabad, authenticated by Taxonomist Dr. A. K. S. Rawat and the voucher specimen (No. CIF-RB-2-126-2) was deposited in the departmental herbarium of National Botanical Research Institute Lucknow, India for future reference. All the other chemicals used were of analytical and highest purity grade from standard companies.

2.1.1. Macroscopic & microscopic evaluation

The Macroscopical characters of seeds like shape, size odour, taste and colour were observed. The seeds 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. The specimens were passed into paraffin blocks[10].

2.1.2. Sectioning

The paraffin embedded specimens were sectioned to a thickness of 10–12 μm and stained with toluidine blue[11-12]. For studying the epidermal trichomes, the seeds were immersed in hot water for few min and the trichomes were removed by scrapping the surface of the seed with the scalpel. The scrapped material was mounted in a drop of glycerin and sealed with the cover slip. Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnification were taken with Nikon Lab photo 2 microscopic unit.

2.2. Physicochemical parameters

Physicochemical parameters viz. moisture content, ash values, extractive values with various reagents were determined as per the Indian Pharmacopoeia[13]. The fluorescence characters of the powder with different reagents were observed under visible light and UV light as per the standard procedure[14].

2.3. Preparation of extract and phytochemical screening

The powder was prepared by grinding the dried seeds in a blender. 500g seed powder was macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 50% ethanol for 3 days (3 X 3L) by cold percolation method and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Heto Drywinner, Thermo Scientific, USA) under reduced pressure and thus 215.0 g of solid residue (yield 21.5 % w/w) was obtained. The extract was subjected to preliminary phytochemical screening for the identification of various active constituents[15-16].

2.4. HPTLC fingerprint profile

2.4.1. Selection of mobile phase

Non polar and polar solvents in different ratios Ethyl acetate: acetic acid: formic acid: water in the ratio (10:1:1:1v/v), Toluene: Ethyl acetate: formic acid: methanol in the ratio (3:3:0.8:0.2 v/v), Chloroform: Ethyl acetate: formic acid in the ratio (5:4:1 v/v)] were tried as the mobile phase for the extract of *S. potatorum* and for the quantification of Quercetin. The optimum mobile phase for the separation of constituents from extract was found to be Toluene: Ethyl acetate in the ratio (7:3v/v) and for Quercetin is Toluene: Ethyl acetate: Formic acid in the ratio (5: 6: 2v/v).

2.4.2. HPTLC profile of the total extract

2gms of extract of *S. potatorum* was weighed and dissolved in 25ml of methanol in a eppendrof tube (Solution A). 10 µl of solution A was taken in a syringe and applied on pre-coated silica gel 60 F_{254} plate of thickness 0.2 mm. The plates were air dried and developed in twin trough chamber using Toluene: ethyl acetate (7:3v/v) as mobile phase. After development, the plates were dried and densitometrically scanned at 376 nm.

2.4.3. Quantification of Quercetin

10 µl of the solution A was applied on plate, the plate was then developed in the solvent system (Toluene: Ethyl acetate: Formic acid in the ratio (5: 6: 2v/v) as mobile phase. The plate was dried and scanned densitometrically at 368 nm.

3. RESULTS

3.1. Morphological study

Macroscopically, the seed of *Strychnos potatorum* is spherical in outside. No definite odor or taste is marked. The seeds are tough and sturdy, become soft on extended boiling. The seeds are yellowish white in colour and measure 6-7 mm in diameter, and 4-5 mm in thickness. The surface of the dry seed exhibited fine reticulate marking.

3.2. Microscopic characters

The seed coat or testa comprises two different region: the outer region 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 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.

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. The transverse section of testa showed horizontally oriented trichomes.

3.3. Physicochemical parameters

Physicochemical parameters like foreign matter, percentage of moisture content, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were determined and depicted in Table 1**.** The results of fluorescence analysis of the powder drug are mentioned in Table 2.

Table 1: Physicochemical parameters of S. potatorum

Parameter	*% w/w**
Foreign matter	Nil
Moisture content	7.47
Total ash	2.23
Water soluble ash	-
Acid insoluble ash	0.08
Alcohol soluble extractive value	5.65
Water soluble extractive value	10.22

Table 2: Fluorescence analysis of S. potatorum powder

S.No.	*Reagents*	*Color of the powdered drug*	
		Day light	*Ultraviolet light (254nm)*
1.	Saturated picric acid	Yellow	Dark Bluish
2.	50% Nitric acid	Orange	Greenish
3.	Hydrochloric acid	No change	Light green
4.	Sulphuric acid (80%)	Yellow	Light green
5.	Glacial acetic acid	No change	Green
6.	Iodine solution (N/20)	No change	Dark green
7.	Powder as such	Whitish brown	Dull brown
8.	Methanol	No Change	Dark green

3.4. Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of carbohydrate, fixed oil, saponins, phenolic compounds, phytosterols, alkaloids, flavonoids in 50% ethanolic extract of seeds. (Table 3)

3.5. HPTLC profile of total extract

The HPTLC profile of extract in selected solvent system revealed the presence of thirteen spots at R_f 0.05 (8.54%), 0.26 (4.88%), 0.34 (10.26%), 0.37 (6.35%), 0.44 (18.60%), 0.48 (15.01%), 0.55 (6.07%), 0.59 (3.96%), 0.63 (8.50%), 0.71 (2.55%), 0.73 (2.80%), 0.78 (3.18%), 0.81 (3.92%) and 0.93 (5.39%), the maximum percentage were found to be of components 5 and 6 at R_f 0.44 (18.60%) and 0.48 (15.01%), respectively. (Fig.1)

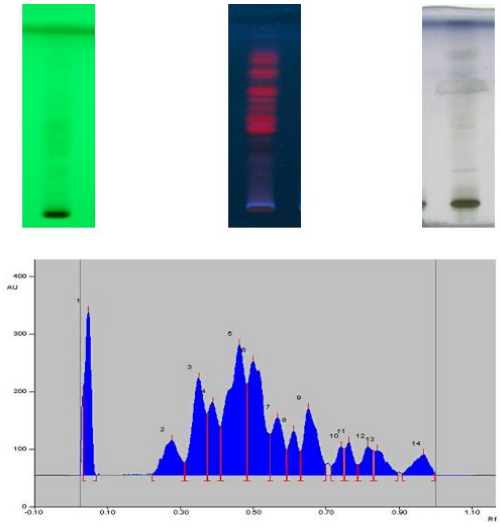

Figure.1: HPTLC fingerprint profile of Strychnos potatorum in solvent system Toluene: Ethyl acetate (7:3) and scanned at 376nm.

Table 3: Preliminary phytochemical screening of the 50 % ethanolic extract of S. potatorum

S. No.	*Constituents*	*Tests*	*50% Ethanolic extract*
1.	Carbohydrate	Molish's test	+
		Fehling's test	-
2.	Fixed oil & fats	Spot test	+
		Saponification test	+
3.	Proteins & amino acids	Millon's test	-
		Ninhydrin test	-
		Biuret test	-
4.	Saponins	Foam test	+
5.	Phenolic compounds	$FeCl_3$ test	+
		Pot. permanganate test	–
		Lead acetate test	+
6.	Phytosterol	Salkowiski test	+
		Liebermann Burchard test	+
7.	Alkaloids	Dragendorff test	+
		Mayer's test	-
		Wagner's test	+
		Hager's test	-
8.	Gum & mucilage	Swelling test	–
9.	Flavonoids	Aqueous NaOH test	+
		Con. H_2SO_4 test	+
		Shinoda's test	-

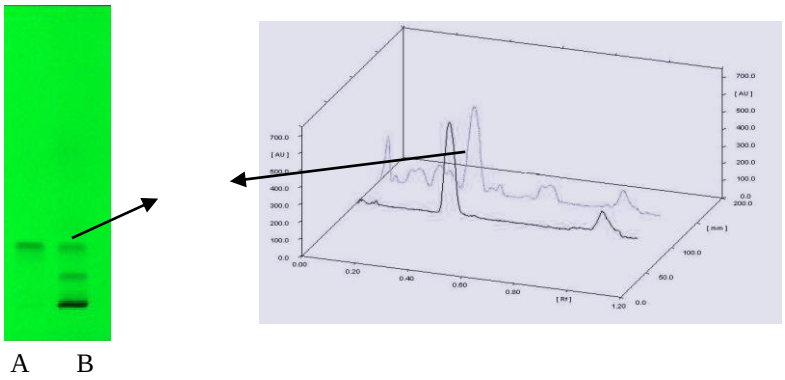

Figure 2&3: Photograph of chromatograms obtained, at 368 nm, from Quercetin standard (A) and extract (B) of the seeds of Strychnos potatorum

3.6. Quantification of Quercetin in extract

A band (Rf = 0.44) corresponding to marker compound (Quercetin) was visible in both reference solution and test solution tracks. The percentage of Quercetin was found to be 10.5 % (w/w). (Fig. 3)

4. DISCUSSION

Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. The macro and micro morphological features of the seed described, distinguishes it from other members of the genera. The percent extractives in different solvents indicated the quantity and nature of constituents. The fluorescence analysis of the powdered drug in various solvents shows the change in color indicated presence of chromophore and fluorescent compounds. Phytochemical investigation of the secondary plant metabolites are known to possess various pharmacological effects and may be responsible for the various actions. HPTLC profile of the extract shows thirteen spots qualitatively whereas Quercetin present in the extract was found to be 10.5% w/w at R_f value of 0.44. As there is no pharmacognostic anatomical work on record for this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Macro and micro morphological standards and HPTLC profile discussed here can be considered as identifying parameters to substantiate and authenticate the drug.

5. CONCLUSION

It can be concluded that the present study on seeds of *S. potatorum* can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material available in the market. The determination of these characters will aid future investigators in their pharmacological analyses of this species.

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