Preparation and Standardisation of Mother Tincture from *Strychnos potatorum*: A New Drug Source in Homoeopathy

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Abstract

Strychnos potatorum L.f. is a deciduous tree of 12 metre height, belongs to Loganiaceae family under genus *Strychnos* and is commonly distributed throughout India. Its flowering time is from February to May and fruiting time from October to March. This plant contains diaboline as active compound and besides it, terpenoid, cardiac glycoside and phenolic compounds as other phytochemical constituents. Recent study has revealed that the plant's seed has antidiabetic, nephroprotective, hepatoprotective, antipyretic, antineoplastic, antimicrobial and contraceptive properties. Hence different systems of medicine like Ayurveda, Unani and Siddha have included this plant in their pharmacopoeial preparation and use it according to their own principles. Ayurvedic system uses the seed against skin disease, gonorrhoea, fever and inflammation of the eyes. Unani system uses it for treatment of urinary complaints whereas Siddha system uses it for treatment of chronic obstructive pulmonary disease. Genus *Strychnos* is a well-known genus to homoeopathy system. Drugs like *Ignatia* and *Nux vomica* belong to this genus, but no data till today regarding the above-stated plant are available in the homoeopathic pharmacopoeia of India. So, the objective of this physiochemical study is to search the scope of inclusion of this plant as new medicinal source in homoeopathic system of medicine for the purpose of drug proving. Keeping this objective in our mind, chemical analysis of the ethanolic extract of the seed was done by thin layer chromatography and ultraviolet-visible spectrophotometry, which was followed by preparation and standardisation of mother tincture.

Keywords
- homoeopathy
- mother tincture
- new drug
- *Strychnos potatorum*
- thin layer chromatography

Introduction

Strychnos potatorum (SP) is a medicinal plant of Loganiaceae family, under genus *Strychnos*. This plant is commonly known as *kataka*, *nirmali* or *clearing nut*. Seeds of SP are solitary and orbicular. Morphological study of seed describes that its testa is composed of two to three layers of thick, walled, elongated lignified sclerenchymatous cells, covered with numerous cylindrical unicellular lignified trichomes having basal portion ramified. Outer endosperm is composed of three to eight layers of thick, walled, elongated palisade-like cells around in rows, and inner endosperm is

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composed of thin, walled, oval to polygonal parenchyma cells having numerous small aleurone grains and oil globules.[1,2] Chemical analysis of seed has revealed the presence of different alkaloids, phenolic compounds, terpenoids etc., which are genus specific. The most abundant alkaloid of this plant is found to be diaboline,[3] which is a glycine receptor antagonist and, like strychnine, it is a convulsant. Recent biochemical study also shows that diaboline has the capacity to produce hypotension.[4] The plant is rich in medicinal properties and thus used by different systems of medicine according to their own principles. Literature review shows that Ayurveda, Unani and Siddha systems use the seed of SP to prepare medicine. Ayurveda system uses the seed against skin disease, gonorrhoea, fever, inflammation of the eyes etc. Unani system uses it for treatment of urinary complaints whereas Siddha system uses it for treatment of chronic obstructive pulmonary disease.[4] Contemporary research on SP revealed that the seed has antimicrobial activity against *Mycobacterium tuberculosis*, *Pseudomonas* sp., *Vibrio cholerae* etc.[5–7] It also shows antidiabetic,[4] antipyretic,[4,8] antiarthritic,[4,8] hepatoprotective[4,8] and nephroprotective activities.[5–10]

Homoeopathy is a branch of medical science discovered by Master Samuel Hahnemann, which is based on the following law: 'A weaker dynamic affection is permanently extinguished in the living organism by a stronger one, if the latter (whilst differing in kind) is very similar to the former in its manifestations' (Aphorism 26 of *Organon of Medicine*).[11] Considering this law, we treat the sick by that substance (medicine) that is capable of producing similar symptoms to the diseased individual. The identification of the disease-curing power of a drug (disease-producing power of drug) can be known when it is introduced in healthy human being of both sexes and of different ages and constitutions (prover) for human pathogenetic trial or drug proving (Aphorisms 105–145 of *Organon of Medicine*).[11]

The plant kingdom acts as the richest source of drug substances and in the plant kingdom, the family Loganiaceae includes large number of medicinal plants, subdivided into several genus. Moreover, genus *Strychnos* has wide range of Indian species (~12 species are available) and this genus is used in different systems of medicine like Ayurveda, Unani, Siddha and Homoeopathy. Among the Indian species of above-stated genus, only *Nux vomica* is used as drug source in homoeopathy system, whereas *S. potatorum* has good therapeutic activity in other systems of medicine, but the same is not used in homoeopathic system yet.[4]

Every species of plants are different from every other species by virtue of their life process such as growth, structure and smell. So, the action of each plant substances on healthy human being will be different. Each plant substance will make the alteration of the state of health by its own peculiar way (Aphorism 119 of *Organon of Medicine*).[11] Hence the effect of *S. potatorum* upon the state of health will not be same or similar to the effect produced by other *Strychnos* species like *Nux vomica*.

Thus, the objective of this physiochemical study is to search the scope of inclusion of *S. potatorum* as new medicinal source in homoeopathic system of medicine for the purpose of human pathogenetic trial (drug proving). Keeping this objective, we made extract of seed, which was followed by preparation and standardisation of mother tincture. This mother tincture will act as source for preparation of potencies, and potentised medicine will be used for human pathogenetic trial (drug proving) in future.

Material

All the chemicals and chemical materials that were used in this experimental process were in purest and finest form and obtained from Merck Millipore India Pvt. Ltd.

Collection of the Seed

Seeds were collected from central part of India by a well-known herb supplier of Hahnemann Publishing Company Private Limited (HAPCO), India. Furthermore, the collected seeds were authenticated and verified by Botanical Survey of India, Shibpur (Certificate No. BSI/PLANT CHEM/0001–2021/ Date 18.01.2021).

Phenotype Study of the Seed

The morphological features of seeds were evaluated and its macroscopic study was done by photographing the sample. Ten seeds of SP were taken randomly. Their diameter (in mm) and thickness (in mm) were measured with the Metric Vernier Slide Caliper and its arithmetic mean was calculated. After the macroscopic study, the microscopic study of the seeds was done. For this purpose, seeds were warmed in water for half an hour to soften the tissue. Softened seeds were taken from hot water and dried. The epidermal trichomes were then removed by scrapping from the surface of the seed with the scalpel. Then sectioned material was placed over glass slide, two to three drops of distilled water were poured over the cross-section and examined under compound microscope at ×10 magnification.

Ethanolic Extraction of the Seed and Filtration

Hard seeds were first crushed into powder form in a pulveriser and then taken in five equal volume conical flasks. Then 50 mL aqueous ethanolic solutions of five different strengths, that is, 30, 50, 60, 70 and 80% of ethyl alcohol, were prepared according to ►**Table 1**. Solvent extraction was done according to the method of Maceration given in *Homoeopathic Pharmacopoeia of India*, Vol. I.[12] Then 10 g of powdered drug substances was taken and mixed with each 50 mL of alcohol–water solution. Then these suspensions were kept in cool, dry and dark place for 21 days with daily stirring. After 21 days, the solutions of different concentrations of ethanolic samples were pressed and filtered separately and volumes were adjusted to 50 mL.[12]

Measurement of Total Dissolved Solid (TDS), Specific Gravity and pH

All the parameters were measured according to the protocol mentioned in *Homoeopathic Pharmacopoeia of India*, Vol I.[12] In short, TDS and specific gravity were measured for all five

Table 1 Preparation of different strengths of aqueous ethanolic solution

Strength of C_2H_5OH (in %)	Volume of C_2H_5OH required to make 50 mL solution (in mL)	Volume of distilled water required to make 50 mL solution (in mL)
30	15.46	34.54
50	25.67	24.33
60	30.92	19.08
70	36.08	13.92
80	41.23	8.77

samples and computed accordingly. For measuring pH, we used Systronics Digital pH Meter Model 335 for the sample.

Chemical Analysis of 70% Ethanolic Extract

After physical investigation of 70% ethanolic extract, following chemical analyses were performed:

(1) Qualitative analysis for the presence of different active phytochemical constituents

Identification tests of different phytochemicals, that is, terpenoids, alkaloids, tannin, cardiac glycosides, flavonoids, phlobatannins and phenolic compounds, were performed following methodology as described in different research journals.[13–16]

(2) Quantitative measurement of total alkaloid

Total alkaloid was quantitatively estimated by spectrophotometric method as described by Marozzi et al.[17] For our study, we made a standard scale of quinine of different concentration, and the alkaloid concentration of our ethanolic extract was plotted against this standard and thus concentration of total alkaloid was computed.

(3) Chemical analysis of 70% ethanolic extract by thin layer chromatography

The thin layer chromatography (TLC) plate of 20 × 5 cm size was prepared with silica gel G6 and activated at 105 to 110°C for 30 minutes. The dried plate was divided into two equal parts. The TLC plates were spotted with *S. potatorum* and ethanolic solutions of TLC grade pure strychnine and diaboline. Then the plate was ran with the mobile phase, ethyl alcohol:chloroform (9:1),[18,19] in a saturated chamber and run to a distance of 15 cm. Visualisation was done through iodine staining and ultraviolet (UV)-visible spectrophotometry of 366 nm and the respective R_f values of each spot were calculated.

(4) Chemical analysis of 70% ethanolic extract by UV-visible spectrophotometry

UV-visible spectroscopy of the sample was done against blank (ethanolic) solution using Shimadzu-1800. UV-visible spectrophotometry and absorbance maxima with corresponding wavelength were computed.

Result

It was observed from the macroscopic view of the SP seed that seeds were circular and bluntly lenticular. Seeds were cream in colour and shiny with apprised silky hairs (as evident in ►**Fig. 1A**). It was also observed that a prominent ridge was present around the border with presence of hilum and micropyle, which were marked in picture (►**Fig. 1B**). Average diameter of the control seed was 12.08 mm and thickness was 7.53 mm (as mentioned in ►**Table 2**). After boiling, when seed was touched, a slimy sensation was felt in finger.

From the microscopic observation of the seed transverse section under ×10 magnification, it was found that tiny hairy projections came out from the epidermis (trichomes; marked with black arrowhead, ►**Fig. 2A**). Trichomes were elongated, lignified, unicellular and having basal portion ramified (as shown by black arrowhead, ►**Fig. 2B**). The outer endosperm consisted of layers of thick walled, elongated palisade-like cells arranged in rows. The inner endosperm consisted of thin walled, oval to polygonal, parenchyma cells, having numerous small aleurone grains and oil globules (shown by red arrowhead in ►**Fig. 3A** and **B** and by black arrowhead in ►**Fig. 4A** and **B**).

It was found from the filtration process that 30% ethanolic solution produced nonturbid filtrate, whereas turbid filtrate

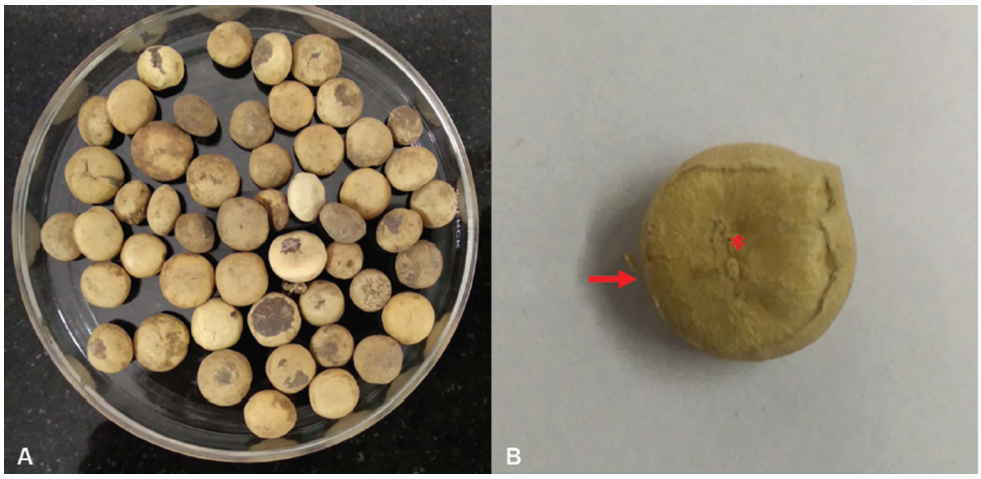

Fig. 1 *Strychnos potatorum* seed. (**A**) Seed in Petri dish. (**B**) Seed marked with mycropyle with red arrow and hilum with red star.

Table 2 Measurement of *Strychnos potatorum* seed

Seed	Diameter (in mm)	Thickness (in mm)
Seed 1	12.54	8.20
Seed 2	13.92	7.94
Seed 3	12.40	8.14
Seed4	11.08	7.80
Seed 5	11.92	7.14
Seed 6	10.82	7.22
Seed 7	12.58	7.04
Seed 8	12.10	7.96
Seed 9	13.48	7.00
Seed 10	10.04	6.90
Arithmetic mean	12.08	7.53

was produced by 50 and 60% solution. But an oily and transparent filtrate was obtained from 70 to 80% aqueous ethanolic solution (as evident from ►**Table 3** and ►**Fig. 5A** and **B**).

Filtrate with different strengths of ethanol produced different TDS values (calculation expressed in ►**Table 4**).

From the standardisation process of the SP mother tincture, it was found that specific gravity of sample was 0.88309, pH of the sample was 6.61 and colour of the mother tincture was light yellow (►**Table 5**).

From the qualitative analysis of ethanolic extract of SP, different active biochemical components were found in the extract and their presence is expressed in ►**Table 6**.

Quantitative analysis of total alkaloid in SP extract with the aid of standard alkaloid solution by spectrophotometry resulted in 1.34 mg of total alkaloid in 1 g of SP seed powder.

TLC study showed that SP produced seven spots when developed with iodine, and the TLC profile of the same was compared with TLC profile of pure strychnine and diaboline (►**Fig. 6**, lanes 1 and 2) and also their R_f values were computed, which are expressed in ►**Table 7**. Same TLC plate was also observed under UV-visible spectrophotometry of 366 nm (►**Fig. 6**, lanes 3 and 4). Both spots of strychnine and diaboline gave fluorescence (►**Fig. 6**, lane 3) but only one fluorescence spot appeared for SP (►**Fig. 6**, lane 4). After computing the *Rf* values, it was evident that SP contains only diaboline as active alkaloids and strychnine was absent in the extract (shown in blue marked box in ►**Table 7**).

From the UV-visible spectrophotometry study, it was found that λ_{max} of the sample was found to be 287.4 nm and 325.00 nm (►**Fig. 7**).

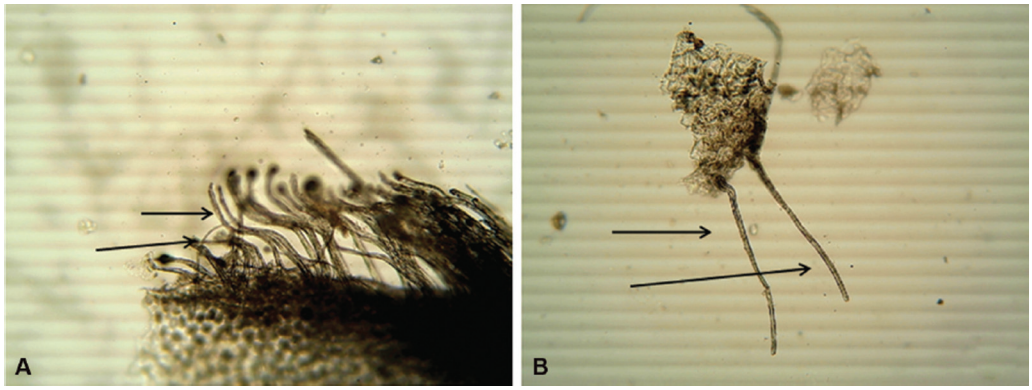

Fig. 2 Transverse section of *Strychnos potatorum* seed observed under light microscope at ×10 magnification. (**A**) Transverse section of seed with attached trichomes marked with black arrow. (**B**) Individual trichomes marked with black arrow.

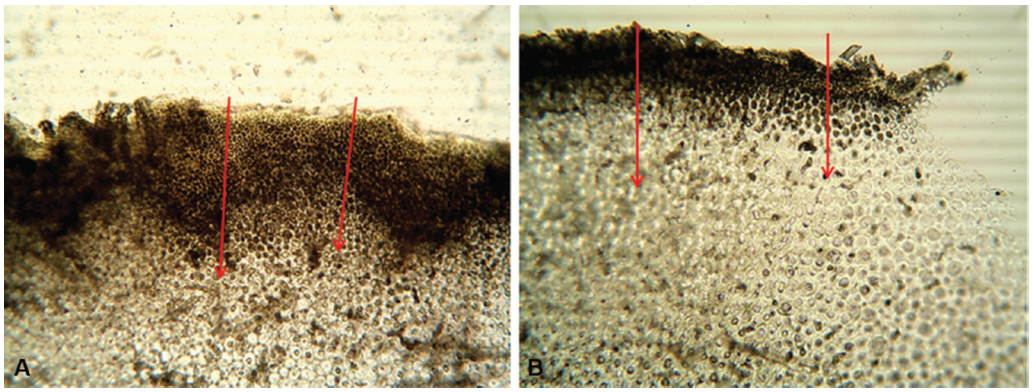

Fig. 3 Transverse section of *Strychnos potatorum* seed observed under light microscope at ×10 magnification. (**A**) Endosperm of seed stained with iodine solution (red arrow) observed under ×10 magnification of compound microscope. (**B**) Endosperm of unstained seed's transverse section (red arrow) observed under ×10 magnification of compound microscope.

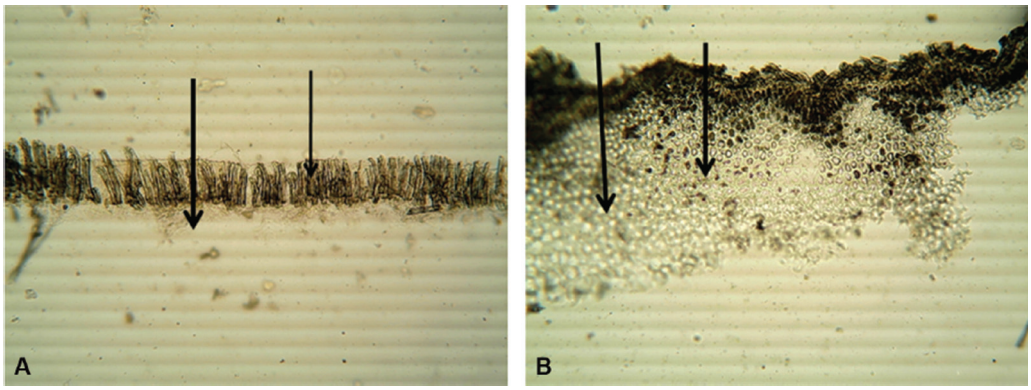

Fig. 4 Transverse section of *Strychnos potatorum* seed observed under light microscope at ×10 magnification. (**A**) Palisade cells (black arrow) of outer endosperm of seed observed under ×10 magnification of compound microscope. (**B**) Outer endosperm and inner endosperm (both marked with black arrow) of seed observed under ×10 magnification of compound microscope.

Table 3 Result of filtration

Concentration of ethanol (%)	Filtration rate	Character of filtrate
30	Slow	Nonturbid and transparent
50	Medium	Turbid
60	Medium	Turbid
70	Fast	Oily and transparent
80	Fast	Oily and transparent

Table 4 Result of measurement of total dissolved solid value

Ethanolic strength (%)	Initial weight (W_1) (gram)	Final weight (W_2) (gram)	Value of TDS (gram)
30	38.1033	38.1370	0.674
50	39.7023	39.6560	0.926
60	44.6958	44.7434	0.952
70	41.5797	41.6180	0.766
80	31.6451	31.6770	0.638

Abbreviation: TDS, total dissolved solid.

Table 5 Result of standardisation of *Strychnos potatorum* L.f. mother tincture made with 70% aqueous ethanolic solution

Parameter	Value
Alcoholic strength	70% (122.5° O.P.)
% of total dissolved solid	0.766
Specific gravity	0.88309
pH	6.61
Colour	Light yellow

Discussion

In search for the scope of inclusion of *S. potatorum* as new medicinal source in homoeopathic system of medicine for the purpose of human pathogenetic trial (drug proving), we found that the genus and the species of the seed of SP that were collected from an outside vendor were authentic as tested by the Botanical Survey of India, Government of India. Furthermore, macroscopic and microscopic analyses of the seed at analytical laboratory of HAPCO also strengthens the data, which corroborates the findings obtained from the other literatures[1,2] also.

Softening of the seed by boiling produces a slime that directed us towards the maceration process as the choice of extraction.[12] During the standardisation of solvent for extraction, we found that 70 and 80% aqueous ethanolic solution produces clear transparent extract (marker of proper solution) in comparison with other turbid extracts (i.e. 30, 50

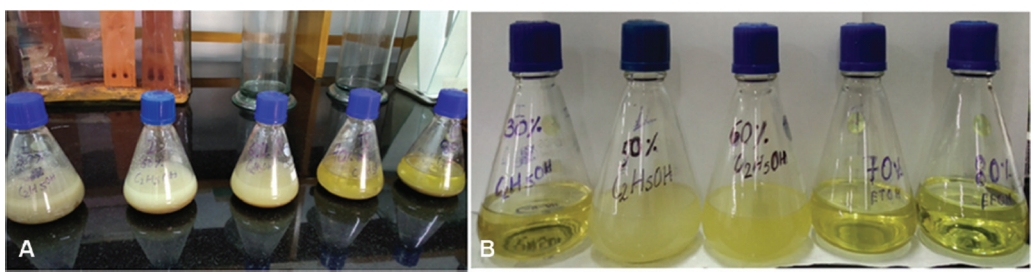

Fig. 5 Extraction and filtration of seed under different concentrations of aqueous ethanolic solution. (**A**) Extraction under different concentrations of aqueous ethanolic solution. (**B**) Filtration under different concentrations of ethanol.

Table 6 Presence of different active biochemical components in ethanolic extract of *Strychnos potatorum* seed

Phytochemical components	Ethanolic extract
Terpenoid	+
Alkaloid	+
Tannin	–
Cardiac glycoside	++
Phlobatannins	–
Phenolic compound	++
Flavonoid	++

and 60%). Furthermore, TDS measurement showed that 70% aqueous ethanolic solution has highest amount of dissolved solid than 80% aqueous ethanolic solution. So, for the preparation of mother tincture, 70% aqueous ethanolic solution was taken as our choice of solvent of extraction. The measurement of pH, specific gravity and colour matching of the SP mother tincture was done because these parameters would act as definite identifying mark of the mother tincture.

As the result shows, the total alkaloid content of SP was found to be almost 1.4%. Literature review shows that *Nux vomica*, a taxonomical neighbour of SP, usually contains ~1.8 to 5% of total alkaloid among which major members are strychnine and brucine and little amounts of loganine.[1,2] In-depth chemical analysis reveals that seeds of SP contain no strychnine,[19]but it has been reported to contain trace amounts of diaboline and its acetyl derivatives. This result corroborates our TLC data.

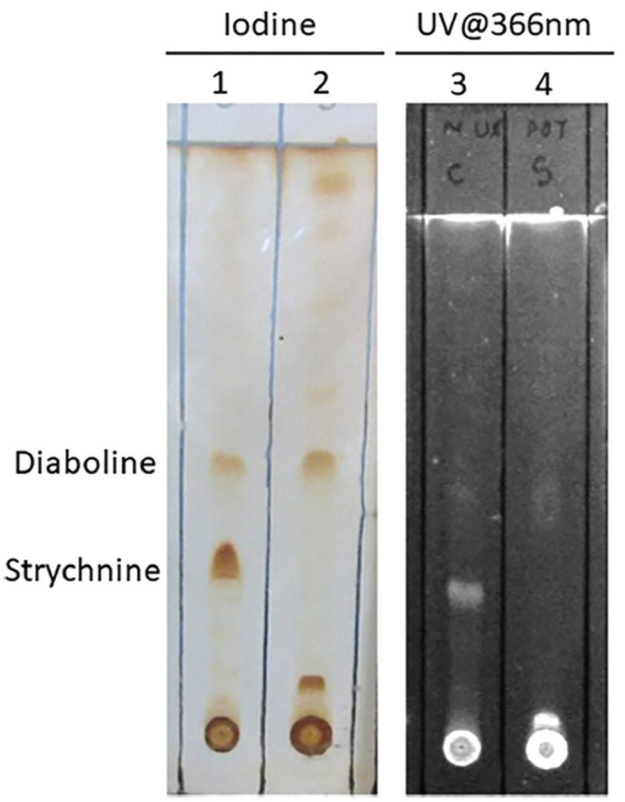

Fig. 6 Thin layer chromatography of the *Strychnos potatorum* seed (lanes 2 and 4) with standard strychnine and diaboline (lanes 1 and 3) developed in iodine chamber (left panel, lanes 1 and 2) and visualised under ultraviolet-visible spectrophotometry of 366 nm (right panel, lanes 3 and 4).

Conclusion

Homoeopathic pharmacopoeia of India has not mentioned any information regarding this plant *S. potatorum.* No data on human pathogenetic trial of *S. potatorum* are available in the homoeopathic system of medicine to date. Hence, *S. potatorum* L.f may be introduced as new drug source in homoeopathy. The mother tincture from the SP seed was prepared and standardised by the above-stated procedure in the HAPCO laboratory. The mother tincture will be used as source for preparation of potentised medicine and the

Table 7 Result of R_f value of thin layer chromatography

Pure reference compounds	R_f of reference compound after development with iodine	R_f of SP after development with iodine	R_f of reference compound under UV-visible spectrophotometry at 366 nm	R_f of SP under UV-visible spectrophotometry at 366 nm
Strychnine	0.28	0.94	0.28	0.45
Diaboline	0.45	0.87	0.45	0.09
		0.81		
		0.75		
		0.59		
		0.45		
		0.09		

Abbreviations: SP, *Strychnos potatorum*; UV, ultraviolet.

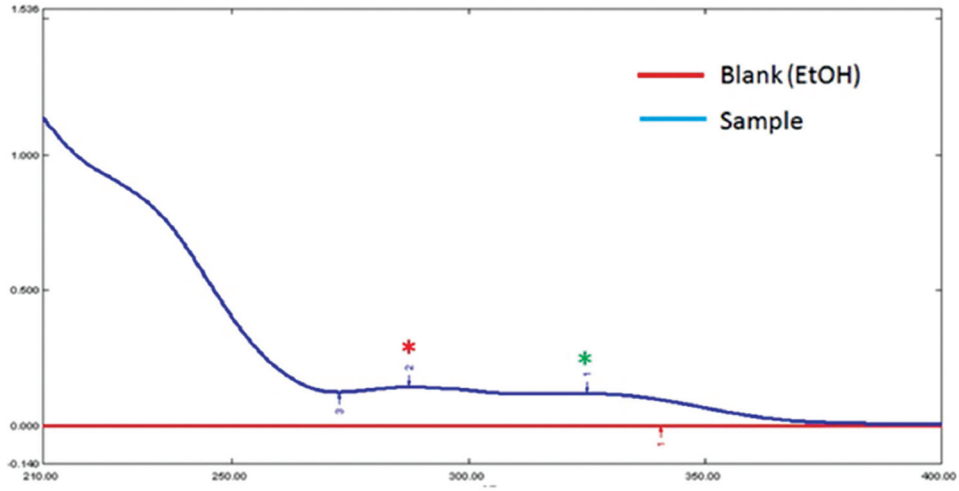

Fig. 7 Spectrophotometry graph showing absorbance maxima (λ_{max}) of the sample to be 287.4 nm (marked with red star) and 325.00 nm (marked with green star).

potentised medicine will be used for human pathogenetic trial (drug proving) in future.

Conflict of Interest

There are no conflicts of interest present between the authors.

Acknowledgement

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