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BIOAUTOGRAPHY SCREENING OF *Strychnos potatorum* LEAVES AND BARK

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ABSTRACT

Strychnos potatorum leaf and bark were tested for their antibacterial properties against some pathogenic gram positive and gram negative bacteria. The growth of *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Escherichia coli* were significantly inhibited. The maximum zone of inhibition were found in *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. These findings have confirmed the use of this plant in treating of several bacterial infections both traditional and folk medicine in India.

Key words: *Strychnos potatorum*, Pathogens, Antibacterial activity.

INTRODUCTION

Strychnos potatorum is a medium sized glabrous and deciduous tree occurs both in the tropic and sub – tropics of north and south – east parts of Africa and India, Peninsula, Srilanka and Myanmar of Asia. The bark is cracked and scaly, leaves are opposite simple entire, stipules are absent (Mallikharjuna PB and Seetharam YN, 2009). Inflorescence is an axillary lax or congested thyrsuse. Flowers are bisexual ovary superior, fruits is a globose berry with 10 to 25 mm diameter (Sanmugapriya E and Venkataraman S, 2007). 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, gonorrhea and kidney troubles in Unani and for the leucorrhoea tuberculosis, venereal diseases and acute diarrhea in siddha medicine. 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.

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MATERIAL AND METHOD

Plant Sample Collection

The plant samples were collected from Velur, Pudukottai district. The leaves and bark were separated from the collected plant and dried under shade. After drying it was powdered and used for our studies.

Preparation of Plant Extract

The different parts of *Strychnos potatorum* plant bark leaf were collected and dried at temperature for 2 – 3 days and further dried at 60° C. The dried bark leaf was extracted with solvents. Ethanol, Chloroform separately and incubated at room temperature for 48 hours with stirring at regular interval. The extracts were filtered with the Whattman filter paper 41 and then dried by using rotary evaporator. The filtrate was stored in screw cap bottle at -20° C for further use.

Microbial Strains Used

Different microbial strains were used to evaluate the antimicrobial effect of which two were gram positive bacterial strains (i.e.) *Staphylococcus aureus, staphylococcus epidermidis*, and three strains were gram negative bacterial strains (i.e.) *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae*. The strains were obtained from Jamal Mohamed College, Trichy, Tamil Nadu, India and maintained agar slants (Venkatesh A and Silambujanaki P, 2011).

Disc Diffusion Method

Disc diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant extract (Ravikumar, 2011; Kim, 1999; Nusrat S, 2008). Muller Hinton agar (MHA) plates were prepared. Overnight nutrient broth culture of test organisms were seeded over the MHA plates using sterile cotton swab so as to make lawn culture. The discs which had been impregnated with aqueous extracts of leaf were placed on the MHA with the control disc and subjected to antibacterial screening. The plates were then incubated at 37° c for 18 to 24 hours depending on the species of bacteria used in this test. After the incubation, the plates were examined for inhibition zone.

Chi – Square Test (x^2)

In this study chi - square test (x^2) was applied. The purpose of chi – square test (x^2) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002).

RESULTS

The result of the antibacterial activity of the different parts of the *Strychnos potatorum* solvent extracts is given in Table 1, 2, 3, and 4. The solvent were prepared as different concentrations compared with all the concentration 128 µg/ml. Concentration gave the maximum zone of inhibition for all the extracts. Among the different parts of the *Strychnos potatorum*, the leaf and bark produced best antibacterial activity. Polar extracts like aqueous and ethanol to be the best solvent for extraction antibacterial compounds from the *Strychnos potatorum* plants. The maximum zone of inhibition was found in leaf extract and least with bark extract.

Best zone of inhibition was produced by the ethanol leaf extract *Pseudomonas aeruginosa* (30 mm), and least was produced against *Klebsiella sp.,* (16 mm). The aqueous extract revealed that maximum zone of inhibition was produced Pseudomonas aeruginosa (35 mm), and least was produced against *Klebsiella sp.*, (17 mm). Non – polar extracts like chloroform and petroleum ether to be the best solvent for extraction of antibacterial compounds from the plants. The maximum zone of inhibition was found in petroleum ether leaf extract when compared with chloroform leaf extract. Best zone of inhibition was by petroleum ether leaf extract *Pseudomonas aeruginosa* (40 mm), and least was produced against *Klebsiella sp.,* (18 mm). The chloroform leaf extract revealed that maximum zone of inhibition was produced *Pseudomonas aeruginosa* (32 mm), and least was produced against *Klebsiellasp.,* (16 mm).

Best zone of inhibition was produced by ethanol bark extract *Escherichia coli* (18 mm), and least was produced against *Klebsiella sp.,* (16 mm). The aqueous extract revealed that maximum zone of inhibition was produced *Staphylococcus epidermidis*(21 mm), and least was produced against *Pseudomonas aeruginosa* (16 mm). The chloroform bark extract revealed that maximum zone of inhibition was produced *Staphylococcus epidermidis* (26 mm), and least was produced against *Klebsiella sp.,* (18 mm). The maximum zone of inhibition was found in petroleum ether bark extract Proteus vulgaris (22 mm), and least was produced against *Staphylococcus aureus* (18 mm).

Overall, the antibacterial activity of the *Strychnos potatorum* revealed that the best antibacterial activity was produced by leaf extract followed by bark extract. All the extracts produce better zone of inhibition against *Pseudomonas aeruginosa* (40 mm) and *Staphylococcus epidermidis* (26 mm) and the least zone of inhibition against *Klebsiella sp.,* (16 mm). In the present study to analyse the solvent extracts using both polar and non – polar extracts. The bark gave the maximum zone of inhibition in the non – polar extracts (chloroform). The leaf gave the maximum zone of inhibition in the non – polar extracts (petroleum ether).

DISCUSSION

In earlier studies Mallikarjuna *et al.*, reported the antimicrobial activity in alkaloid fractions isolated from *Strychnos potatorum* seeds against some pathogenic gram positive, gram negative, 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 anti - microbial activity of this plant seeds.

Table 1. Antibacterial activity polar extract of *Strychnos potatorum* leaf powder (zone of inhibition in mm)

S. No	Sample	µg/ml	Bacterial strains used	Aqueous		$X^{2=\Sigma}$ [(O-E)2]/E	Ethanol		$X^{2=\Sigma}$ [(O-E)2]/E
				Standard value	Observed value		Standard value	Observed value	
1	*Strychnos potatorum* leaf powder	128µg	*E. coli*	22	18	0.727	22	18	0.727
2			*Klebsiella sp.,*	22	17	1.136	22	16	1.636
3			*P. vulgaris*	22	22	0	22	21	0.045
4			*P. aeruginosa*	22	35	7.681	22	30	2.909
5			*S. aureus*	22	20	0.181	22	20	0.181
6			*S. epidermidis*	22	20	0.181	22	19	0.409

Table value x^2 (0.05) = 3.841, Chi – square value significance at 5% level

Table 2. Antibacterial activity Non - polar extract of *Strychnos potatorum* leaf powder (zone of inhibition in mm)

S. No	Sample	µg/ml	Bacterial strains used	Chloroform		$X^{2=\Sigma}$ [(O-E)2]/E	Petroleum ether		$X^{2=\Sigma}$ [(O-E)2]/E
				Standard value	Observed value		Standard value	Observed value	
1	*Strychnos potatorum* leaf powder	128µg	*E. coli*	22	18	0.727	22	20	0.181
2			*Klebsiella sp.,*	22	16	1.636	22	18	0.727
3			*P. vulgaris*	22	20	0.181	22	25	0.409
4			*P. aeruginosa*	22	32	4.545	22	40	14.727
5			*S. aureus*	22	18	0.727	22	23	0.045
6			*S. epidermidis*	22	18	0.727	22	22	0

Table value x^2 (0.05) = 3.84, Chi – square value significance at 5% level

Table 3. Antibacterial activity of polar extract of *Strychnos potatorum* bark powder (zone of inhibition in mm)

S. No	Sample	µg/ml	Bacterial strains used	Aqueous		$X^{2=\Sigma}$ [(O-E)2]/E	Ethanol		$X^{2=\Sigma}$ [(O-E)2]/E
				Standard value	Observed value		Standard value	Observed value	
1	*Strychnos potatorum* bark powder	128µg	*E. coli*	22	20	0.181	22	16	1.636
2			*Klebsiella sp.,*	22	17	1.136	22	14	2.909
3			*P. vulgaris*	22	21	0.045	22	16	1.636
4			*P. aeruginosa*	22	16	1.636	22	17	1.136
5			*S. aureus*	22	20	0.181	22	17	1.136
6			*S. epidermidis*	22	18	0.727	22	18	0.727

Table value x^2 (0.05) = 3.841, Chi – square value significance at 5% level

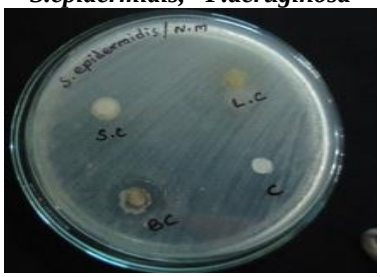
Table 4. Antibacterial activity of Non - polar extract of *Strychnos potatorum* bark powder (zone of inhibition in mm)

S. No	Sample	µg/ml	Bacterial strains used	Chloroform		$X^{2=\Sigma}$ [(O-E)2]/E	Petroleum ether		$X^{2=\Sigma}$ [(O-E)2]/E
				Standard value	Observed value		Standard value	Observed value	
1	*Strychnos potatorum* bark powder	128µg	*E. coli*	22	18	0.727	22	22	0
2			*Klebsiella sp.,*	22	18	0.727	22	20	0.181
3			*P. vulgaris*	22	24	0.181	22	22	0
4			*P. aeruginosa*	22	20	0.181	22	20	0.181
5			*S. aureus*	22	22	0	22	18	0.727
6			*S. epidermidis*	22	26	0.727	22	20	0.181

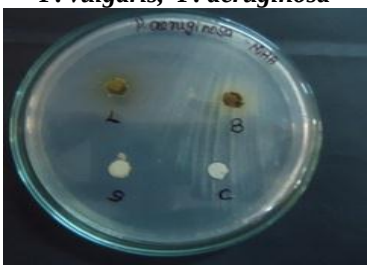
Table value x^2 (0.05) = 3.84, Chi – square value significance at 5% level

Figure 1. Zone Inhibition formed by Polar and Non – polar extract of *Strychnos potatorum* Leaf and bark

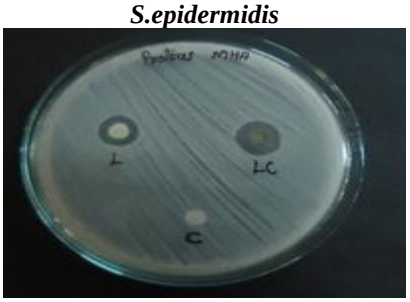
S.epidermidis, P.aeruginosa



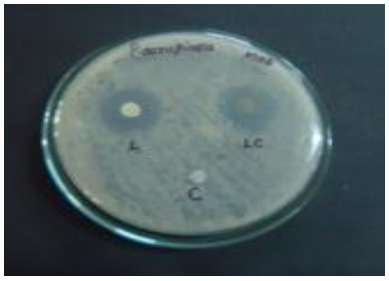
P. vulgaris, P. aeruginosa



S.epidermidis



E.coli



Followed by Panduraju *et al.,* reported the antidiabetic activity in different solvent extracts of seeds and leaves of *Strychnos potatorum*. The administration of methanolic extracts of seeds and leaves of *Strychnos potatorum* by oral route at doses; 200 and 400 mg/kg b.w at every 3 hour interval reduced blood glucose levels by 24.23%, 25.67%, 16.47%, and 17.88% , respectively in alloxan – induced diabetic rats.

In previous research Sanmugapriya and Venkataraman (2007) reported the anti – ulcer activity of *Strychnos potatorum* seeds on aspirin plus pyloric

ligation (Aspirin+PL) induced gastric ulcer model. The result indicate that seed powder and aqueous extract exhibit anti ulcerognic activity by both antisecretory and mucoprotective actions.

In the present study to analyse the solvent extracts using both polar and non – polar extracts. The bark gave the maximum zone of inhibition in the non – polar extracts (chloroform). The leaf gave the maximum zone of inhibition in the non – polar extracts (petroleum ether). The petroleum ether leaf extract gave the maximum zone of inhibition against *Pseudomonas aeruginosa* (40 mm) and the chlorofrom bark extract gave the maximum zone of inhibition against the *Staphylococcus epidermidis* (26 mm).

CONCLUSION

The present study showed the antibacterial activity of the leaf and bark extracts from various solvents of *Strychnos potatorum* against pathogenic organisms. Hence this plant can be used to cure the infection caused by the treated strains. Further studies are needed to isolate pure compounds from this plant extract and to establish the mode of action of the isolated compounds.

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