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In Vitro Antimicrobial Screening of Alkaloid Fractions from *Strychnos potatorum*

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Abstract: Alkaloid fractions isolated from *Strychnos potatorum* L.f. (Loganiaceae) seed were tested for their antimicrobial properties against some pathogenic gram positive, gram negative and acid-fast bacteria and fungi. These fractions have shown considerable antimicrobial activity against both bacteria and fungi at the tested concentrations (100 & 200 µg/mL). Further, the growth of *Proteus vulgaris*, *Staphylococcus aureus, Salmonella typhimurium, Vibrio cholerae, Mycobacterium tuberculosis, Aspergillus niger* and *Candida albicans* were significantly inhibited. These findings have confirmed the use of this plant in treating of several microbial infections both in traditional and folk medicine in India.

Keywords: Alkaloids, Antimicrobial activity, Acid-fast bacilli, Diaboline, Indoles, *Strychnos potatorum* Zone of growth inhibition.

Introduction

Plants contain numerous biologically active compounds, many of which shown to have antimicrobial activities[1]. Plant derived medicines have been a part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases. Given the alarming incidence of antibiotic resistance of pathogenic microbes in particular, there is a constant need for discovering new and effective therapeutic agents[2].

Strychnos potatorum L.f. is a medium sized (~ 15 m) glabrous deciduous tree occurs both in the tropic and sub-tropics of north and south-east parts of Africa and Indian peninsula, Sri Lanka and Myanmar of Asia. The seed, besides its bark and root, is primarily used in the Indian traditional systems of medicine for treating various diseases including microbial infections. It is used in Ayurveda for treating the eye and urinary tract infections[3], gonorrhoea and kidney troubles in Unani and for the leucorrhoea, tuberculosis, venereal

diseases and acute diarrhoea in Siddha medicine[4,5]. Alkaloids, the prime source of secondary metabolites isolated from several *Strychnos* species are known for their therapeutic importance. Therefore, an attempt has been made in order to give the experimental basis for its wide therapeutic use in traditional medicine.

Experimental

The seed material of *Strychnos potatorum* were collected from the Karpakpalli forest, Bidar district of Karnataka, India in December 2002. The plant was identified by Prof. Y.N. Seetharam and a herbarium specimen is deposited in the department of Botany Herbarium, Gulbarga University, Gulbarga with a voucher number HGUG-214[6].

Extraction and fractionation of alkaloids

The pulverized powdered seed wetted with aqueous NH_4OH and lixiviated overnight with (2.5 L) ethyl acetate at room temperature. The filtered organic phase was separated and basified with NH_4OH (pH 11-12). This is extracted with (2.5 L) chloroform (3X) and passed through anhydrous Na_2SO_4 and evaporated to yield 14.3 g of a brown sticky substance.

The crude alkaloid mixture (14 g) was fractionated on Silica gel H column chromatogram (60-120 mesh) eluting with $CHCl_3$, followed by gradient mixtures of $CHCl_3$-CH_3OH (19:1), $CHCl_3$-CH_3OH (9:1), $CHCl_3$-CH_3OH (1:1) and CH_3OH. These fractions were collected and pooled according to their similarity by TLC eluting with EtOAc:*iso*PrOH:NH_4OH (80:15:5). The harvested seven alkaloid fractions from preparative TLC were partially purified by dissolving in EtOH-HCl and precipitated with EtOAc. The UV, FT-IR, ^{1}H NMR and FAB-MS spectra of these compounds were recorded.

Biological activity

Escherichia coli (clinical isolate), *Klebsiella* sp., *Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Vibrio cholerae* (clinical isolate), *Mycobacterium tuberculosis* (clinical isolate), *Mycobacterium tuberculosis* H37Ra, *Mycobacterium smegmatis, Aspergillus niger, Aspergillus fumigatus, Candida albicans* and *Microsporum gypseum* were used. Agar-well diffusion assay for both gram positive and gram negative bacteria and fungi[7] and minimum inhibitory concentration assay for acid-fast bacilli were adopted[8]. For gram bacteria Müeller-Hinton agar, Rosebengal agar for *Aspergillus* sp. and for *C. albicans* and *M. gypseum* Sabauroud agar plates were used. While, *in vitro* antimycobacterial activity of alkaloid fractions ($\leq$100 µg/mL) were determined using two-fold serial broth dilution technique. Streptomycin, nystatin, rifampicin and ethambutol were used as standard antibiotics and brucine, an alkaloid for comparison.

Statistical analysis

Values are expressed for the zone of growth inhibition diameter of gram positive and negative bacteria and fungi as mean of triplicates ± SE. The significance of difference of data obtained statistically from student's paired *t*-test ($P \leq 0.05$) and was evaluated using Duncan's multiple range test[9].

Results and Discussion

Seven alkaloid fractions (PB-I to PB-VII) were isolated from *Strychnos potatorum* seed and their UV, FT-IR, ^{1}H NMR and FAB-MS spectra have revealed that these are indole type of alkaloids with the basic strychnine skeleton, the prime type of alkaloid of *Strychnos* species. Further, from co-TLC with an authentic sample (kindly provided by Dr. Michel Frederich, Liege University, Belgium) and the literature available[10-13] fraction PB-IV was identified as diaboline (1-acetyl-19,20-didehydro-17,18-epoxycuron-17-ol), the most abundant alkaloid of *S. potatorum* (Figure 1).

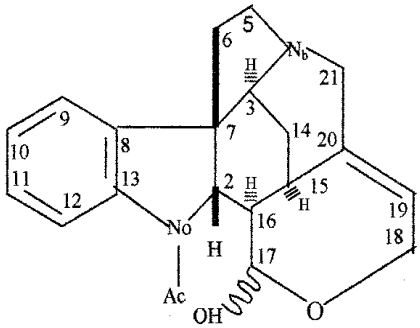

Figure 1. The structure of Diaboline.

However, the structures of remaining alkaloids are to be established. These alkaloid fractions have exhibited considerable antimicrobial activity against the tested bacteria and fungi. Marked zone of growth inhibition of gram positive bacterium *S. aureus* and gram negative bacteria *P. vulgaris*, *S. typhimurium*, and *V. cholerae* by the fractions including diaboline (PB-IV) is observed (Table 1). Further, they have exhibited significant ($P \leq 0.05$) antifungal activity at higher concentration (200 µg/mL) especially against *A. niger* and *C. albicans* (Table 2). Furthermore, these alkaloid fractions showed considerable minimum inhibitory concentrations (IC_{50}) against *M. tuberculosis* (clinical isolate) at ≤100 µg/mL concentration (Table 3).

Table 1. Antibacterial activity of *Strychnos potatorum* alkaloid fractions.

Drug, 100 µg/mL	Zone of inhibition diameter (mm) of bacteria						
	E. coli	*S. aureus*	*P. vulgaris*	*P. aeruginosa*	*S. typhimurium*	*Klebsiella* sp.	*V. cholerae*
Streptomycin	18.33 (± 0.33)*^{c}	13.67 (± 0.33)^{a}	15.33 (± 0.33)^{c}	14 (± 0.58)^{b}	14.67 (± 0.33)^{a}	15.33 (± 0.67)^{b}	15 (± 0.58)^{a}
Brucine	12.67 (± 0.33)^{a}	17 (± 0.58)^{c}	17 (± 0.58)^{d}	12.33 (± 0.33)^{a}	16 (± 0.58)^{b}	15.67 (± 0.33)^{b}	17 (± 0.58)^{b}
Total alkaloids	14.67 (± 0.33)^{b}	17 (± 0.58)^{c}	15.33 (± 0.33)^{c}	14 (± 0.58)^{b}	15.67 (± 0.88)^{b}	16.67 (± 0.33)^{c}	17.67 (± 0.33)^{b}
PB - I	14 (± 0.58)^{b}	17 (± 0.58)^{c}	14 (± 0.58)^{b}	13.67 (± 0.33)^{a}	16 (± 0.58)^{b}	12.33 (± 0.33)^{a}	18.33 (± 0.33)^{b}
PB - II	12 (± 0.33)^{a}	16 (± 0.58)^{b}	11 (± 0.58)^{a}	14.33 (± 0.33)^{b}	14.33 (± 0.33)^{a}	13 (± 0.0)^{a}	18.33 (± 0.33)^{b}
PB - III	11.67 (± 0.33)^{a}	15.33 (± 0.33)^{b}	14 (± 0.58)^{b}	14 (± 0.58)^{b}	16.33 (± 0.33)^{b}	14 (± 0.58)^{b}	19.67 (± 0.33)^{c}
PB-IV, Diaboline	12.33 (± 0.33)^{a}	16.67 (± 0.33)^{b}	17.67 (± 0.33)^{d}	14.67 (± 0.33)^{b}	16.67 (± 0.33)^{b}	15.67 (± 0.33)^{b}	19.33 (± 0.33)^{c}
PB - V	12.67 (± 0.33)^{a}	17.33 (± 0.33)^{c}	19 (± 0.58)^{e}	14.33 (± 0.33)^{b}	17 (± 0.58)^{c}	14.33 (± 0.33)^{b}	20 (± 0.58)^{c}
PB - VI	12 (± 0.0)^{a}	17.33 (± 0.67)^{c}	18.67 (± 1.20)^{d}	13 (± 0.58)^{a}	17 (± 0.58)^{c}	12.33 (± 0.33)^{a}	17 (± 0.58)^{b}
PB - VII	11.67 (± 0.33)^{a}	19 (± 0.58)^{d}	18.67 (± 0.67)^{d}	12.67 (± 1.33)^{a}	19 (±1.15)^{c}	13.33 (± 0.33)^{a}	20.33 (± 0.33)^{c}

**Values are the mean of three replicates (± SE) at 100 µg/mL concentration. Means within the column followed by the same letter do not differ significantly as determined by Duncan's multiple range test (P<0.05) among the treatments*

Table 2. Antifungal activity of *S. potatorum* alkaloid fractions.

Drug	Concentration	Zone of inhibition diameter, mm of fungi			
		A. niger	*A. fumigatus*	*C. albicans*	*M. gypseum*
Nystatin	I	24 (±0.58)*[e]	17 (±0.58)[e]	20.33 (±0.88)[c]	14 (±0.58)[b]
Brucine	I	18 (±0.58)[b]	12.33 (±0.33)[b]	13.67 (±0.33)[a]	19.33 (± 0.88)[d]
Total alkaloids	I	16.67 (±0.33)[a]	13.33 (±0.33)[c]	14.67 (±0.33)[a]	17.67 (± 0.88)[c]
PB-I	I	15.33 (±0.33)[a]	11.67 (±0.33)[b]	14 (±0.58)[a]	-
	II	21 (±0.58)[d]	14.33 (±0.33)[c]	20.67 (±0.67)[c]	14.67 (± 0.88)[a]
PB-II	I	16 (±0.58)[a]	14.33 (±0.33)[c]	14 (±0.58)[a]	13 (± 0.58)[a]
	II	20.33 (±0.33)[c]	17.67 (± 0.88)[e]	17.67 (±0.33)[b]	16.67 (±0.33)[c]
PB-III	I	16.67 (±0.33)[a]	15.33 (± 0.33)[d]	13 (± 0.58)[a]	12.67 (± 0.33)[a]
	II	20 (± 0.58)[c]	17 (±0.58)[e]	19 (±0.58)[c]	16 (± 0.58)[c]
PB-IV, Diaboline	I	16.33 (±0.33)[a]	9 (±0.58)[a]	13.67 (± 0.33)[a]	14.67 (± 0.33)[b]
	II	19.33 (±0.33)[c]	14.33 (±0.67)[c]	18.33 (±0.88)[b]	16.67 (± 0.33)[c]
PB-V	I	15.33 (±0.33)[a]	11.33 (±0.33)[b]	14 (±0.58)[a]	16 (± 0.58)[c]
	II	17 (± 0.58)[b]	14.33 (± 0.33)[c]	19.33 (±1.20)[b]	21 (± 0.58)[e]
PB-VI	I	16 (± 0.58)[a]	12.33 (± 0.33)[b]	13.67 (±0.67)[a]	12.33 (± 0.33)[a]
	II	17.67 (±0.33)[b]	14.33 (± 0.33)[c]	19 (±0.58)[c]	14.67 (± 0.33)[b]
PB-VII	I	16.67 (±0.33)[a]	13.33 (±0.33)[c]	14.67 (±0.33)[a]	-
	II	20 (± 1.0)[c]	15.33 (±0.33)[d]	21 (±0.58)[d]	17.33 (± 0.33)[c]

I: 100 μg/mL; II : 200 μg/mL (w/v) ; '- ' No inhibition.

Table 3. *In vitro* antimycobacterial activity of *S. potatorum* alkaloid fractions.

Drug	Minimum inhibitory concentrations, μg/mL		
	M. tuberculosis	*M. tuberculosis* H37Ra	*M. smegmatis*
Ethambutol	ND	50	-
Rifampicin	6.25	3.125	25
Brucine	25	50	100
Total alkaloids	25	25	100
PB – I	50	100	100
PB – II	50	50	-
PB – III	25	100	-
PB – IV, Diaboline	12.5	50	50
PB – V	12.5	50	100
PB – VI	50	-	-
PB - VII	100	-	-

'ND': Not done '-' : No inhibition.

Diaboline (PB-IV) and fraction PB-V were proved to be more effective against *M. tuberculosis* (12.5 μg/mL). On the other hand, the total alkaloid extract exhibited variable effect on the tested microorganisms. It may be because of intrinsic synergistic and/or antagonistic behaviour of these fractions when they are in a mixture.

Conclusion

It is evident from literature that alkaloids isolated from plants have considerable biological activity[1,2]. Similar observations were made by Caron *et al.*[14] of indole alkaloids for their antimicrobial properties[14] and antifungal properties[15], isolated from various *Strychnos* species. Further, dolichantoside and palicoside, the glucoindole alkaloids of *S. melladora* have

shown significant antimycotic activity against *Candida* species[16]. The mode of action of indole alkaloids is understood to some extent, and has studied the action of cryptolepine, serpentine and matadine, which stimulate topoisomerase II mediated DNA cleavage, where these alkaloids tightly bind to DNA specifically at GC-rich sequences and behave as typical intercalating agents[17,18]. These findings have further consolidated the wide use of *S. potatorum* seed in treating of various microbial infections both in traditional (Ayurveda, Unani and Siddha) and folk medicine. From the results obtained, it may be concluded that the antimicrobial activity of these alkaloid fractions has a broad spectrum and is relatively species specific.

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References

1. Cowan M M, *Clin Microbiol Rev.,* 1999, **12,** 564 - 582.
2. Okunade A L, Elwin-Lewis M P F and Lewis W H, *Phytochemistry,* 2004, **65**, 1017-1032.
3. Bisset N G, The Asian species of *Strychnos* Part III: The Ethnobotany *Lloydia.,* 1974, **37,** 62-107.
4 Annalakshmi G, *Amruth*, 2003, **7,** 35-36.
5. Kirtikar K R and Basu B D, Indian Medicinal Plants. 3rd Ed., Vol. **7.** Published by Sri Satguru Publications, Delhi, India, 1998, 2265-2269.
6. Seetharam Y N, Kotresha K and Uploankar S B, Flora of Gulbarga District, Gulbarga University Press, Gulbarga., 2000,160.
7. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare, Published by the Controller of Publications, Delhi, 1996, **2**. 218.
8. Inderieid C B and Salfinger M, Antimicrobial agents and susceptibility tests, mycobacteria In: Manual of Clinical Microbiology. Edited by Murray PR *et al.,* (6th Ed.), ASM Press, Washington, D. C.1995, 1385 - 1404.
9. Duncan D B, *Biometrics,* 1955, **11,** 1- 42.
10. Singh H, Kapoor V K, Phillipson J D and Bisset N G, *Phytochemistry,* 1975, **14**, 587-588.
11. Kapoor V K, Sharma S K, Chagti K K and Singh M, *Indian J Chem.*, 1988, **27,** 641- 644.
12. Massiot G, Thepenier P, Jacquier M J, Men Oliver L L, Delaude C and Massiot G, *Phytochemistry,* 1992, **31,** 2873- 2876.
13. Mallikharjuna P B, Rajanna L N, Seetharam Y N and Sharanabasappa S K, *E J Chem.,* 2007, **4,** 510-518.
14. Caron C, Hoizey M J, Le Men Oliver L, Massiot G, Zeches M, Choisy C, Le Magrex E and Verpoorte R, *Planta Med.,* 1988, **54**, 409-412.
15. Quetin-Leclercq J, Favel A, Balansard G, Regli P and Angenot L, *Planta Med.,* 1995, **61**, 475-477.
16. Brandt V, Tits M, Penelle J, Frederich M and Angenot L, *Phytochemistry,* 2001, **57**, 653.
17. Bonjean K, De Pauw Gillet M C, Defresne M P, Colson P, Houssier C, Dassonneville L, Bailly C, Gremiers R, Wright C, Quetin-Leclercq J, Tits M and Angenot L, *Biochemistry,* 1998, **37,** 5136-5146.
18. Dassonneville L, Bonjean K, DePauw-Gillet M C, Colson P, Houssier C, Quetin-Leclercq J, Angenot L and Bailly C, *Biochemistry,* 1999, **38**, 7719 - 7726.