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Pharmacognostical and phytochemical standardization of seeds of *Strychnos potatorum* Linn. (Loganiaceae)

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Abstract
In traditional system of medicine, plants with medicinal activity play a major role in curing diseases. Now-a-days, usage of herbal medicines is increasing globally for the treatment of diseases. Plants have been evaluated for their potential benefits. In the present study, pharmacognostical and phytochemical standardization of seeds of *Strychnos potatorum* Linn. (Loganiaceae) was carried out. The pharmacognostical evaluation was performed for proper identification and to detect the adulterants. Physicochemical analysis like ash value (Total ash – 1.98% w/w, acid-insoluble ash – 0.5% w/w, water soluble ash – 0.99% w/w, sulphated ash – 1.02% w/w), extractive value (water soluble - 9.26% w/w, alcohol soluble - 6% w/w), loss on drying (7.11% w/w) and iron content (1.85% w/w) was estimated. Phytochemical study characterizes the phytoconstituents present in this plant. Evaluation of the seed of *Strychnos potatorum* ensures identity and ascertains quality and purity of this drug.

Keywords: *Strychnos potatorum*, Loganiaceae, pharmacognostical, phytochemical standardization

Introduction
In all traditional system of medicine, plants with medicinal activity play a major role in curing diseases. In Indian system of medicine, Ayurveda, Unani, Siddha and Homeopathy herbal drugs play a major role for treating the various acute and chronic conditions. Herbal medicine is widely used now-a-days for the treatment of diseases when compared with modern medicine. Advantage of using herbal medicine is because of fewer side effects, low cost and easily available. Herbal drugs with one or more active ingredients from the various parts of the same or different plants were used to treat diseases. Plants have been evaluated for their potential in the treatment of various diseases. *Strychnos potatorum* Linn belongs to the family Loganiaceae was selected for the study. Aim of the present study is to evaluate the pharmacognostical and phytochemical analysis on the seeds of *Strychnos potatorum*. Synonym for *S. poatatorum* is *Strychnos heterodoxa, Strychnos stuhimannii*. Common name is clearing nut tree. In Tamil, it is called as Tetan-kotai, tettran, Tetta and Tettamaram. It is present in West Bengal, Central and South India, grown up to 1200 m altitude [1, 2].

Taxonomy

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Super division	:	Sperrmatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Asteridae
Order	:	Gentianales
Family	:	Loganiaceae
Genus	:	*Strychnos*
Species	:	*potatorum*

A tall tree of 13 m height with opposite branches. Seeds are one or two, circular, 8 mm diameter, bluntly lenticular, not compressed, shining with silky hairs. Seeds are yellow in colour. Seeds are used for clearing muddy water. Seeds are used as antidiabetic, astringent, aphrodisiac, antidysentric, diuretic, gonorrhea, stomachic and demulcent. Antidote to snake poison [3, 4, 5].

Materials and Methods

Plant Collection

Seeds of *Strychnos potatorum* was procured from local pharmacy, Chennai, Tamil Nadu, India.

Identification and Authentication

Procured seeds of *Strychnos potatorum* was identified and authenticated by Dr. Sunil Kumar, Research Officer-Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai, Tamilnadu, India. Number is S17100503C. A voucher specimen of the plant was deposited in the herbarium for reference.

Preparation of powder

Collected seeds were made into coarse powder using a grinding mill. Then the powder was stored in an air tight container.

Chemicals

Ethanol, formaldehyde, acetic acid, safranin, phloroglucinol, hydrochloric acid, glycerine and all other chemicals used were of analytical grade.

Macroscopical Evaluation

Maroscopical evaluation is the preliminary evaluation of drugs which can be observed with the naked eye and sensory feelings. Shape, size, surface, colour, odour and taste was observed [6, 7].

Microscopical Evaluation

Various parts of the plant possess a characteristic features and they can be studied from the cells, tissues and their arrangements with suitable staining technique. Seeds of *Strychnos potatorum* is kept in the fixative solution FAA (Formalin 5 ml + Acetic acid 5 ml + 70% ethyl alcohol 90 ml) for more than 48 hours. Then the specimens were cut into thin transverse section and stained with safranin. Photographs were taken with Nikon ECLIPSE E200 trinocular microscope attached with digital camera under bright field light [8, 9, 10].

Powder Microscopical Evaluation

A pinch of powdered material was stained with glycerine and mounted for studying the characters of powdered crude drugs [11, 12].

Quantitative Microscopy

Length and width of stone cells and trichomes were measured. These values are most important to identify whether the powdered parts of the plant are adulterated or substituted with inferior quality [13, 14].

Physicochemical Analysis

Physicochemical analysis was performed as per the standard procedures of WHO guidelines and Indian Pharmacopoeia. Ash values, extractive values, loss on drying and foaming index were performed [15, 16, 17, 18, 19, 20].

Fluorescence Analysis

Fluorescence analysis of powdered drug with suitable reagents was performed as per Chase and Kokoski methods [21, 22].

Determination of heavy metal contamination

Limit tests for heavy metals like arsenic and lead was performed as per Indian Pharmacopeia 1996 [17].

Test for arsenic

Test for arsenic was carried out using the apparatus of 100 ml conical flask closed with glass stopper, through which a glass tube is passed. The glass tube is placed in a position of atleast 3 mm below the lower surface of the stopper. Second tube has placed to contact with the first tube. 50 – 60 mg of lead acetate cotton is loosely packed and a small square of mercuric chloride paper is placed to cover the orifice of the tube. To the conical flask, 1 ml of test solution, 10 gm of zinc AST and 5 m1 of 1 M potassium iodide was added. Close the container. Repeat the procedure for standard 1 ml of arsenic diluted to 50 ml with water. After 40 minutes, any stain produced on the mercuric chloride paper of sample is compared with the standard mercuric chloride paper.

Test for lead

Standard solution: 1 ml of standard lead solution is taken in a 50 ml Nessler cylinder and dilute with water to make up to the volume 25 ml. Adjust the pH between 3 and 4 with dilute acetic acid or dilute ammonia solution. Then dilute to 35 ml with water and mix.

Test solution: Dissolve the seed extract with water to 25 ml and transferred to a 50 ml Nessler cylinder. Adjust the pH between 3 and 4 with dilute acetic acid or dilute ammonia solution. Then dilute to 35 ml with water and mix.

Procedure: Add 10 ml of freshly prepared hydrogen sulphide solution to both standard and sample. Mix well and dilute to 50 ml with water. Allow to stand for five minutes and observe the colour produced in both the standard and test. The colour produced in the test solution is not more than the colour produced in the standard solution [23, 24, 25].

Qualitative and quantitative estimation of inorganic elements

Presence of inorganic elements like sodium, phosphate, potassium, nitrate, iron, magnesium, calcium, sulphate, chloride and carbonate were qualitatively evaluated as per the standard procedure present in Indian Pharmacopeia 1996 [17].

Quantitative estimation of Iron

Iron content was quantitatively determined as per the method described by Sethi [26].

Preparation of standard

To 50 mg of the ferrous fumarate, add 20 ml of 10% sulphuric acid and heated on a water bath for 30 minutes. Shake well and cool. Then dilute the solution to 100 ml with water. Take 2.5 ml of the resulting solution and dilute to 50 ml with water.

Preparation of sample

Sample solution is prepared similar to standard solution with 50 mg of the seed of *Strychnos potatorum.*

Procedure

To each 5 ml of standard and sample solutions, add 2.5 ml of ascorbic acid solution and 5 ml of acetate buffer solution of pH 4.7. It is kept aside for 15 minutes at room temperature. To this solution add 2.5 ml of 3% w/v bipyridyl solution. Shake well and adjust it to 25 ml with water. Absorbances are measured for both the standard and sample at 523 nm against reagent blank and calculate the results.

Determination of microbial contamination
Total viable aerobic count for bacteria and fungi was determined by plate count method [16, 17].

Preliminary Phytochemical Screening
Preliminary phytochemical screening was carried out to find out the active constituents present in this plant as per standard procedure [27, 28, 29].

Results
Macroscopy: Seeds are circular, shiny with short, appressed silky hairs, 8 mm in diameter. Cream-white in colour with a slightly prominent ridge round the border. Odour is characteristic and taste is nil. (Fig. 1).

Fig 1: *Strychnos potatorum*

Microscopy
TS of seed showed testa, with two to three layers of thick-walled, elongated, lignified sclerenchymatous cells. These cells are covered with cylindrical, unicellular trichomes with swollen basal portion numerously. There is a collapsed cell layer below which, followed by outer endosperm composed of 3 to 8 layers of thick walled, elongated palisade like cells arranged in rows. The inner endosperm composed of oval to polygonal, thin walled, parenchymatous cells having numerous small aleurone grains and oil globules (Fig. 2, 3, 4).

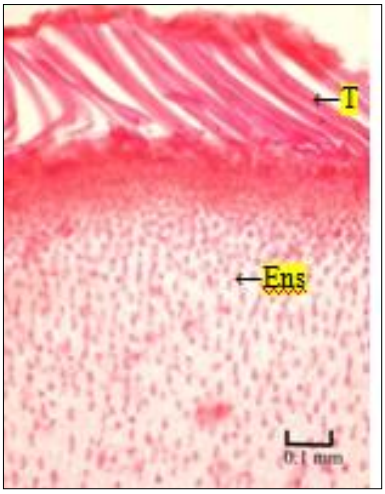

Fig 2: TS of seed

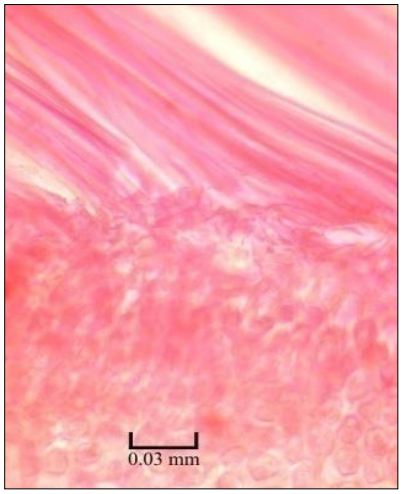

Fig 3: Trichomes

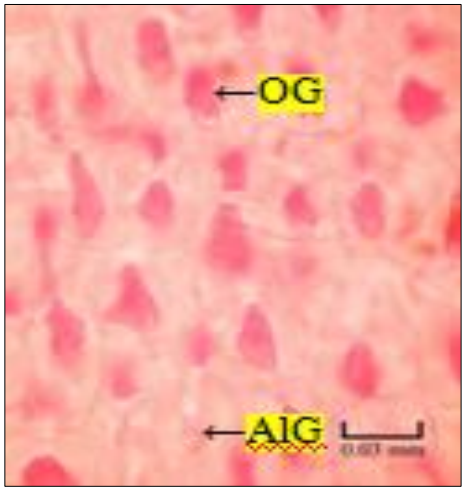

Fig 4: Endosperm cells

AlG- Aleurone grain, Ens – Endoperm, OG – Oil globule, T - Trichomes

Powder Microscopy
Powder microscopical analysis showed fragments of testa, groups of trichomes, endosperm tissues having plasmodesmata, tiny stone cells, oil globules and aleurone grains (Fig 5, 6, 7, 8).

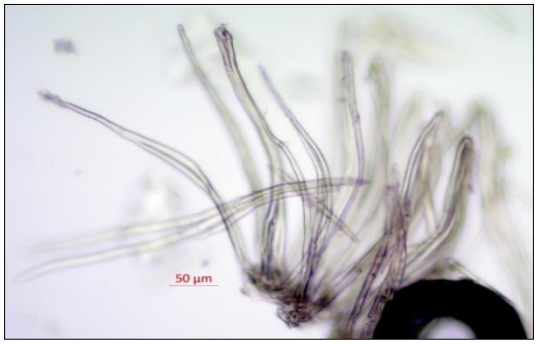

Fig 5: Trichomes

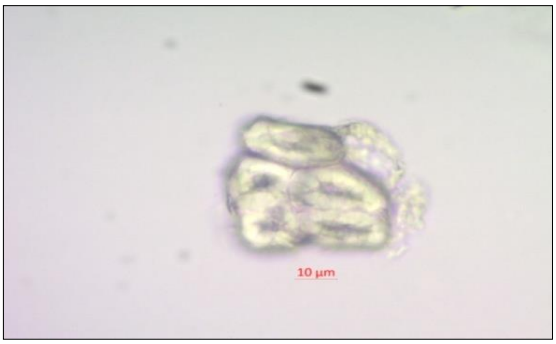

Fig 6: Stone cells

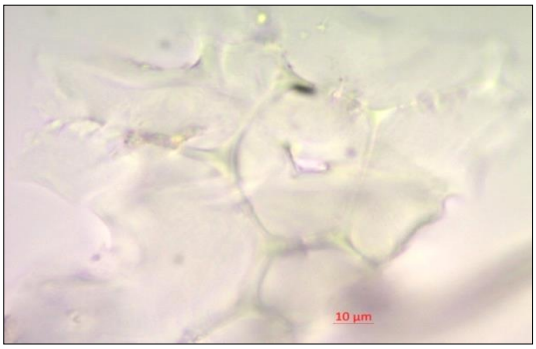

Fig 7: Plasmodesmata

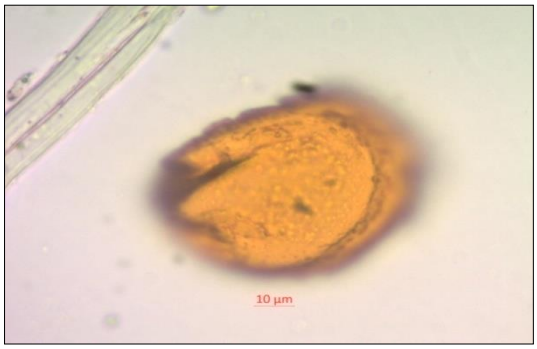

Fig 8: Oil cell

Quantitative Microscopy

The results of length and width of the stone cells and trichomes were presented in the table 1.

Table 1: Determination of length and width of stone cells and trichomes of *S. potatorum*

S. No	Parameter	Length (µ)			Width (µ)		
		Max	Avg	Min	Max	Avg	Min
1	Stone cells	130	78	65	78	52	26
2	Trichomes	78	65	39	39	26	26

Physicochemical analysis

The results of physicochemical analysis like ash values, extractive values, loss on drying and foaming index were presented in the tables 2 and 3.

Table 2: Determination of ash values and extractive values

S. No.	Name of the Drug	Ash values (% W/W)				Extractive values (% W/W)	
		Total ash	Acid insoluble ash	Water soluble ash	Sulphated ash	Water soluble	Alcohol soluble
	Strychnos potatorum	1.98	0.5	0.99	1.02	9.26	6

Table 3: Determination of loss on drying and foaming index

S. No.	Name of the Drug	Loss on Drying (%W/W)	Foaming Index
1.	*Strychnos potatorum*	7.11	Less than 100

Fluorescence Analysis

The Fluorescence analysis of *Strychnos potatorum* was presented in the following table no. 4. With ferric chloride and nitric acid, green flouresence was observed in UV at 254 and 365nm.

Table 4: Fluorescence analysis of *Strychnos potatorum*

S. No	Treatment	Day light	Short UV (254nm)	Long UV (365nm)
1	Powder	Pale green	Green	Brown
2	Powder + water	Light brown	Light green	Light black
3	Powder+1N Alc. NaOH	Yellowish brown	Dark brown	Greenish black
4	Powder +1N Alc. KOH	Yellowish brown	Greenish brown	Reddish brown
5	Powder + 1N H_2SO_4	Brown	Dark brown	Black
6	Powder + 1N HCl	Dull white	Yellowish green	Brown
7	Powder + 1N HNO_3	Yellowish brown	Greenish black	Fluorescence green
8	Powder + 1N NaOH	Yellowish brown	Yellowish green	Dark green
9	Powder + 1N KOH	Light brown	Greenish brown	Dark brown
10	Powder + Acetic acid	Pale brown	Green	Brown

11	Powder + Ammonia	Dull white	Greenish brown	Reddish brown
12	Powder + Ethanol	Pale brown	Green	Brown
13	Powder + $FeCl_3$	Yellowish green	Fluorescence green	Yellowish brown
14	Powder + Iodine	Bluish black	Bluish green	Dark blue

Determination of heavy metal contamination

Limit tests for arsenic present in the seed of *Strychnos potatorum* showed that the stain produced in the sample is less than that of the standard and limit tests for lead showed that the colour produced in the sample is less than that of the standard. The results of determination of heavy metals showed that the seed of *Strychnos potatorum* is less than that of given standard ppm and free from heavy metal contamination.

Qualitative and quantitative estimation of inorganic elements

The qualitative estimation of inorganic elements showed the presence of potassium and iron.

Quantitative estimation of Iron

Quantitative estimation of iron in the seeds of *Strychnos potatorum* was found to be 1.85% w/w.

Determination of microbial contamination

Determination of total viable count for bacteria and fungi in the seeds of *Strychnos potatorum* showed that there was no bacterial and fungal growth.

Preliminary Phytochemical Screening

Preliminary phytochemical screening showed the presence of active constituents like alkaloids, glycosides, sterols, phenolic compounds, tannins, saponins**,** flavonoids and terpenoids.

Discussion

The practice and usage of crude drugs are globally increasing for the past two decades. This is due to the awareness of herbal medicines because of their less toxicity, affordable cost and more toxicity of synthetic drugs. Pharmacognosy is a study of crude drugs including plant, animal and mineral origin. It deals with history, distribution, cultivation, collection, identification, macroscopical and microscopical characters, preservation, adulteration, biochemical and biological evaluation of crude drugs [30].

Herbal materials are mostly supplied to the market in dried form without trade name and proper identification. These drugs can be easily adulterated or substituted with inferior quality of the same parts or other parts of the same or different plants. To ensure safety, efficacy and reproducible quality of the herbal drug, proper identification and quality assurance of the crude drug is essential [31].

Vernacular names of this medicinal plants present in the literature is useful to identify the botanical name of this plant available in different areas. Parameters like macroscopical and microscopical characters gives us the knowledge about the identification of medicinal plant. Quantitative microscopy of length and width of stone cells and trichomes ensures the quality of this plant *S. potatorum*.

Physicochemical constants like ash values and extractive values are important for the detection of adulterants and helpful in providing standards for crude drug. Ash values are useful to evaluate the quality and purity of the drug. Ash value gives the knowledge about inorganic constituents and other impurities like salts and silica. Evaluation of extractive values for the crude drugs gives an idea about the phytoconstituents present in the crude drug. Crude drug was evaluated for fluorescence characters by using day light and ultraviolet light at 254 and 366 nm.

During the growth of the plants, there may be a chance to contaminate with heavy metals from the soil, fertilizers or from environment. If the presence of heavy metal content is more in the plants, it may leads to toxicity. Quantitative estimation of iron content showed that this plant contains sufficient iron content and hence, this plant will also act as a health supplement. During storage of crude drugs for a long time there will be a chance for absorbing moisture and may be contaminated by bacterial and fungal growth. These studies are directly related to stability and shelf life of crude drugs. Less moisture content means there will be a less chance of microbial growth and higher will be the stability of crude drug.

Identification of phytoconstituents gives a representation about the pharmacological active metabolites present in the plant. Phytochemical studies on *S. potatorum* is useful to investigate further pharmacological studies of this plant.

The parameters evaluated are useful to identify, authenticate and ensure the quality of traditional medicinal plant *Strychnos potatorum.* This will help us to prepare the monograph and pharmacopoeial standards emphasized by WHO.

Conclusion

Pharmacognostical studies and preliminary phytochemical screening of seeds of *Strychnos potatorum* may help in standardization, identification, quality and to detect the adulterants. This study may also be helpful to the researchers to carry out further research in the seeds of *Strychnos potatorum* and the herbal formulations containing seeds of *Strychnos potatorum*.

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Conflict of interest

We declare that we have no conflict of interest.

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