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Phytochemical analysis and *in-vitro* antibacterial activity of aqueous methanolic extract of *Strychnos nux vomica* leaves

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Abstract

Strychnos nux vomica is a non edible tree with a strong content of two poisonous alkaloids, strychnine and brucine. Qualitative phytochemical analysis and antimicrobial activity of the aqueous methanolic extract of its leaves were studied. Alkaloids, triterpenes, oils & fats, phenol, tannins and flavonoids were present. *Escherichia coli* Enterobacter, *Staphylococcus aureus*, and *Pseudomonas* showed susceptibility but *Salmonella typhimurium* survived the inhibition. Minimal inhibitory concentration of the extract for *E. coli* after an overnight incubation was found to be 0.25 mg. In the present study the antibacterial activity of *Strychnos nux vomica* leaves showed inhibitory effects on selective bacteria which could be used to control these microbes.

Keywords: *Strychnos nux vomica*, antibacterial activity, qualitative analysis.

1. Introduction

Phytochemicals occur in plants are responsible for the colour, flavor and smell of the plants. They bear therapeutic values for human health as reported in the indigenous system of medicine [1]. Plant extracts provide the innumerable opportunities for developing newer drugs for the existing as well as emerging diseases. Traditional use of plant remedies for number of ailments is seen across nations. *Strychnos nux vomica* is an evergreen tree native to South East Asia and India belonging to the family Loganiaceae. It is medium sized tree found mostly in open habitats. Two poisonous alkaloids, Strychnine and brucine are found in this tree [2]. Qualitative phytochemical analysis and antibacterial activity of the methanolic extract of leaves of *Strychnos nux vomica* was studied and its minimal inhibitory concentration against a specific bacterium was also documented.

2. Materials and methods

2.1 Plant collection

Strychnos nux vomica leaves were collected from Ammapettai village near 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.

2.2 Preparation of leaf extract

The fully matured plant leaves collected were separated and washed thoroughly with tap water, air dried, homogenized with aqueous methanol, filtered and stored in test tubes and covered with foil sheets.

2.3 Plant extract

100 grams of air dried leaves are homogenized with 200 ml of aqueous methanol, filtered and stored.

2.4 Phytochemical analysis

To know the compounds present in the aqueous methanol plant extract, the following test are conducted.

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2.4.1 Alkaloid test

Plant extract was treated with dil.HCL and filtered .The filtrate was taken to perform the following tests.

a. Wagner's reagent

A fraction of extract was treated with 3-5 drops of Wagner's reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] and observed for the formation of reddish brown precipitate (or colouration).

b. Hager's test

To the one ml of filtrate few drops of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate formed indicates the presence of alkaloids

2.4.2. Glycosides test

50 mg of the extract was hydrolyzed with dil. HCl, filtered and the filtrate is used to perform the Glycosides test.

a) Modified Borntragers test ^[3]

To the one ml of extract FeCl₃ solution is added and kept in boiling water bath for 5 minutes. The mixture was cooled, and equal volume of benzene was added and shaken well. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour indicates glycosides.

2.4.3. Saponin test

a. Foam test

To 2 ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

2.4.4. Phytosterol test

Extracts were treated with chloroform and filtered. The filtrate is used to perform the test.

a) Salkowski's test

The filtrate is treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Golden yellow colour develop indicates the presence of triterpenes.

2.4.5. Fixed oils and fats

a) Stain test

Small drops of extracts were pressed in between two filter papers. An oily stain on filter paper indicates fixed oils and fats.

2.4.6. Resin test

a) Acetone water test

Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

2.4.7. Phenol test

Ferric Chloride Test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.4.8. Tannin test

To the extract, 1% gelatin solution was added. White precipitate formation indicates the presence of tannin.

2.4.9. Flavonoids

a) Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

2.4.10. Protein and amino acids

a) Ninhydrin test

To the extract 0.25% ninhydrin reagent was added and heated in boiling water bath, blue colour develops indicating the presence of amino acids.

a) Biuret test

To the extract 1 ml of 10% NaOH was added and kept boiling in water bath for 5 minutes, to this added 0.7% Cu₂SO₄ solution. Formation of purplish violet colour indicates the presence of proteins.

2.5 Selection of bacterial cultures

2.5.1 Antibacterial activity assay

Antimicrobials are compounds that, at low concentrations, exert an action against micro-organisms and exhibit therapeutic toxicity towards them. These can be substances of natural, synthetic, or semi synthetic origin that may kill microorganisms including bacteria, fungi, protozoa, and viruses. The antimicrobial activity of compounds can be detected by observing the growth of various micro-organisms. If the compounds inhibit the growth of the test organism, and general toxic effects are not present, then the compounds can potentially be used to combat diseases caused by these pathogens. The antimicrobial activities of compounds have formed the basis of many applications, including pharmaceuticals, alternative medicine and natural therapies.

2.5.2 Bacterial strains

The test bacterial species included as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Enterobacter*. These bacteria belong to the most important pathogenic bacteria of human diseases. Bacterial strains were maintained on Nutrient agar. Bacterial cultures were sub cultured (1% inoculum) in nutrient broth at 35 °C for at least two to four days before being used in the screening assays.

2.5.3 Procedure

a) The extract was screened for antibacterial activity using the agar well diffusion method with sterile cork borer of size 6.0 mm. The cultures are of 24 hours old, grown on nutrient broth was used for inoculation of bacterial strain on Muller Hinton agar plates.

b) The extracts, after concentration, were weighed and dissolved in DMSO (1mg in 1ml). Each microorganism was diluted in sterile saline solution and adjusted to 0.1 OD reading.

c) The above said microorganisms were then flooded on the surface of the pre sterilized Muller Hinton Agar plate.

d) Two wells, each 10 mm in diameter, were cut from the agar and 100 microliters (100 ppm) of each compounds was loaded in to one well and antibiotic of same concentration was loaded into the other well.

e) The plates were incubated for 24 hours at 37 °C. The complete antibacterial analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out

in triplicates.

2.6 Minimal Inhibitory concentration

The extracts were evaluated for their *in vitro* growth inhibitory activity against Gram-negative bacteria *Escherichia coli* (ATCC 25922). Antibacterial activities of the compounds were tested by the agar-diffusion method under standard conditions. The bacteria was seeded into the nutrient broth and kept for incubation for 24 hours.

a) A set of sterilized petri dishes was taken and to the petri dish, sterilized, molten Muller Hinton agar medium was poured and allowed to cool and solidified.

b) After solidification, with a sterile cork borer, 6 wells are plucked. Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO). Further dilutions were performed with distilled water. The concentration range of the tested

compounds was between 0.1 – 6 mg /ml.

A control using DMSO without any test compound was included. The MIC values of the compounds to be tested were obtained as mg ml⁻¹.

c) 50 microliters of each solution was placed in the well plucked in the pre-sterilized petridish. After incubation for 24 h at 25–27 °C, the diameters of the inhibition (sterile) zone (including disc) were measured (in mm).

d) Every test was performed in triplicate.

3. Results and Discussion

Qualitative screening of phytochemicals in the leaves are presented in table 1. of the 10 phytochemicals screened for 6 were present and they include the alkaloids, triterpenes, oils & fats, phenol, tannins and flavonoids. Remarkably, proteins, resins, glycosides and saponins are absent in them.

Table 1: Qualitative Phytochemical analysis of aqueous methanolic *Strychnos nux vomica* leaf extract.

Sl. NO	Phytochemical test	Remarks	Results
1	Alkaloid test		
	Wagner's test	Presence of Reddish brown precipitate	Presence of alkaloids
	Hager test	Presence of yellow precipitate	
2	Glycoside test		
	Bornfrager's test	No change observed	Absence of Glycosides
3	Saponin test		
	Froth test	No frothing & foaming	Absence of Saponins
4	Phytosterols		
	Salkowski's test	Appearance of Golden yellow	Presence of Triterpenes
5	Oils and fats		
	Stain test	Stain is observed	Presence of oils & fats
6	Resins test		
	Acetone water test	No turbidity appeared	Absence of Resins
7	Phenols Test		
	Phenol test	Appearance of Black colour	Presence of phenols
8	Tannin test		
	Gelatin test	white precipitate formed	Presence of Tannins
9	Flavanoids test		
	Alkaline test	Intense yellow appeared	Presence of flavonoids
10	Protein test		
	Ninhydrin test	No blue colour	Absence of proteins

In the present investigation antibacterial activity of aqueous methanolic extracts of the leaves of *strychnos nux vomica* were tested against five human pathogenic bacteria. The anti-microbial activity was compared against the antibiotic ciprofloxacin by antibiotic sensitivity test.

Antibacterial activity of methanolic extract of *Strychnos nux vomica* leaves was tested against, *Staphylococcus aureus*,

Escherichia coli, *Enterobacter*, *Pseudomonas*, and *Salmonella typhimurium*. Methanolic extract of the *Strychnos nux vomica* showed maximum inhibition zone against *E. coli* (1.8 mm) and *Pseudomonas* (1.8 mm). But the extract never showed any inhibition for *Salmonella typhimurium*.

Table 2: Antibacterial activity of methanolic extract of *Strychnos nux vomica* leaves.

S. No	Extract 1	Organisms studied				
		<i>E. coli</i>	<i>Pseudomonas</i>	<i>Enterobacter</i>	<i>S.aureus</i>	<i>S. typhimurium</i>
1	R1	1.6 cm	1.6 cm	1.2 cm	1.4 cm	-
2	R2	1.8 cm	1.2 cm	1.4 cm	1.5 cm	-
3	R3	1.8 cm	1.8 cm	1.6 cm	1.7 cm	-
4	Ciprofloxacin	2 cm	2.1 cm	1.8 cm	2 cm	2 cm

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation. It was found to be 0.25 mg of the methanolic extract at which

the zone of inhibition was 0.8 mm which occurred for the standard antibiotic ciprofloxacin even at a concentration of 0.1 mg (Table 3).

Table 3: Minimal inhibitory concentration exhibited by methanolic extract of *Strychnos nux vomica* leaves for *E. coli*.

S. No	<i>E. coli</i>						
	Extract	0.1 mg	0.25 mg	0.5 mg	1.5 mg	3.00 mg	6.00 mg
R1	---	0.8 mm	1 mm	1.4 mm	1.8 mm	2.1 mm	
R2	---	---	0.8 mm	1.2 mm	1.6 mm	1.6 mm	
Ciprofloxacin	0.8 mm	1 mm	1.2 mm	1.8 mm	2.2 mm	2.3 mm	

The present investigation was carried out with the aqueous methanolic extract of *Strychnos nux-vomica* leaves for qualitative phytochemical analysis and the antimicrobial activity. Antimicrobial potential of plant extract was compared according to their zone of inhibition against the several pathogenic organisms [4]. Resistance against various antibiotic drugs by the pathogenic bacteria has become a serious concern these days, and hence there is a continuous search for control agents from plants and related sources is being undertaken [5, 6]. Non edible plants and their products are found to be suitable for antimicrobial activities against pathogenic bacteria [7].

4. Conclusion

The present study indicated a significant antibacterial effect of the methanolic leaf extracts of *Strychnos nux vomica* and exposes the existing potential of the other parts of the tree to be explored for other medicinal benefits of the human kind.

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