



Research Article

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Antibacterial and antifungal activity of *Strychnos nux vomica* seed extract

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ABSTRACT

Antimicrobial activity of the ethanolic extract of *Strychnos nux vomica* seeds was studied. The invitro antimicrobial activity was performed by agar disc diffusion method. The most susceptible bacteria gram positive bacteria identified was *Staphylococcus aureus* and gram negative bacteria's were *Escherhia coli* and *Klebsiella pneumoniae*. All the fungal species showed resistance to the extract. The effects of extract on these microbes were compared against the standard antibiotics such as ampicillin and amphotericin with 20µg/disc. In this study, *Strychnos nux vomica* seed extracts showed maximum inhibition against the gram negative bacteria and less or no inhibition against the fungal organisms tested. From this study it was identified that ethanolic seed extracts of this tree could be used to control the above said microbes.

Keywords: antimicrobial activity, *Strychnos nux vomica*

INTRODUCTION

Eighty percentage population of the developing countries use traditional medicine for their primary health care needs as reported by World Health Organization[1].Rural India is known for its use of raw plant products and its juices because of it availability and popularity as medicine in practices of traditional medicine system[2].About 45,000 species of wild plants, out of which 7500-8000 species of medicinal plants are used in tribal healthcare needs whereas only 1500 plants are in use in Indian Ayurvedha, Unani and Siddha systems of medicine [2].It is time to evaluate the plant products used by our people in traditional medicine scientifically to prove its efficacy and use. As deforestation for reasons like, a) increasing human invasion on the forests for settlement, b) forest fires c)construction of Dams are on the rise, continual survey for record of traditional medicinal knowledge is to be done to safeguard the knowledge earned by our ancestors towards the cure of disease.

Resistance of pathogenic bacteria to antibiotics is of great clinical concern these days. Evolution of multidrug resistant bacteria like *Staphylococcus aureus* has attracted concern from people in healthcare who donot have a single drug to treat patients infected with these microbes. Drug resistant strains of *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Neisseria*, *Salmonella*, *Haemophilus* and few more have evolved[3].Failure of drugs in treatment of these multi drug resistant strains of bacteria has caused a global search for new drugs from natural resources like plants and plant products [4,5].

Strychnos nux vomica belongs to the family Loganiaceae. It is an energetic poison affecting the central nervous system. It's a medicine for paralysis and nervous debility generally [6]. Traditionally it is used for treating acute diarrhoea, mixed with lemon juice and made into pills and taken orally during dysentery, arthritis, rheumatism and piles [7].

This paper is anticipated to provide with the scientific evidence of the ability of the *Strychnos nux vomica* seeds as an antibacterial and antifungal agent especially against few gram negative and gram positive bacteria.

EXPERIMENTAL SECTION

Plant material

Strychnos nux vomica seeds were collected from Tiruporur, Tamilnadu state in the month of July, 2013. Botanical identification was done by Prof. P.Jayaraman, Director, Plant Anatomy Research Centre, Medicinal plant research unit, West Tambaram, Chennai.

Sterilization of plant materials

About 25 grams of healthy seeds were taken and cleaned in running tap water and then soaked in 0.1% mercuric chloride for few seconds. Then the seeds are washed with distilled water three times and shade dried.

Preparation of seed extract

Extraction procedure

The dried seeds were then subjected to size reduction to get coarse powder of desired particle size. The coarse powder was then stored in a clean dry air tight container. The powdered material was first subjected to defatting by Soxhlet apparatus using petroleum ether for 15 hrs. Then the maceration was subjected to extraction by Soxhlet apparatus with ethanol for 48 hrs. The obtained extract was finally dried under reduced pressure in a rotary evaporator. Crude powder was obtained and used to prepare suspensions.

Microbial strains:

The following human pathogenic bacteria species *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Proteus*, *Salmonella typhi*, *E.coli* and fungal species of *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* were collected from Lifeteck research centre, Vadapalani, Chennai.

Antibacterial activity assay

Preparation of Inoculum

Stock cultures were maintained at 4°C on Nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated for 24hrs at 37°C. The assay was performed by agar disc diffusion method.

Agar disc diffusion method

Antibacterial activity of the extract was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium was poured in to the petriplates. After the medium has solidified, the inoculums were spread on the solid plates with a sterile swab moistened with the bacterial suspension. The discs were placed in MHA medium and added 20 µl of ampicillin in one disc, extract sample of concentrations with 1000µg, 750µg, 500µg in another three discs, and DMSO in the fifth disc. The plates were incubated for 24 hrs, at 37°C. Then the antimicrobial activity was determined by measuring the diameter of the zone of inhibition. Single sample was made with the different microorganisms such as *Staphylococcus aureus*, *Salmonella typhi*, *E.coli*, *Proteus*, *Klebsiella pneumoniae* and *Bacillus Subtilis*. Standard antibiotic Ampicillin (20µg/disc) served the control.

Antifungal activity assay

Preparation of Inoculum

Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing Sabourad Dextrose broth, that were incubated at 24hrs at 37°C. The assay was performed by agar disc diffusion method.

Agar disc diffusion method

Antifungal activity of the extract was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured into the petriplates. After the medium has solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The discs were placed in SDA plates and added 20 µl of Amphotericin, extract sample concentrations of 1000µg, 750µg, 500µg in another 3 discs and DMSO in the fifth disc. The plates were incubated for 24 hrs, at 37°C. Diameter of zone of inhibition determined the antifungal activity. Single sample was made with the three different fungi such as *Aspergillus Niger*, *Aspergillus Flavus*, and *Candida albicans*. Standard drug Amphotericin-B (20µg/disc) served the control.

RESULTS

Table 1 explains the antibacterial activity of the ethanolic seed extracts of *strychnos nux vomica* with differences in dose dependency. In comparison with that of the standard antibiotic used, ethanolic extract of the plant showed better zone of inhibition with a concentration of 1000 $\mu\text{g/ml}$ on the three bacterial samples namely *E.coli*, *Staphylococcus aureus* and *Klebsiella*.

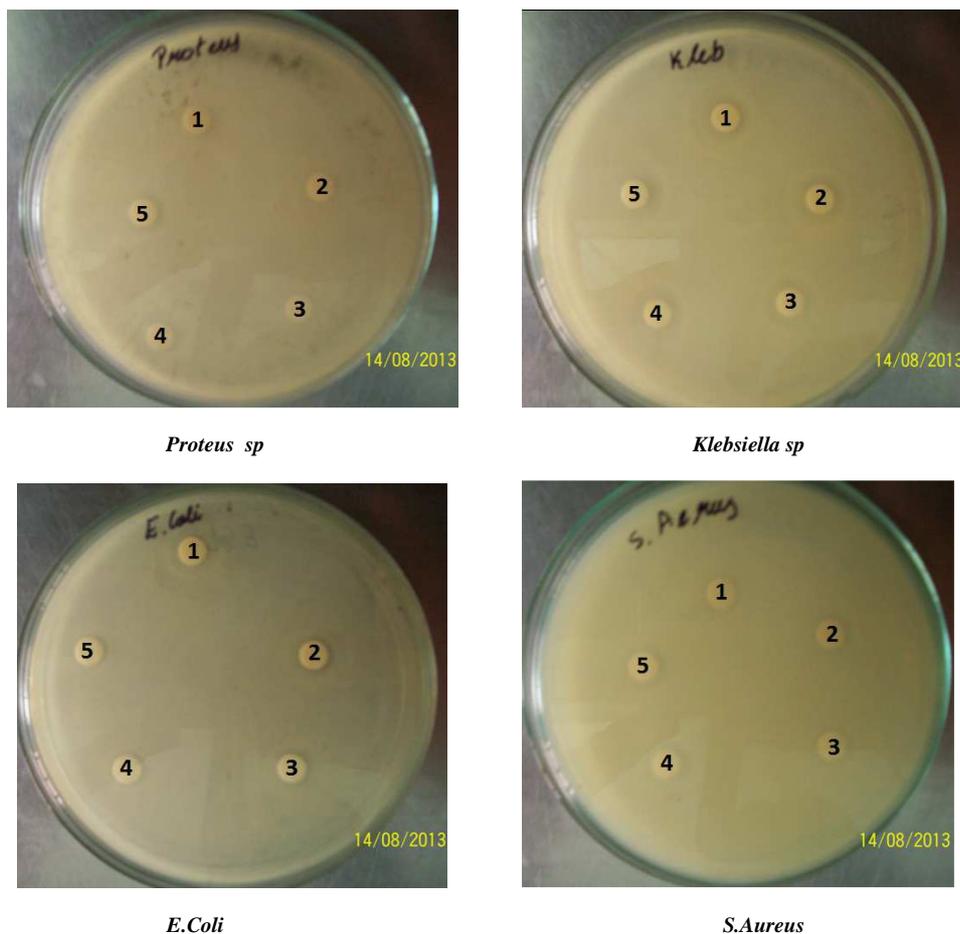
Table 2 showed the ineffectiveness of the plants extract on fungi with literally no antifungal activity.

Figure 1 shows the samples of bacterial strains in disc diffusion method with the crude extract loaded discs with different concentrations of it and the zone of inhibition it has exerted on the bacterial strains.

Table 1. Antibacterial activity

Sl.No	Microorganisms	Zone of inhibition in(mm)				
		Concentration ($\mu\text{g/ml}$)			Std	DMSO
		1000	750	500		
1.	<i>E.coli</i> g-	5.5	5	4.5	5	-
2.	<i>Salmonella typhi</i> g-	-	-	-	5	-
3.	<i>Proteus</i> g-	-	-	-	6	-
4.	<i>Staphylococcus aureus</i> g+	5	5	5	5	-
5.	<i>Klebsiella pneumoniae</i> g-	6	5.5	5	6	-
6.	<i>Bacillus Subtilis</i> g+	-	-	-	4	-

Figure 1. Antibacterial activity of ethanolic extract of *Strychnos nux vomica* seeds against selected bacterial samples



*S.Typhi**B.Subtilis***Denotations**

- 1- Sample of 1000ug conc.
- 2- Sample of 750ug conc.
- 3- Sample of 500ug conc.
- 4- Ampicillin-B (20 μ g/disc)
- 5- DMSO

Table 2. Antifungal activity

Sl.No	Microorganisms	Zone of inhibition in(mm)				
		Concentration (μ g/ml)			Std	DMSO
		1000	750	500		
1.	<i>A.Niger</i>	-	-	-	8	-
2.	<i>A.Flavus</i>	-	-	-	5	-
3.	<i>Candida albicans</i>	-	-	-	4	-

DISCUSSION

In this study we tried to explore the antibacterial and antifungal property of the ethanolic seed extracts of the *Strychnos nux vomica* seeds. Results showed antibacterial activity and poor antifungal activity by the ethanolic seed extract. Our study contradicted the earlier reports of Gnanavel et al (2012) in which herbal extracts inhibited only the gram positive bacteria[8]. Whereas in our study we got better inhibition of the seed extract over gram negative bacteria, which marks an important finding as we have proved it again that this plant has got lot of antibacterial activity. R. Mahalingam et al (2011) has proved the exhibition of antibacterial activity of ethyl-acetate and n-butanol root extracts of *Strychnos nux vomica* in which the zone of inhibition ranged from 13 -16mm against tested pathogens [9]. Antibacterial activity effect of ethanolic extract of bark of the *Strychnos nux vomica* was reported earlier, in which *Escherichia coli* was the organism which showed inhibition which was similar in our study also[10]. *Staphylococcus aureus*, *Acinetobacter* sp, *Klebsiella* sp, *P.aeruginosa*, *S.typhi*, *V.cholerae* microbial organisms have shown inhibition to ethanolic and water extracts of *Strychnos nux vomica* leaves and bark whereas *C.fereundii*, *C.violeceum*, *E.coli*, *Proteus* species didn't showed any inhibition as reported in an earlier study[7]. In our results we didn't see any antimicrobial activity of the ethanolic extract against *Bacillus subtilis*, *Salmonella typhi* and *Proteus*.

Antifungal activity against fungal samples like, *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* was not promising as we didn't see any inhibition activity. Previous reports on the same fungi using methanolic and aqueous extracts of *Strychnos nux vomica* leaves showed no antifungal activity, however n-butanol extract have showed promising results [8].

CONCLUSION

The potential of the seeds of *strychnos nux vomica* for developing antibacterial drugs is highly appreciated and it can lead to the development of phytomedicines for various strains of both gram positive and gram negative bacteria. Plant based antibacterial drugs have lesser side effects than synthetic. We conclude that the ethanolic extract of *strychnos nux vomica* seeds could be used as antimicrobial drugs. Future studies on this should be carried out to explore the other potential use of this plant product.

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