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Phytochemical Studies of *Strychnos potatorum* L.f.- A Medicinal Plant

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Abstract: The present paper deals with the phytochemical screening of therapeutic importance from *Strychnos potatorum* L.f., an important medicinal plant. This study involves the preliminary screening, quantitative determination and the qualitative thin layer chromatographic separation of secondary metabolites from the root, stem bark and seeds (collected and market) of *S.potatorum*. Further, HPLC alkaloid profile of the seed has been studied. The generated data has provided the basis for its wide use as the therapeutant both in the traditional and folk medicines.

Keywords: Phytochemical, *Strychnos potatorum*, Medicinal plant.

Introduction

Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odour (terpenoids), pigmentation (tannins and quinines), and flavour (capsacin). However, several of these molecules possess medicinal properties¹. *Strychnos potatorum* L.f. is an important medicinal plant, used in Ayurveda, Unani, Siddha, and in folk medicine for treating several ailments including microbial infections, diarrhoea and diabetes.

Alkaloids mainly diaboline, and four triterpenes *viz.*, isomotioli, sitosterol, stigmasterol and campesterol were reported from seeds and leaves of *S.potatorum*, respectively²⁻³. Further, twenty four alkaloids including diaboline were reported from the root of *S.potatorum*⁴. Where as, Adinolfi *et al.* (1994) have studied galactomannan and galactan (1:1.7), the mixture of the main polysaccharide component of seed⁵.

In the present study, we have concentrated on the preliminary screening, quantitative determination, and the qualitative separation of secondary metabolites from the root, stem bark, and seeds of *Strychnos potatorum*.

Experimental

Collection and identification of plant

The plant material *viz.*, root, stem bark, and seeds of *Strychnos potatorum* L.f., belongs to the family Loganiaceae, were collected from the Karpakpalli forest, Bidar district, Karnataka in December 2002. The plant was identified with the help of *The Flora of Presidency of Madras*⁶ and *The Flora of Gulbarga district*⁷ and a voucher specimen is deposited in the Herbarium, Department of Botany, Gulbarga University, Gulbarga (HGUG-214). Further, another set of seeds were procured from the M/s Jajee Ayurvedic stores, Gulbarga.

Preliminary screening of Secondary Metabolites

The shade dried plant material is powdered using mixer grinder, and subjected to Soxhlet extraction with petroleum ether, chloroform, 95% ethanol, and distilled water for 18h in the order of increasing polarity of solvents. The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids (Iodine, Wagner and Dragendorff's tests), flavonoids (Pew's, Shinoda and NaOH tests), glycosides (Keller-Kiliani, conc. H₂SO₄, and Molish tests), Lignins (Labat and Lignin tests), phenols (ellagic acid and phenol tests), saponins (foam and haemolysis tests), sterols (Lieberman-Burchard, and Salkowski tests), tannins (gelatin test) were carried out⁸⁻⁹.

Quantitative estimation of secondary metabolites

The presence of secondary metabolites from root, stem bark, and seeds of *S.potatorum* were quantitatively determined by adopting standard protocols. Alkaloids by Ikan's method¹⁰, flavonoids by Swain and Hillis method¹¹, phenols by Bray and Thorpe method¹², tannins by Folin-Denis method¹³, and saponins by Sanchez method¹⁴.

Separation of secondary metabolites by thin layer chromatography

For the thin layer chromatography studies of secondary metabolites, precoated Alugram[®] Sil G/UV_{254nm} (Machery – Nagel GmbH, Germany) aluminum plates (20 X 20cm) were used.

TLC study of alkaloids

The powdered root, stem bark, and seeds of *S.potatorum* were wetted with a half diluted NH₄OH and lixiviated with EtOAc for 24h at RT. The organic phase is separated from the acidified filtrate and basified with NH₄OH (pH 11-12). It is extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1). The colour and R_f values of the separated alkaloids were recorded both under ultraviolet (UV_{254nm}) and visible light after spraying with Dragendorff's reagent¹⁵.

TLC study of flavonoids

One gram powdered root, stem bark and seeds of *S.potatorum* were extracted with 10ml methanol on water bath (60°C/ 5min). The filtrate was condensed by evaporation, added a

mixture of water and EtOAc (10:1 mL), and mixed thoroughly. The EtOAc phase thus retained is used for chromatography. The flavonoid spots were separated using chloroform and methanol (19:1) solvent mixture. The colour and hRf values of these spots were recorded under ultraviolet (UV_{254nm}) light¹⁵.

TLC study of glycosides

The powdered root, stem bark, and seeds of *S.potatorum* were extracted with 70% EtOH on rotary shaker (180 thaws/min) for 10h. 70% lead acetate is added to the filtrate and centrifuged at 5000rpm/10 min. The supernatant was further centrifuged by adding 6.3% Na₂CO₃ at 10000 rpm/10min. The retained supernatant is dried, redissolved in chloroform and used for chromatography. The glycosides were separated using EtOAc-MeOH-H₂O (80:10:10) solvent mixture. The colour and hRf values of these spots were recorded by observing under ultraviolet (UV_{254nm}) light¹⁶.

TLC study of phenols

The powdered root, stem bark and seeds of *S.potatorum* were lixiviated in methanol on rotary shaker (180 thaws/ min) for 24h. The condensed filtrate was used for chromatography. The phenols were separated using chloroform and methanol (27:0.3) solvent mixture. The colour and hRf values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent heating at 80°C/10min¹⁶.

TLC study of saponins

Two grams of powdered root stem bark, and seeds of *S.potatorum* were extracted with 10 ml 70% EtOH by refluxing for 10 min. The filtrate is condensed, enriched with saturated *n*-BuOH, and thoroughly mixed. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and hRf values of these spots were recorded by exposing chromatogram to the iodine vapours¹⁵.

TLC study of sterols

Two grams of powdered root, stem bark, and seeds of *S.potatorum* were extracted with 10ml methanol in water bath (80 °C/15 min).The condensed filtrate is used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and hRf values of these spots were recorded under visible light after spraying the plates with anaisaldehyde-sulphuric acid reagent and heating (100°C/6 min)¹⁵.

HPLC study of alkaloid profile of S.potatorum seed

Extraction and preparation of sample

The total alkaloids from the powdered collected seed of *S.potatorum* were extracted as described by Nuzillard *et al.* (1996)¹⁷. This extract was redissolved in 5mL absolute ethanol (analar grade) and filtered through Whatman filter paper No.1.The filtrate is used for high performance liquid chromatography study.

HPLC instrumentation

An isocratic HPLC (Shimadzu HPLC class VP series) with two LC – 10 AT VP pumps (Shimadzu), variable wavelength programmable photodiode array detector SPD MIOA VP (Shimadzu), CTO-IOAS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and a reverse phase Luna 5 µC18 (2) Phenomenex column (250 mm x 4.6 mm)

was used. The HPLC system was equipped with software class VP series version 6.1 (Shimadzu). The mobile phase components acetonitrile: water (1:3) were filtered through 0.2 µ membrane filter before use and pumped from the solvent reservoir to the column at a flow rate 1 mL/min which yielded a column backpressure of 16-165 Kgf/cm². The column temperature was maintained at 27°C. 20 µL of sample was injected using Rheodyne syringe (Model 7202, Hamilton).

Results and Discussion

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc.* The successive extracts of root, stem bark, and seeds of *Strychnos potatorum* have revealed the presence of alkaloids, flavonoids, glycosides, lignins, phenols, saponins, sterols, and tannins (Table 1). Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The lignan-glycosides vanprukoside, strychnoside, and glucopyranoside isolated from *Strychnos vanprukii* have shown significant antioxidant property¹⁸.

Table 1. Preliminary screening of secondary metabolites from *S. potatorum*

Secondary metabolite	Name of the test	Root				Stem bark				Collected seed				Market seed			
		I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Alkaloids	Iodine test	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
	Dragendorff's test	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+
	Wagner's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	Pew's test	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-
	Shinoda test	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-
	NaOH test	-	+	+	+	-	+	+	+	-	-	+	+	-	-	+	+
	Kellar-	+	+	-	+	+	+	-	+	+	+	-	+	-	+	-	+
Glycosides	Kiliani test																
	Conc. H ₂ SO ₄	+	+	-	+	+	+	-	+	+	+	-	+	-	+	-	+
	Molisch test	+	+	-	+	+	-	-	+	-	-	-	+	-	-	-	+
Lignin	Labat test	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	Lignin test	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
Phenols	Ellagic acid test	-	-	+	+	-	-	+	+	-	+	+	-	-	+	+	+
	Phenol test	-	-	+	+	-	-	+	+	-	-	+	-	-	-	+	-
	Foam test	-	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Saponins	Haemolysis test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	Libermann-Burchard test	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-
Sterols	Salkowski test	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-
	Gelatin test	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

'+' Present, '-' Absent, I : Petroleum ether, II : Chloroform, III: Ethanol, IV: Aqueous extracts.

The data of quantitative determination of secondary metabolites is tabulated in the Table 2. Among the five groups of phytochemicals determined from the root, stem bark, and seeds of *S.potatorum*, tannins were found to be the most abundant one followed by saponins and alkaloids. While, phenols and flavonoids were low in concentration.

Table 2. Quantitative estimation of phytochemicals of *S.potatorum*

S. No.	Metabolites	Percent of secondary metabolites / dry weight			
		Root %	Stem bark %	Collected seed %	Market seed, %
1	Alkaloids	1.7	2.2	1.4	1.3
2	Flavonoids	0.140	0.374	0.021	0.021
3	Phenols	0.209	0.167	0.039	0.059
4	Tannins	12.22	20.00	18.00	18.00
5	Saponins	1.535	0.465	5.135	4.741

The data of qualitative separation of alkaloids from root, stem bark, and seeds of *S.potatorum* by thin layer chromatography is tabulated in the Table 3.

Table 3. Qualitative separation of Alkaloids *S.potatorum* by TLC(UV254nm)

S.No	Colour of the spot	hRf values	Plant parts tested				Authentic sample
			Root	Stem Bark	Collected seed	Market seed	
1	Light green	5.26	+	+	+	+	
2	Blue	8.42	+	+	+	+	
3	Green	12.63	+	+	+	-	
4	Bluish green	14.74	+	+	+	-	
5	Light pink	18.95	+	+	+	-	
6	Pink	21.05	-	-	+	-	
7	Intense pink	24.21	+	+	+	+	
8	Blue	28.42	-	-	+	-	
9	Light blue	31.58	+	+	+	+	
10	Pink	34.74	+	+	+	-	Diaboline (31.58)
11	Green	40.00	+	+	-	+	
12	Blue	46.32	+	+	+	-	
13	Light blue	52.63	+	+	-	-	
14	Pinkish green	57.89	+	+	-	-	
15	Blue	63.16	-	-	+	-	
16	Light green	68.42	+	+	-	+	
17	Pale pink	78.95	+	+	+	-	
18	Light green	88.42	+	+	-	-	
19	Blue	92.63	+	+	-	-	
20	Light green	94.74	+	+	+	-	

Twenty quenching and fluorescing alkaloids were reported from the various parts of the plant. However, more number of alkaloids were found in the root and stem bark (17 spots). Further, the collected seed has possessed more alkaloids (13 spots) compared to the market seed (6 spots). This may be perhaps due to the inter conversion of these compounds into other derivatives owing to the prolonged period of storage and method of processing in case of the market seed. Similar observation is made in *Strychnos melladora* where alkaloids

are transformed into glucoindole alkaloids dolichontaside and palicoside¹⁹. The most abundant alkaloid diaboline is found in all samples of *S.potatorum* with hRf value 34.74 (light blue) and identified by matching with authentic sample. Further, four flavonoid spots were found to be common for all samples with hRf values 5.26, 8.42, 24.21 and 31.08.

Alkaloids are the lead molecules of therapeutic importance from *Strychnos* species. These are heterocyclic indole compounds which have proved to be have pharmacological properties such as hypotensive activity²⁰, anticonvulsant activity²¹, antiprotozoal, antimicrobial and antimalarial activities²²⁻²³.

The data of thin layer chromatography of flavonoids of *S.potatorum* is tabulated in the Table 4. Three flavonoid spots were reported in the root, collected and market seed samples however with different colour and hRf values. While, in the stem bark four alkaloid spots were observed. Flavonoids are the phenolic substances and are the largest group of phenols. These generally occurs as a C6-C3 unit linked to an aromatic ring.

Table 4. Qualitative separation of flavonoids of *S.potatorum*

S.No	Colour of the spot	hRf values	Plant parts tested			
			Root	Stem Bark	Collected seed	Market seed
1	Light blue	23.33	-	+	-	-
2	Dark blue	30.00	-	+	-	-
3	Light blue	36.76	+	-	-	-
4	Tapioca	33.67	-	-	+	+
5	Dark blue	42.65	+	-	+	+
6	Touch of lemon	51.47	+	-	+	+
7	Yellow	66.67	-	+	-	-
8	Blue	83.33	-	+	-	-

The data of glycosides of *S.potatorum* by thin layer chromatography is tabulated in the Table 5. Three glycosides with similar colour and hRf values 8.06, 16.13 and 35.48 were observed both in the root and stem bark. Where as, four glycosides were observed in both seed samples with hRf values 28.69, 46.09, 73.04 and 91.30.

Table 5. Qualitative separation of glycosides of *S.potatorum*

S.No	Colour of the spot	hRf values	Plant parts tested			
			Root	Stem Bark	Collected seed	Market seed
1	Intense blue	8.06	+	+	-	-
2	Pink	16.13	+	+	-	-
3	Yellow	28.69	-	-	+	+
4	Light green	35.48	+	+	-	-
5	Pink	46.09	-	-	+	+
6	Pink	73.04	-	-	+	+
7	Yellow	91.30	-	-	+	+

The data of phenols of *S.potatorum* by thin layer chromatography is tabulated in the Table 6. The highest number of phenols (9 spots) were reported in the market seed. However, five phenolic spots with similar color and hRf values (6.67, 16.67, 44.0, 58.67, and 90.0)

were found both in root and stem bark. Further, it is observed that five spots were found to be common for root, stem bark, and collected seed. Plants have the limitless ability to synthesize phenols or their derivatives. The presence of phenols in all types of tissues is a characteristic feature of plants.

Table 6. Qualitative separation of phenols of *S.potatorum*

S.No	Colour of the spot	hRf values	Plant parts tested			
			Root	Stem Bark	Collected seed	Market seed
1	Blue	6.67	+	+	+	-
2	Dark blue	16.67	+	+	+	-
3	Blue	33.33	-	-	-	+
4	Light blue	38.67	-	-	-	+
5	Intense blue	44.00	+	+	+	+
6	Light blue	49.33	-	-	-	+
7	Intense blue	58.67	+	+	+	+
8	Light blue	66.67	-	-	-	+
9	Blue	70.67	-	-	+	+
10	Blue	80.00	-	-	+	+
11	Intense blue	90.00	+	+	+	+

The data of saponins of *S.potatorum* by thin layer chromatography is tabulated in the Table 7. It has revealed the presence of five yellow to intense yellow coloured saponins with hRf values 69.39,71.43,79.59,85.71,and 96.94 in all parts of the plant. Saponins are glycosides of both triterpenes and sterols generally possessing five sugar units and gluconic unit as a component. The occurrence of saponins has been reported in over seventy families of higher plants²⁴.

Table 7. Qualitative separation of saponins of *S.potatorum*

S.No	Colour of the spot	hRf values	Plant parts tested			
			Root	Stem Bark	Collected seed	Market seed
1	Light yellow	69.39	+	+	+	+
2	Intense yellow	71.43	+	+	+	+
3	Intense yellow	79.59	+	+	+	+
4	Intense yellow	85.71	+	+	+	+
5	Intense yellow	96.94	+	+	+	+

The data of sterols of *S.potatorum* by thin layer chromatography is tabulated in the Table 8. It has revealed the presence of four sterols in the stem bark and seeds. While three spots of sterols were found in the root. These are of may be isomotioli, sitosterol, stigmasterol and compesterol as ealier reported³.

The qualitative HPLC alkaloids profile of *S.potatorum* seed were detected at a wavelength of 270 nm due to sharpness of the peaks and proper baseline and recorded its retention time (R_t min), percent area and heights in the Table 9 and Figure 1. The HPLC chromatogram has shown 61 peaks. However, 18 peaks were prominent with significant percent area and height (>0.5%). The most abundant peak with 36.68 percent area and 15.96 percent height is observed at the retention time 31.104 (R_t ,min), which is probably diaboline ,the prominent alkaloid of this plant as reported earlier^{2,4}.The other prominent peaks were reported with retention time 2.624,3.893,8.117,9.003 and 12.832 (R_t ,min) respectively.

Table 8. Qualitative separation of sterols of *S.potatorum*

S.No	Colour of the spot	hRf values	Plant parts tested			
			Root	Stem Bark	Collected seed	Market seed
1	Greenish black	40.82	+	+	+	+
2	Greenish black	56.12	+	+	+	+
3	Intense black	74.49	+	+	+	+
4	Pinkish black	89.90	-	+	+	+

Table 9. HPLC alkaloid profile of *S.potatorum* seed

S.No.of the peak	Retention time Rt,min	Peak area %	Peak height %
1	2.624	16.96	23.22
2	3.456	0.21	1.43
3	3.893	3.86	5.33
4	4.256	1.44	3.26
5	4.629	0.90	2.33
6	5.152	2.34	2.86
7	5.781	0.42	0.88
8	6.667	2.06	2.90
9	7.211	1.29	2.76
10	8.117	3.55	6.38
11	9.003	6.07	3.38
12	10.891	6.20	2.97
13	12.832	8.49	13.70
14	14.283	0.59	0.69
15	18.123	1.27	0.89
16	25.035	1.78	1.45
17	29.515	4.04	2.74
18	31.104	36.68	15.96

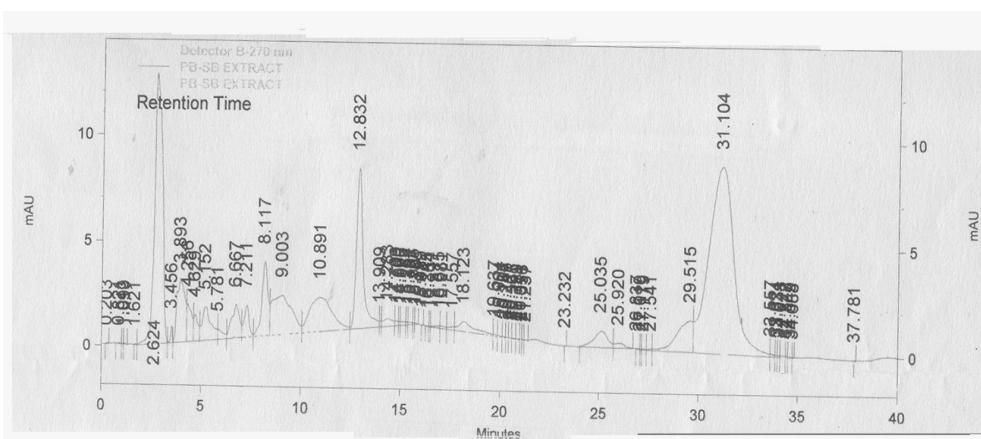


Figure 1. HPLC alkaloid profile of *Strychnos potatorum* seed

Therefore, the data generated from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. However, there is need to further carry out advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds. Furthermore, this data may be handy in probing of biochemistry of this plant in the future.

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