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REGULAR ARTICLE

PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *STRYCHNOS WALLICHIANA* STEUD EX DC

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SUMMARY

A preliminary phytochemical screening of seed and leaf of *Strychnos wallichiana*, an Asiatic woody liana species belongs to the family Loganiaceae revealed the presence of alkaloids, phenols, glycosides, flavonoids, saponins and sterols. Further, the HPLC chromatogram of alkaloid profile of seed has shown twelve prominent alkaloid peaks. Furthermore, the methanol and aqueous extracts were evaluated for *in vitro* antimicrobial properties against the selected bacteria and fungi at the concentration 2 mg/ml and 4 mg/ml, respectively using agar well diffusion assay. The findings have revealed considerable to significant inhibition activity of pathogens tested.

Key words: *Strychnos wallichiana*, Loganiaceae, Phytochemicals, HPLC alkaloid profile, Antimicrobial activity.
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1. Introduction

Plants, especially medicinal plants, offer a vast resource of novel natural compounds, often with exciting activities and biological properties. Therefore, the systematic screening of plant extracts or plant derived substances still remains an interesting strategy to find new lead compounds. Structure-activity relationship studies of these leads preferentially combined with computer-graphic model building should result in molecules with optimal activity, better bio-availability, fewer side effects and an acceptable therapeutic index and consequently good candidates for the development of new drugs (Vlietinck, 1998). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments (Nascimento *et al.*, 2000; Rios and Recio, 2005)

Strychnos wallichiana, often known as Snakewood (English), Nagamusti (Kannada) of the family Loganiaceae, is an Asiatic large woody liana species, generally occurs in Sri Lanka, south and north-east India, Andaman & Nicobar Islands of India, north Vietnam and south China at an altitude about 1000 m (Bisset, 1974). Though it is a rare but a very important medicinal plant generally used in folk medicine especially the root as a remedy for the bites of venomous snakes, to alleviate pain, and also to remove swellings. A decoction of the root is also being given to treat rheumatism, ulcers, elephantiasis, fever and epilepsy (De and Bisset, 1988).

2. Material and Methods

Collection of Plant material

The seed and leaf material of *Strychnos wallichiana* were collected from the evergreen forest near Agumbe, Western Ghats, Karnataka, India during November 2004 and a voucher specimen of the same is deposited in the Herbarium, Department of Botany, Gulbarga University (HGUG-216), Gulbarga

(Figure 1). The shade dried and powdered plant material were extracted using methanol and distilled water solvents by cold extraction (at 25±2 °C) and thus extracts were condensed to dryness *in vacuo* at 40^{0}C and preserved at 4^{0}C until further use.

Figure1. *Strychnos wallichiana* - a) a fruiting twig & b) seed material

Phytochemical analysis

The preliminary screening tests, for the detection of phytochemicals, were carried out for all the extracts of *S. wallichiana* by adopting standard methods. Five hundred milligram of each extract was dissolved in 100 ml of the respective solvent and filtered through Whatman No.1 filter paper. Thus, the filtrates obtained were used as test solutions for the detection of alkaloids (Iodine, Dragendorff and Wagner tests), flavonoids (Pews and Shinoda tests), glycosides (Kellar – Kiliani and Molisch tests), lignins (Labat and Lignin tests), phenols (Ellagic acid and phenol tests), saponins (Foam and Haemolysis tests), sterols (Liberman-Burchard and Salkowiski tests), tannins (gelatin test) (Evans, 1989; Gokhale *et al*., 1993; Harborne, 1998)

HPLC-alkaloid profile of S. wallichiana seed

The total alkaloids were extracted from the seed of *S.wallichiana* as described by Nuzillard *et al.,* (1996). 10 g seed material is wetted with 50 ml of half diluted aqueous NH_4OH and lixiviated overnight with 1000 ml ethyl acetate. The filtered organic solution was extracted with 2% (v/v) H_2SO_4. The resulted organic phase was separated using separating funnel and basified with NH_4OH (pH 11-12). This is extracted with 1000 ml $CHCl_3$ (3X), and dried over Na_2SO_4 and evaporated to dryness at 40 °C *in vacuo*. The dried extract was re-dissolved in 5 ml of absolute ethanol (Analar grade) and filtered through Whatman No.1 filter paper. 20µl is loaded into an isocratic High Performance Liquid Chromatography (Shimadzu HPLC class VP series). The mobile phase components acetonitrile: water (1:3) were filtered through 0.2µ membrane filter before use and pumped from the solvent reservoir to the column at a flow rate 1 ml/min which yielded a column back pressure of 16-165 kgf/cm^2. The column temperature was maintained at 27 °C. The peaks of alkaloids were detected at a wavelength of 270 nm due to sharpness of the peaks and proper baseline and recorded its retention time (R*t*) and percent area of peaks.

Antimicrobial studies

The methanol and water extracts of seed and leaf samples of *S.wallichiana* were screened for antimicrobial activity against *Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus, Aspergillus niger* and *Mucor* sp. *in vitro* by adopting the agar well diffusion technique (Indian Pharmacopoeia, 1996).

Drug solutions and Media used: All the drugs i.e., crude extracts, streptomycin sulphate, and catacanazole were dissolved in 5% (v/v) dimethyl sulfoxide (DMSO) and the final concentrations of these drug solutions were obtained mg/ml. For bacteria

Muller–Hinton Agar and Martin's Rose Bengal Agar for fungi (Hi- Media, Mumbai, India) were used.

Agar well diffusion assay: Twenty milliliters of sterile molten Mueller-Hinton Agar medium was poured into a set of sterile petri dish under aseptic conditions and was allowed to solidify. Likewise a series of agar plates were prepared. Then, each plate was inoculated with 200 µl of 18 h old bacterial culture and was evenly spread with a sterile bent glass rod. Similarly the fungal culture is lawned with a cotton swab on MRB agar plates. A few number of agar wells (8mm, diameter) were made equidistantly using sterile cork borer in order to load test solutions. Of these, one of the well is loaded with 100 µl of DMSO as negative control, the second one with 4 mg/ml (w/v) streptomycin or catacanazole antibiotic solutions with the help of a micropipette (Hi-Media Lab. Ltd., Mumbai). Whereas, the remaining two wells were loaded with 100 µl of the plant crude extracts of 2 mg/ml and 4 mg/ml crude extracts, respectively. The zone of inhibition was observed and recorded after 48 h and 72 h for bacteria and fungi, respectively. The data obtained was the mean of triplicates ± standard error.

3. Results

The methanol and aqueous extracts of both seed and leaf of *S.wallichiana* were screened qualitatively for the occurrence of various phytochemicals. This plant is rich in alkaloids, flavonoids, glycosides, phenols, saponins, sterols as mentioned in the Table 1. However, the methanolic extracts of seed are positively responded for lignins and tannins.

Table 1. Preliminary screening of secondary metabolites of *S. wallichiana*

Types of Phytochemicals	**Name of the test**	**Seed Extracts**		**Leaf Extracts**	
		Methanol	**Aqueous**	**Methanol**	**Aqueous**
	Iodine test	+	+	+	-
Alkaloids	Dragendorff's test	+	+	+	-
	Wagner's test	+	+	+	+
Flavonoids	Pew's test	+	+	+	+
	Shinoda test	+	+	+	-
Glycosides	Kellar-Kiliani test	+	+	-	+
	Molisch test	-	+	-	+
Lignin	Labat test	+	-	-	-
	Lignin test	+	-	-	-
Phenols	Ellagic acid test	+	+	+	+
	Phenol test	+	+	+	+
Saponins	Foam test	+	+	-	+
	Haemolysis test	-	+	-	+
	Libermann-Burchard test	+	-	+	+
Sterols	Salkowski test	+	+	+	+
Tannins	Gelatin test	+	-	-	-

' +' : Present '-' Absent

Further, the HPLC chromatogram alkaloid profile of *S.walichiana* seed extract has revealed a wide range of peaks with differential per cent area and height as shown in the Figure 2 and Table 2. However, it is evident that only 12 peaks were significant based on their percent area and percent height. Furthermore, the most prominent alkaloid peaks were observed with retention time (R*t*, min) 2.19 min (40.08% and 46.3759%), 2.27 min (41.5218% and 33.7028%), 2.56 min (6.1413% and 2.33) and 6.65 min (6.4973% and 8.0195%), respectively.

Figure 2. HPLC chromatogram showing the alkaloid profile of *Strychnos wallichiana* seed.

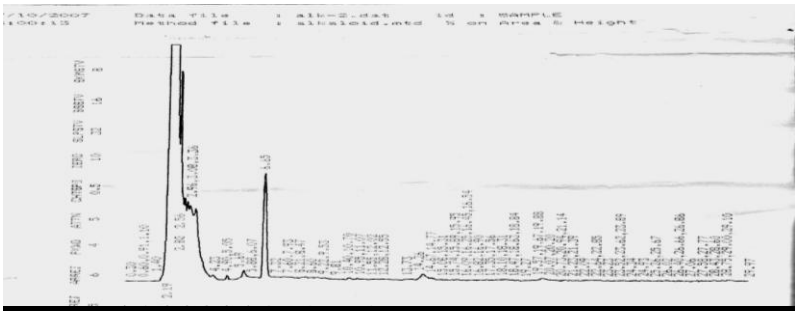

Table - 2. Showing the HPLC alkaloid profile of *S.wallichiana* seed.

Sl. No. of the peak	Retention time in min.(R*t*)	Percent Height of the peak	Percent Area of the peak
1	2.19	40.0804*	46.3759*
2	2.27	41.5218*	33.7028*
3	2.56	6.1413*	2.3311*
4	2.80	0.7169	0.2157
5	2.96	0.8980	0.5223
6	3.08	1.0317	1.3270
7	3.26	1.7311	1.2947
8	4.23	0.1790	0.1999
9	4.90	0.2773	0.2210
10	5.69	0.5558	1.0379
11	6.65	6.4973*	8.0195*
12	14.26	0.3684	0.9221

* Significant peaks of alkaloids

Antimicrobial activity of the crude extracts i.e., methanol and aqueous extracts of *S.wallichiana* seed and leaf were studied against *Escherichia coli, Bacillus* sp., *Staphylococcus aureus, Aspergillus niger* and *Mucor* sp. at 2 mg/ml and 4 mg / ml concentrations by adopting standard agar well diffusion assay are mentioned in the Table 3. All the four extracts have exhibited considerable to significant antimicrobial activity against these microorganisms. However, the methanolic extracts of both seeds and leaves have shown relatively greater activity than that of the aqueous extracts. Further, it is noticed that the increase in the zone of inhibition is corresponding to the increase of the drug concentration (4 mg/ml). Thus, the results obtained above are comparable to the standard drugs i.e., streptomycin and catacanozole in terms of the zone of inhibition against these microorganisms.

4. Discussion

The phytochemicals such as alkaloids, phenols, saponins and terpenoids may have potentially significant application against microorganisms (El-Mahmood, 2009). The preliminary phytochemical screening of *Strychnos wallichiana* has revealed the presence of secondary metabolites of therapeutic importance. It is evident from the literature that the genus *Strychnos*, which is consisting about 200 species, is known for therapeutic role especially the primary, secondary, tertiary, and quaternary type indole alkaloids as antimicrobials (Verpoorte *et.al*., 1983, Quetin-Laclercq *et al*., 1995, Mallikharjuna and Seetharam, 2009), antimalarials (Frederich *et al*.,2002), antitumor properties (Frederich *et al*., 2003) and others. Some of these alkaloids are strychnine, brucine, diaboline, novicine, toxiferine, icajine, isopentamine and others with their derivatives (Penelle *et al*., 1999; 2001).

Table 3. Showing antimicrobial activity of *Strychnos wallichiana* extracts.

Name of pathogen	Concentration of the drug (mg/ml)	Zone of inhibition in mm				Standard drug (4mg/ml)
		Seed Extracts		Leaf Extracts		
		Methanol	Aqueous	Methanol	Aqueous	Streptomycin
Escherichia coli	2.0	30.0*±0.58	29.0±0.58	27.0±1.16	25.3±0.33	-
	4.0	37.3± 0.88	36.0± 0.58	31.7± 0.67	28.3± 0.88	35.3± 0.33
Bacillus subtilis	2.0	26.0± 0.58	25.7± 0.33	31.0± 2.082	29.0± 0.58	-
	4.0	31.0± 0.58	31.0± 0.58	38.0± 0.58	32.0± 0.58	30.0 ± 0.58
Staphylococcus aureus	2.0	24.00± 0.58	26.7± 0.33	25.7± 0.33	25.0± 0.58	-
	4.0	36.0± 0.58	35.0±0.58	31.7± 1.45	30.7± 0.88	33. 7± 0.88
Aspergillus niger	2.0	26.0± 0.58	25.0±0.58	23.3± 0.67	24.0± 0.58	Catacanazole
	4.0	32.3±0.88	31.0±0.58	38.0±1.15	37.0± 0.58	34.7± 0.66
Mucor sp.	2.0	18.0± 0.58	21.0± 0.58	20.3± 0.33	19.0± 0.58	-
	4.0	27.0± 0.58	27.0± 0.58	28.0±0.58	27.0± 0.58	29.3± 0.33

* Values are the mean of triplicates ± standard error

The HPLC chromatogram has revealed the presence of twelve alkaloid fractions from the seed material of *S. wallichiana*. Two prominent alkaloids, icajine and novicine were reported by Bisset and Choudhury (1974) from the leaf of *S. wallichiana* collected from Bangladesh. Further, De and Bisset (1988) reported brucine and brucine –*N*-oxide as the major alkaloid fractions besides vomicine, β-colubrine, novicine from the leaf material of *S. wallichiana* collected from the Andaman, India.

Several authors have reported the antimicrobial activity of crude extracts of various plants (Nwolobi *et al*., 2007; Oyeleke *et al*., 2008, Shilpa Bhat *et al*., 2009). Similar kinds of observations were found for the species of *Strychnos*. Verpoorte *et al.* (1983) have screened the ethanolic extracts of various species of *Strychnos* for antibacterial activity against *S. aureus, B.subtilis* and *Pseudomonas aeruginosa*. They reported that majority of extracts were active against Gram+ve bacteria i.e., *S.aureus* and *B.subtilis* compared to Gram -ve *P.aeruginosa*. Similar observations are made by Mc Gaw *et al.* (2000) for the hexane, ethanol and aqueous extracts of *Buddleia salvifolia* and *Strychnos spinosa*. Recently, Mallikharjuna and Seetharam (2007) have observed moderate to significant antimicrobial activity of the successive organic solvent extracts of *Strychnos potatorum* against *E.coli, S.aureus, Vibrio cholerae, Salmonella typhimurium, P.aeruginosa, Candida albicans, Microsporum gypseum, Aspergillus niger,* and *A. fumigatus* at 2 mg/ml concentration.

In summary, the antimicrobial activity of the extracts of *Strychnos wallichiana* may be attributed to the presence of various phytochemicals such as alkaloids, phenols, glycosides, steroids, sterols owing to their synergistic action and/or the action exhibited by a single group of molecules such as alkaloids. However, there is a need to further investigate to establish the structure – activity relationship, so our efforts are in progress on these lines.

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