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In Vitro Antibacterial activity and Phytochemical screening of *Strychnos potatorum* seed extract

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ABSTRACT

Biologically active compounds in plant extracts play an important role in herbal medicine through their antimicrobial properties. Strychnos potatorum belongs to the family Loganiaceae. The seed is primarily used in the Indian traditional systems for several treatments including microbial infections. It is used in Ayurveda for treating the eye and urinary tract infections and in Unani for gonorrhoea and kidney troubles ,leucorrhoea, tuberculosis, venereal diseases and in Siddha medicine for acute diarrhea. Present study was to determine the In Vitro antibacterial activity of different seed extracts of Strychnos potatorum and test for the presence of phytochemical constituents. Cold, hot and ethanol seed extracts of Strychnos potatorum were tested for In Vitro antibacterial activity against Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 , E coli ATCC 25922 and Enterococcus faecalis ATCC 29212 by using the standard agar well diffusion method. Distilled water and ethanol as solvent were used as control. Qualitative phytochemical analysis of extracts was also carried out for the presence of bioactive compounds using standard procedures. Results indicated that Cold, hot and ethanol extracts of Strychnos potatorum seed exhibited antibacterial activity among the tested bacteria. Cold seed extract failed to inhibit the growth of Staphylococcus aureus and Pseudomonas aeruginosa where as hot seed extract did not inhibit only the growth of Pseudomonas aeruginosa. But ethanol seed extract inhibited the growth of gram positive and all gram negative bacteria. Growth of Enterococcus faecalis(35.67±1.15) and E.coli (21.67±0.354)was signif icantly inhibited by ethanol seed extract rather than the growth of Staphylococcus aureus(16.24±0.653). Hot se ed extract significantly inhibited the growth of Enterococcus faecalis(22.67±1.15). Phytochemical analysis indica ted that Terpenoids and Saponins were present in all three extracts. Steroids and Flavanoids were only absent in hot extract. But ethanol extract contained all the tested phytochemical constituents. Further studies on sequential extraction and purification of antibacterial compounds in seeds could be developed.

Key words: *Strychnos potatorum* seed, Antibacterial activity, agar well diffusion method

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INTRODUCTION

Biologically active compounds in plant extracts play an important role in herbal medicine through their antimicrobial properties. *Strychnos potatorum* belongs to the family Loganiaceae, which are medium sized (~15m) and much branched glabrous tree[1]. Seeds are alterative tonic, stomachic, demulcent, mild expectorant, astringent to the bowels, and also have diuretic, aphrodisiac, emetic properties[1,2]. The seed is primarily used in the Indian traditional systems for several treatments including microbial infections. It is used in Ayurveda for treating the eye and urinary tract infections[2], and in Unani for gonorrhoea and kidney troubles ,leucorrhoea, tuberculosis, venereal diseases and in Siddha medicine for acute diarrhoea [3,4]. The ripe seeds are used for clearing muddy water. Moreover, alkaloids, the prime source of secondary metabolites isolated from several *Strychnos* species are known for their therapeutic importance. The seeds are non poisonous which are taken in various forms as pickle, sampol, decoction & involve in other preparation methods for preventive and curative and palliative treatment[7]. In traditional preparations water is mainly used as solvent. Objective of this study was to determine the *In Vitro*

antibacterial activity of different seed extracts of *Strychnos potatorum* and test for the presence of phytochemical constituents.

MATERIALS AND METHODS

Preparation of seed extracts
The healthy plant seeds were collected from botanical garden of the Unit of Siddha Medicine, University of Jaffna, Sri Lanka. They were dried in shade and ground into fine powder.

Hot extract preparation
40 gm of powered seed was transferred into a conical flask with 640ml of distilled water(16 times). Solution was boiled well until the volume was reduced to 80ml(1/8 times). It was allowed to cool at room temperature.

Cold extract preparation
4 gm of powered seed was transferred into 50ml of distilled water in a conical flask. It was mixed well by the shaker with 400 rpm for 5minutes and filtered by Whatman No 1 filter paper. Filtrate was collected in airtight bottle[5].

Preparation of direct ethanol extract
20gm powered seed was soaked in 60ml of ethanol in airtight conical flask for two days on an orbital shaker and it was filtered through double layered muslin cloth followed by Whatman No 1 filter paper. Filtrate was collected in airtight bottle. The above process was repeated twice with fresh ethanol and the filtrates were pooled together. Finally ethanol was removed from the filtrate at 40^oC by keeping in an oven[6].

Test microorganisms
Staphylococcus aureus ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 , *E coli* ATCC 25922 *and Enterococcus faecalis* ATCC 29212 were obtained from the culture collections of the Department of Botany, University of Jaffna, Sri Lanka.

Determination of antibacterial activity by agar well diffusion method
Young bacterial cultures were obtained by subculturing on Nutrient Agar medium and bacterial suspensions were prepared separately by using sterile saline solution. Bacterial cell concentration was adjusted to 10^8cells /ml with a help of the MacFarland 0.5M standard solution. Pour plate technique was carried out to prepare nutrient agar plates with 1ml of bacterial suspension separately. After complete solidification, 9mm diameter wells were made using a sterile cork borer. Cut wells were filled with different extracts separately with a help of a sterile micro pipette. Distilled water and ethanol were used as controls. Plates were incubated at 37^oC for 24 hours and zones of inhibition were measured(mm). Replicates were also made[8,9].

Phytochemical analysis
Qualitative analysis were carried out using standard procedures to identify the following components [10, 11,12,13].

Test for Tannins
About 0.01 g of the crude extract was boiled in 20 ml of water in a boiling tube. Few drops of 0.1% of $FeCl_3$ were added. Formation of brownish green or a blue black coloration indicated the presence of tannins.

Test for Saponins
About 0.01g of the crude extract was boiled in 20 ml of distilled water in a water bath. Then it was mixed with 5 ml of distilled water and it was shacked well. Stable persistent forth indicated the presence of saponins.

Test for Phlobatanins
About 0.01 g of the crude extract was boiled with 1% aqueous hydrochloric acid. A deposition of a red precipitate indicated the presence of phlobatanins.

Test for Flavanoids
About 0.01 g of the crude extract was dissolved in 2 ml of ethanol solvent. Con.HCl and Mg turnings were added. A yellow coloration in extract indicated the presence of flavanoids.

Test for Steroids
About 0.01g of the crude extract was dissolved in 2ml of ethanol solvent. 2ml of acetic anhydride and 2ml of con.H_2SO_4 were added. The colour change from violet to blue or green indicated the presence of steroids.
Test for Cardiac glycosides

0.01g of crude extract was dissolved in 2ml of ethanol and then 2ml of glacial acetic acid which contained one drop of $FeCl_3$ solution was added. This was underplayed with 1ml of con H_2SO_4. A brownish ring of the interface indicated the presence of cardiac glycosides.

Test for alkaloids
About 0.01 g of crude extract was dissolved in 2ml of ethanol and it was divided into two parts. Few drops of Wagner's reagent along the wall of the test tube were added to one part. Brownish red precipitate indicated the presence of alkaloids.

Few drops of Mayer's reagent were added to the other part. A creamy white precipitate observed in extract indicated the presence of alkaloids.

Test for Terpenoids
5ml of crude extract was treated with 2ml of $CHCl_3$ and 3ml of con H_2SO_4 by adding carefully to from a layer. A reddish brown colouration of interface indicated the presence of terpenoids.

Statistical analysis
Data were analyzed by ANOVA ($P<0.05$) and the mean values were compared by using Least Significant Difference test ($\alpha=0.05$) using software, the SPSS system for windows (Version 13.0).

RESULTS AND DISCUSSION

Table1: Zone of inhibition(mean±SD) of the seed extracts of *Strychnos potatorum* against bacteria

Seed extract	*E.coli* ATTC25922	*S.aureus* ATTC25923	*P.aeruginosa* ATTC27853	*E.faecalis* ATTC29212
Cold extract	10.34±0.234^{c}	-	-	10.57±0.789^{c}
Hot extract	15.33±0.207^{b}	12.33±0.582^{b}	-	22.67±1.15^{b}
Ethanol extract	21.67±0.354^{a}	16.24±0.653^{a}	10.98±0.865	35.67±1.15^{a}
Ethanol	-	-	-	-
Distilled water	-	-	-	-

(-) No activity: Values are mean± SD: Values with different superscript on the same column show significant ($P<0.05$) difference: Zone of inhibition includes the diameter of the well (9 mm in diameter)

Cold, hot and ethanol extracts of *Strychnos potatorum* seed exhibited antibacterial activity among the tested bacteria. Cold seed extract failed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* where as hot seed extract did not inhibit only the growth of *Pseudomonas aeruginosa*. But ethanol seed extract inhibited the growth of gram positive and all gram negative bacteria. Previous study also stated that the alcoholic extract of *Strychnos potatorum* showed antimicrobial action against pathogenic, gram positive, gram negative and acid fast bacteria and fungi[14]. Another study indicated that the seed column compounds inhibited the growth of *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis* and *E.coli* significantly[15].

Significant difference was also observed on the growth inhibition of bacteria among the three seed extracts. The ethanol seed extract showed the highest degree of inhibition on bacterial growth. Methanol leaf extract of *Strychnos potatorum* exhibited antimicrobial activity was also reported[16]. Further alkaloids fractions isolated from seeds significantly inhibited the growth of *Proteus vulgaris, Staphylococcus aureus, Salmonella typhimurium, Vibrio cholera, Mycobacterium tuberculosis, Aspergillus niger* and *Candida albicans*[7]. But in this study growth of *Enterococcus faecalis* and *E.coli* was significantly inhibited by ethanol seed extract rather than the growth of *Staphylococcus aureus*. Hot seed extract significantly inhibited the growth of *Enterococcus faecalis* and the growth of *Pseudomonas aeruginosa* was only inhibited by ethanol seed extract.

Table 2. Qualitative Phytochemical tests for chemical constituents

Test	Seed extracts of *Strychnos potatorum*		
	Cold	Hot	Ethanol
Tannins	_	+	+
Saponins	+	+	+
Phlobatanins	_	+	+
Steroids	_	_	+
Flavanoids	_	_	+
Terpenoids	+	+	+
Alkaloids	_	+	+
Cardiac glycosides	_	+	+

Note: + present ; - absent

Phytochemical analysis indicated the presence of various types of phytochemicals in three different seed extracts of *Strychnos potatorum*. Terpenoids and Saponins were present in all three extracts. Steroids and Flavanoids were only absent in hot extract. But ethanol extract contained all the tested phytochemical constituents. But previous studies on phytochemical screening showed that the methanol extract of leaf of *Strychnos potatorum* had Alkaloids, Glycosides, Saponins and Flavanoids. Seed aqueous extract of *Strychnos potatorum* contained Alkaloids, Glycosides, Saponins ,Flavanoids, Tannins, Phenolic compounds, Protein and Carbohydrates ,but Oil, Fats, Gums and Mucilage were absent. Variation in the results of these compounds was determined by the plant type, plant parts and the mode of extraction[17].

CONCLUSION

This study revealed that different seed extracts exhibited different degree of antibacterial activity among tested bacteria. Cold seed extract failed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* where as hot seed extract did not inhibit only the growth of *Pseudomonas aeruginosa*. But ethanol seed extract inhibited the growth of gram positive and all gram negative bacteria and showed the highest degree of inhibition on bacterial growth. Various types of phytochemicals were present in three different seed extracts of *Strychnos potatorum*. Terpenoids and Saponins were present in all three extracts. Steroids and Flavanoids were only absent in hot extract. But ethanol extract contained all the tested phytochemical constituents. Studies on sequential extraction and purification of antibacterial compounds in seeds could be developed.

REFERENCES

[1] D.M.A.Jayaweera; Medicinal Plants, A National Science Foundation, Sri Lanka, **2006**,3,245.
[2] N.G.Bisset; The Asian species of *Strychnos* Part III, The Ethnobotany Lloydia,**1974**,37,62-107.
[3] G.Annalakshmi,Clearing Nut,An Annotated Bibliography of Indian Medicine, Amruth, **2003**, 7,6**,** 35-36.
[4] K.R.Kirtikar, B.D.Basu;Indian Medicinal Plants. 3rd Edition, Sri Satguru Publications, Delhi, India, **1998**,7**,** 2265-2269.
[5] S.Tharmila, T.Thileepan, A.C.Thavaranjit,R. Srikaran, Sri Lanka Journal of Indigenous Medicine.,**2011**, 1(2),83-85.
[6] E.C. Jeyaseelan, S. Kothai, R. Kavitha, S.Tharmila ,A.C. Thavaranjit, *International Journal of Pharmaceutical & Biological Archives*., **2012**; 3(2),343-347.
[7] P.B. Mallikharjuna, Y.N. Seetharam, *E-Journal of Chemistry*., **2009**,6(4), 1200-1204.
[8] L. Prince, P.Prabakaran, *Asian Journal of Plant Science and Research*., **2011**, 1(1), 84-87.
[9] A.M.Clark, and F.S. El-Feraly, *Journal of Pharmaceutical Sciences*., **1981**, 70. 951-952.
[10] F.J.A. Matos; Introducaoa fitoquimica experimental. Imprensa Universitaria, Universidade Federaldo ceara,**1988**, Folrtaleza,128.
[11] A.E. Sofowara; Medical Plants and traditional medicine in Africa. Part 11, Pitman Press Ltd, London, **1982**,128-146.
[12] G.E.Treas, W.C.Evans; A text book of Pharmacognosy. 12th Edition, Bailliere Tinall Ltd, London,**1989**, 333-337.
[13] J.B.Harbone; Phytochemical methods. A guide to modern Techniques of plant analysis, Chapman and Hall, London, **1973**,199-204.
[14] K.N. Yadav, P.V. Kadam, J.A.Patel, M.J. Patil, *Pharmacogn rev*., **2014**,8(15),61-66.
[15] N. Packialakshmi, C. Suganya, V.Guru, *International Journal of Research in Pharmaceutical and Nano Sciences*.,**2014**,3(5),380-396.
[16] M. Amman, R.Rai, P.V. Samaga, *Medicinal and Aromatic Plant Science and Biotechnilogy*., **2010**,4(1), 69-72.
[17] K. Sathyaa, T. Manoranjan, A.C. Thavaranjit, J.P. Jeyadevan, *Der pharma chemica*., **2015**,7(6), 282-286.