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Pharmacognostic Studies of *Strychnos potatorum* L.f.

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ABSTRACT

Strychnos potatorum (Clearing nut), an important medicinal plant used in the traditional and folk medicine for treating several aliments including microbial infections, diarrhoea and diabetes. Some of its pharmacognostic studies such as fluorescent, organoleptic, ash and mineral contents of root, stem bark and seed (both collected and market) , and GC- alkaloid profiles of seed have been investigated. Considerable colour variations in the fluorescent behaviour of raw drugs were observed. The highest yields were obtained for the aqueous extracts followed by ethanol, petroleum ether and chloroform extracts. The colour of the extracts thus obtained have from ivory to dark brown and bitter to pungent bitter in taste. Higher values of the total ash and insoluble acid contents were recorded for the stem bark, followed by root and seed samples. Considerable amounts of iron and copper found in all parts of the plant. However, lead, a toxic element was found in trace amount in the market seed sample. Further, the GC-alkaloid profiles of seed samples have shown significant variation in terms of percent area of peaks. Furthermore, the co-TLC study has revealed the absence of strychnine and brucine from the seed sample.

Introduction

Strychnos potatorum L.f. (Family: Loganiaceae) is a medium sized (~ 15m) and much branched glabrous tree generally occurs as a pan tropic species in the peninsular India, Burma, Sri Lanka and also in the north and south-east parts of Africa. It is known as the clearing nut (English), kataka or ambuprasada (Sanskrit), nirmali (Hindi), chillibeejagida (Kannada), illam or tettan-kottai (Tamil), chillachettu or indupachettu (Telugu), tettam-parel (Malayalam), kotaku (Oriya) and nirmali (Bengali).

It is an important medicinal plant widely used in the traditional and folk medicines for treating various ailments including microbial infections, anaemia and diabetes, despite its seed use in water purification in vogue since Charaka period (Bisset, 1974). The plant is acrid, bitter, alexiteric, increases appetite and improves taste. Seed is the chief source of therapeutic importance used for treating eye and urinary tract infections and acute diarrhoea (Ayurveda), gonorrhoea (Unani) and venereal diseases including leucorrhoea and piles (Siddha) (Kirtikar and Basu, 1998; Annalakhmi, 2003). Further, the root cures all kinds of leucoderma and the stem bark powder is mixed with some lime juice is said to be effective against cholera (Annalakhmi, 2003). Therefore, the present paper deals with some of its pharmacognostic parameters that help us to standardize its drugs in terms of fluorescent and organoleptic attributes and also to know about the presence or absence of some metals and other secondary metabolites.

Materials and Methods

Collection of Plant material

The plant material *viz.*, root, stem bark and seed of *Strychnos potatorum* were collected from Karpakpalli forest, Bidar district in December 2002. The material was identified and a voucher specimen of the same is deposited in the Herbarium of Botany department, Gulbarga University (HGUG-214), Gulbarga. Further, another seed sample was procured from M/s. Jajee Ayurvedic stores, Gulbarga as the market but processed and stored one for comparison. The plant material thus collected was shade dried at room temperature. The powdered material was preserved in sterilized polythene bags until further use.

Fluorescent studies

Finely particulate (~300µ) plant drugs were tested for their characteristic colours fluoresced both under visible and ultraviolet (UV $_{365nm}$) lights after treating with chemical solvents including alkalis and acids(Chase and Pratt, 1949).

Organoleptic studies

One hundred grams of powdered plant drugs of the root, stem bark and seed of *S. potatorum* were successfully extracted in soxhlet extractor using petroleum ether, chloroform, 95% ethanol and distilled water in the increasing order of polarity for 18h. The obtained extracts condensed in *dry vacuo* (40 ^{o}C) and were determined the organoleptic properties such as colour, taste and yield (Chakraborti *et al*, 1988).

Determination of acid and mineral contents

Two grams of powdered plant material was taken in a sintered crucible and incinerated by keeping in muffle-furnace by gradual increasing its temperature (400 – 500 ^{o}C) for 6h. The obtained ash was calculated and further subjected to acid digestion using 25 % (v/v) HCl at 100 ^{o}C. The retained residue along with Whatman filter paper No.44 (ash less) was ignited in order to determine the acid insoluble ash content (Raghunathan, 1976). Further, four metals *viz*., copper, lead, iron and nickel were quantitatively estimated by atomic absorption spectrophotometric method (Smith-Hieftze 1000, Franklyn MA, USA) (Sahwney and Singh, 2008). Stock solutions were prepared from ash in double distilled water and the amount of

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metals were determined in parts per million (ppm) of five replicates ± SD using standard graph.

GC- Alkaloid profile of seed samples

The total alkaloid extracts of both seed samples *viz*., collected and market seed, were dissolved in 5mL of methanol of analytical grade and filtered through Whatman No.1 filter paper. 20 µL sample was injected into the gas chromatogram column (shimadzu QP-2000, column ULBON HR- with fused silica capillary tube of 0.25mm X50m).The initial temperature was 100 ^{o}C for 6 min and was increased up to 250 ^{o}C with 10 ^{o}C /min. Helium was the carrier gas pumped at 21 mL/min flow rate. All the reagents used were of analytical grade. The resulted number of peaks and their percent area were plotted on the software version 6.2.0.0:b27 and recorded.

Comparative-Thin Layer chromatography

Twenty microlitres of the total alkaloid extracts of the seed of *S. potatorum* and *S. nux-vomica* (procured from M/s Jajee Ayurvedic stores, Gulbarga), the standard alkaloids, brucine and strychnine (Hi-media) were loaded on a precoated Alugram® Sil G/UV $_{254nm}$ (20x20cm) and developed using EtOAc: *iso*PrOH: NH_4OH (80:15:5) mobile phase. The developed chromatogram was observed for the presence of strychnine and brucine in *S. potatorum* seed samples (Wagner and Bladt, 1996).

Results

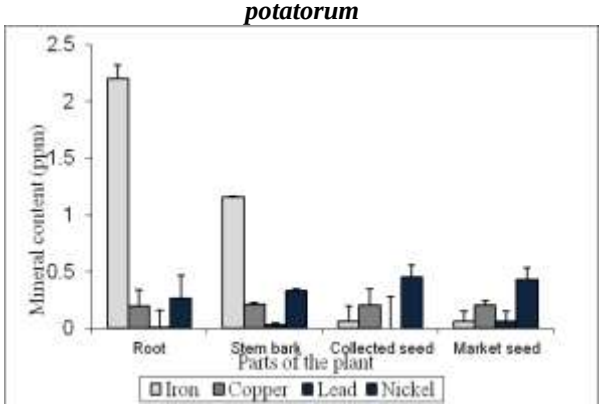
The powdered drugs of *S. potatorum viz*., root, stem bark and seed (collected and market) have exhibited a wide range of characteristic fluorescent colour reactions when treated with various solvents ranging from acids to alkali both under visible and ultraviolet lights (UV $_{365nm}$). These were from ivory to dark black in colour. Further, the market seed samples have exhibited broad range of colours (Table 1).

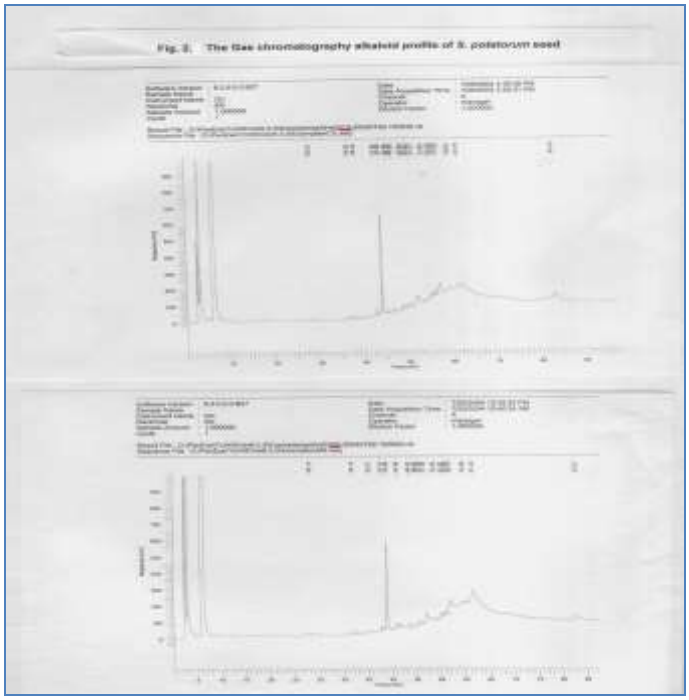
The organoleptic studies of *S. potatorum* have revealed variation in the colour, yield and taste of extracts. It is evident from results, as expected the aqueous extracts are having the highest percent yield owing to the universal soluble nature of water followed by ethanol extracts (Table 2). However, the least percent of yields were recorded for chloroform extracts. Further, the yields of collected seed have greater value. The colours of these extracts were ranging from ivory to dark brown and bitter to pungent bitter in taste. They were amorphous powder to waxy in nature.

The highest percent of total ash and acid contents were obtained for stem bark followed by root and seed samples may be perhaps due to the presence of lignin and tannins. While the seed is rich with carbohydrates especially mannogalactans, on digestion resulted lower yields of ash contents (Table 3). Further, it was observed that uneven distribution of metals in the plant (Figure 1). Higher concentrations of iron were found in the root (2.2 ppm) and stem bark (1.161 ppm). Whereas the copper concentration is however low but uniformly distributed in all parts. Considerable amount of lead, a non-essential but toxic element was reported in the stem bark (0.0283 ppm) and market seed (0.061 ppm). However, the collected seed is completely devoid of lead element. Further, gradual increase of nickel from root to seed is observed.

Although, twenty one alkaloid peaks were reported from the seed samples of *S. potatorum*, considerable variation in their percent areas were observed (Table 4 & Figure 2). The most prominent alkaloid peak in the collected seed was reported with percent area 62.71% at 43.97 retention time (R*t*, min). While, in the market seed two prominent peaks were observed at 43.850 and 61.422 (R*t*, min) with percent area 45.91% and 28.32%, respectively.

Figure 1. Quantity of mineral metals in *S. potatorum*





Discussion

The study of pharmacognostic aspects of medicinal plants in general and their therapeutic drugs in particular is an essential step in order to know their therapeutic value, efficacy, quality of the drug, to check for the adulterants and substitutes, and also for its toxicity. In the present investigation, various pharmacognostic attributes of *S. potatorum* have been studied.

The presence of iron and copper is not only essential for plant growth but also may be useful in treating of anaemia. On the contrary, lead is a toxic element at higher concentration to the biota. The presence of lead more significantly in the market seed may be perhaps due to the method of processing and/or soil contamination where it is grown (Cai *et al*, 1993). The lead poisoning is being associated with carcinogenesis, reduced fertility, miscarriages and spermatotoxicity (Tandon and Suri, 1993).

The longevity of storage of plant drugs generally decreases its bioactive constituents as it is observed in our TLC studies of secondary metabolites including alkaloids (Mallikharjuna *et al*, 2007). However, the method of processing aimed at increase the potency of the drug and minimizing the toxicity is a very essential aspect. For instance, the seed of *Strychnos nux-vomica* are often used in clinical practices after processing in hot sand

(220 ^{o}C for 30 min) which is intended not only to reduce toxicity but also to clean the fine hairs, which cause throat infection thus a better acceptance(Cai *et al*, 1993; Wu *et al*,1994) Further, the comparative thin layer chromatogram (co-TLC) has revealed the absence of strychnine and brucine, from *S. potatorum* however but found in *S. nux-vomica*. On the other hand, further studies reveals that it is rich with diaboline and its derivatives (Mallikharjuna *et al*, 2007). As per the literature available strychnine and brucine were reported from the other Asian *Strychnos* species like *S. nux-vomica*, *S. wallichiana* and *S. ignatti* (Chakraborti *et al*, 1988; Mallikharjuna *et al*, 2010).

Conclusion

The above results of pharmacognostic studies of *S. potatorum* have laid down emphasis on the standardization of its crude drugs, checked for mineral contents, variation in the alkaloid profile and the absence of strychnine and brucine.

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Table 1. Fluorescent studies of *S. potatorum* powdered drugs

Sl. No.	Treatment	Root		Stem bark		Collected seed		Market seed	
		VIS	UV_{365}nm	VIS	UV_{365}nm	VIS	UV_{365}nm	VIS	UV_{365}nm
1	Powder	Ivory	Ivory	Light black	Black	Ivory	Seafoam	Ivory	Ivory
2	+H_2O	Ivory	Dark black	Black	Black	Orange	Spring green	Nile green	Ivory
3	+ HCl	Old gold	Brown	Chocolate	Black	Ivory	Mauve	Copper	Mimosa
4	+ H_2SO_4	Cardinal red	Dark black	Brown	Black	Chocolate	Maroon	Chocolate	Black
5	+ NaOH	Carrot red	Cream	Apricot	Black	Sea foam	Moss green	Burnt orange	Old gold
6	+ $FeCl_3$	Citron	Sea foam	Butter cup yellow	Old gold	Cinnamon	Chocolate	Butter cup yellow	Moss green
7	+ MeOH	Ivory	Sea foam	Black	Black	Ivory	Lettuce green	Chartreuse	Citron
8	+EtOH	Ivory	Sea foam	Black	Black	Ivory	Lettuce green	Chartreuse	Nile green
9	+BuOH	Ivory	Spring green	Black	Black	Ivory	Nile green	Chartreuse	Citron
10	+ Acetic acid	Ivory	Buff	Brickson brown	Sea foam	Ivory	Nile green	Cream	Butter cup yellow
11	+n-Hexane	Ivory	Seafoam	Cinnamon	Black	Ivory	Spring green	Buff	Spring green
12	+ Lactic acid	Ivory	Light brown	Black	Rust red	Ivory	Old gold	Ivory	Old gold
13	+ C_6H_6	Ivory	Light brown	Black	Black	Sea foam	Sea foam	Butter cup yellow	Moss green
14	+ $CHCl_3$	Buff	Citron	Black	Black	Ivory	Spring green	Cream	Sea foam
15	+Pet. ether	Cream	Citron	Black	Black	Ivory	Nile green	Sea foam	Ivory

Table 2. Showing the extractive and organoleptic characters of *S. potatorum* extracts..

Characters	Successive extracts	Plant parts used			
		Root	Stem bark	Collected seed	Market seed
Extractive values (%, w/v)	Pet. ether	1.30	0.50	2.74	2.16
	$CHCl_3$	0.12	0.56	0.34	0.76
	Et-OH	8.90	4.58	6.81	4.79
	Aqueous	5.90	6.97	15.68	10.23
Colour	Pet. ether	Old gold yellow	Mimosa yellow	Cinnamon	Cinnamon
	$CHCl_3$	Dark green	Fern green	Meadow green	Nile green
	Et-OH	Mint leaf green	Apricot	Inca gold yellow	Butter cup yellow
	Aqueous	Dark brown	Dark brown	Ivory	Ivory
Taste	Pet. ether	Pungent bitter	Pungent bitter	Pungent bitter	Pungent bitter
	$CHCl_3$	Pungent bitter	Pungent bitter	Pungent bitter	Pungent bitter
	Et-OH	Bitter	Bitter	Bitter	Bitter
	Aqueous	Bitter	Bitter	Bitter	Bitter
Nature	Pet. ether	Sticky	Sticky	Sticky	Sticky
	$CHCl_3$	Waxy	Waxy	Waxy	Waxy
	Et-OH	Resin	Resin	Resin	Resin
	Aqueous	Amorphous powder	Amorphous powder	Powder	Powder

Table 3. Showing ash and mineral contents of *S. potatorum*

Plant part	Ash content (%, w/w)		Mineral content (ppm)			
	Total ash	Acid insoluble ash	Iron	Copper	Lead	Nickel
Root	14.50	8.35	2.2000* (± 0.125)	0.1972 (± 0.005)	0.0005 (± 0.135)	0.2657 (± 0.091)
Stem bark	20.45	16.52	1.1610 (± 0.145)	0.2155 (± 0.015)	0.0283 (± 0.137)	0.3318 (± 0.042)
Collected seed	3.15	1.25	0.0643 (± 0.161)	0.2065 (± 0.015)	0.0000 (± 0.277)	0.4535 (± 0.088)
Market seed	3.00	1.39	0.0631 (± 0.199)	0.2013 (± 0.014)	0.0607 (± 0.104)	0.4297 (± 0.103)

* Values are parts per million of five replicates (± S. D.)

Table 4. Alkaloid profile of *S. potatorum* seed by Gas Chromatography

Sl. No. of the peak	Collected seed		Market seed	
	Retention time (Rt, min)	Percent area (%)	Retention time (Rt. min)	Percent area (%)
1	28.276	0.32	28.148	0.47
2	36.926	1.08*	36.795	0.89*
3	38.455	0.22	40.275	0.48
4	42.860	2.54*	42.735	1.83*
5	43.987	62.71*	43.252	1.86*
6	45.456	0.74*	43.850	45.91*
7	46.129	0.10	45.989	1.91*
8	46.653	0.70*	46.532	0.77*
9	46.881	1.33*	48.428	1.91*
10	48.885	2.27*	48.762	0.69*
11	49.717	0.59*	49.638	0.07
12	50.482	2.07*	50.000	2.47*
13	51.569	0.88*	51.452	0.07
14	52.125	9.18*	52.011	7.15*
15	53.660	0.68*	53.533	0.53*
16	55.159	0.05	55.065	1.53*
17	55.992	3.89*	55.825	1.50*
18	57.051	9.42*	56.785	1.61*
19	59.623	0.16	59.460	0.17
20	61.729	0.47	61.424	28.32*
21	83.183	0.61*	82.667	0.40

* Indicates the percent area (≥ 0.5%) of peak eluted