International Journal of
Life Science
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Science, 2019; 7 (3):551-556
ISSN:2320-7817(p) | 2320-964X(o)

Original Article Open Access

Preliminary phytochemical analysis and antimicrobial activity of *Strychnos nux – vomica* Linn. (Loganiaceae)

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Manuscript details:

Received: 01.06.2019
Accepted: 23.08.2019
Published: 30.09.2019

Editor: Dr. Arvind Chavhan

Cite this article as:
Vadivalagan A, Kannan R and Balasubramaniam V (2019) Preliminary phytochemical analysis and antimicrobial activity of *Strychnos nux – vomica* Linn. (Loganiaceae), *Int. J. of Life Science*, Volume 7(3): 551-556.

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Available online on
http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

Strychnosnux-vomica Linn. (Loganiaceae) traditionally used as curative purpose for various kinds of diseases. The different use of this plant as herbal remedy in Chinese medicine and described in the vedic literature of Indian system of medicine too. Pharmacologically active phytoconstituents of *Strychnos nux-vomica* are Strychnine and brucine major alkaloids which are used to treat central nervous stimulant. These constituents found to be poisonous when prescribed at high levels. The present analysis of the different polarity of the solvent petroleum ether, acetone and methanol was used to test for the presence of phytochemical constituents of the bark of *S.nux-vomica.* The study revealed the presence of alkaloids, saponins, glycosides, tannins, phenols and resins as major constituents of this plant. The Antimicrobial activity different extracts of *S.nux vomica* bark against *Serratia marcescens*, *Escherichia coli* (gram negative bacterial pathogens) and *Penicillium notatum*, *Fusarium oxysporum* (fungal pathogens) were showed decent zone of inhibition in methanol extract.

Keywords: *Strychnos nux-vomica*, phyto-constituents, antimicrobial activity.

INTRODUCTION

Strychnosnux-vomica is an evergreen tree belongs to the family Loganiaceae. It is native to Southeast Asia and grows in Sri Lanka, India and Australia. The traditional medicinal component of the plant part is seed and in dry form used for the treatment of neurodisorders, arthritis and vomiting. Currently, more than 60 formulations of Indian systems of medicine being uses *S. nux-vomica* out of which 30 preparations are only for imbalance of *vatadosha (*Kumar and Sinha, 2009). The ethnopharmacological and antimicrobial properties of certain medicinal plants extract of 23 crude drug samples used for various skin diseases were assayed for antimicrobial activity against four bacterial and fungal human pathogens (Ram *et al.* 2004).

The various classes of phyto-compounds have been identified in different parts of this plant. Most of them are alkaloids like Indole alkaloids strychnine and brucine which are responsible for a wide range of beneficial prospective and also toxic in nature. This plant is reported extensively for pharmacological and biological activities besides the toxic properties. Considerable progresses have been made on phytochemical, pharmacological and toxicological investigations of this plant over the years. Pharmacological and phytochemical aspects of *S. nux-vomica*, provide a modern scientific source of natural drugs for the development and management of various disorders (Maji and Banerji, 2017).

MATERIALS AND METHODS

Preparation of extracts

The plant parts were collected and shade dried for about two weeks and ground into coarse powder. About 80 g powder of each plant part was separately extracted with 125 ml of petroleum ether using soxhlet apparatus. The same powders were also extracted with acetone and methanol. The extracts were concentrated to dryness to yield crude residue. These residues were used for preliminary phytochemical screening of secondary metabolites and also subjected to antibacterial and antifungal testing.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

In the present study, all preliminary phytochemical screening was carried out following the methodology of Harborne (1973).

Alkaloids

Meyer's reagent (potassium mercuric iodide)

1.36 gm of mercuric chloride was dissolved in 60 ml of distilled water and 5 gm of potassium iodide was dissolved in 10 ml of water. These two solutions were mixed and diluted to 100 ml with distilled water. To 1 ml of the extract, a few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.

Flavonoids

In test tube containing 0.5 ml of extract, 5 to 10 drops diluted HCl and small piece of ZnCl or magnesium were added and the solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour indicates the presence of flavonoids.

Saponins

In a test tube containing about 5 ml of the extract, few drops of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honeycomb like froth was formed and it showed the presence of saponins.

Glycosides

A small amount of extract was dissolved in 1 ml of water and aqueous sodium hydroxide solution was added. Formation of yellow colour indicates the presence of glycosides.

Steroids

To 2.0 ml of extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red colour produced in the chloroform layer shows the presence of steroids.

Resins

To 2.0 ml of extract 5.20 ml of acetic anhydride was added, dissolved by gently heating, cooling and then 0.5 ml of sulphuric acid was added. Bright purple colour indicates the presence of resins.

Phenols

Ferric chloride test:

To 1 ml of the extract 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenols.

Tannins

Lead acetate test:

In a test tube containing about 5 ml of the extract, a few drops of 1 % solution of lead acetate was added. A yellow or red precipitate indicates the presence of tannins.

ANTIMICROBIAL STUDY

The bacterial and fungal strains were obtained from the Department of Microbiology, KMCH Hospitals, Coimbatore. The bacterial strains were maintained in nutrient agar slants and fungal strains in potato dextrose agar slants. Antibacterial and Antifungal studies were carried out by Disc Diffusion methods.

Test Organisms

Bacterial strains

Gram negative bacteria:

- *Serratiamarcescens*
- *Escherichia coli*

Fungal strains

- *Penicilliumnotatum*
- *Fusariumoxysporum*

Composition of Nutrient Agar Medium for bacteria

Peptone	10 g
Beef Extract	15 g
Sodium chloride	3 g
Distilled water	1000 ml
Agar agar	20 g

Composition of Potato Dextrose Agar (PDA) Medium for fungi

Potato tubers	200 g
Dextrose	20 g
Distilled water	1000 ml
Agar agar	20 g

Preparation of Culture Medium and Inoculation

The Petri plates and the nutrient agar medium as well as potato dextrose medium were sterilized for 20 minutes at 120ºC. The rest of the procedure was carried out in laminar air flow. Approximately 20 ml of the media was poured into the sterile Petri plates and allowed to get solidify for 15-20 minutes. After the media gets solidified, the bacterial and fungal organisms were swabbed in respective medium using cotton swabs.

Disc Diffusion method

Antimicrobial activity of the plant extracts were tested using the disc diffusion method according to (Bauer and Kirby, 1996). Sterile nutrient agar plates and potato dextrose plates were prepared for bacterial and fungal strains respectively and inoculated by a spread plate method under aseptic conditions. The filter paper discs of 6 mm diameter (What man's No. 1 filter paper) were prepared and sterilized. The plant extracts to be tested were prepared with various concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml and were added to each disc of holding capacity of 10 microlitre. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Control discs were also placed using respective solvents used for the extraction. All the plates including control plates were incubated at 37ºC for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured.

RESULTS AND DISCUSSION

Adopting the methods of Harborne (1973) the petroleum ether, acetone, methanol extracts were tested for the presence of alkaloids, flavonoids, saponins, phenols, steroids, glycosides, tannins and resins. The detail phytochemical profile of *Strychnos nux-vomica* has not reported comprehensively. It is rich in different classes of phytochemicals of which mostly indole alkaloids with different structural patterns. Last few decades more than 90 chemical compounds have been identified from different parts of this plant (Maji and Banerji, 2017).

Preliminary phytochemical analysis of stem bark revealed the presence of various classes of phyto-chemicals belongs to flavonoids, phenols, alkaloids, carbohydrates, tannins, steroids, triterpenoids and glycosides. Brucine, strychnine, α-colubrine, loganin, mavacurine, vomicine, pseudobrucine, pseudostry-chnine, 16-hydroxycolubrine and caffeic acid ester are some of the compounds present in the stem bark. β-colubrine, brucine, caffeic acid ester, strychnine are present in root bark (Bialaet al., 1998 and Frederich et al., 2009).

Table 1. Preliminary phytochemical analysis of various extracts of bark of *Strychnos nux-vomica*

Sl. No.	Name of the phyto-compound	Name of the Extract		
		Petroleum ether	Acetone	Methanol
1.	Alkaloids	++	++	++
2.	Flavonoids	-	-	-
3.	Saponins	++	++	++
4.	Phenols	+	+++	+++
5.	Steroids	++	++	+
6.	Glycosides	+++	+++	++
7.	Taninns	+++	+	+++
8.	Resins	-	-	+

Recently, 13C NMR and mass spectrometry analysis of stem bark revealed the presence of four new dimericbisindole alkaloids (Jonville et al., 2013). Prior to this root bark of *Strychnos nux-vomica* from Sri Lankan origin were reported to contain nor-macusine B, O-methylmacusine B, 16-epi-O-methylmacusine B, nor-melinonine B, isostrychnine, protostrychnine, strychnine, 10-hydroxystrychnine, 12-hydroxystry-chnine, β-colubrine, 12-hydroxy-11-methoxystry-chnine, brucine, 4-hydroxy-3- methoxystrychnine and 4-hydroxystrychnine (Baser et al., 1979 &Baser and Bisset, 1982). Similarly, our current findings showed the presence of alkaloids, saponins, glycosides, tannins, phenols and resins in the bark of *S. nux-vomica* various from various polarity of the solvent system used (Table 1).

The antimicrobial assay was performed by disc diffusion methods prescribed by Bauer et al 1996. In disc diffusion and minimal inhibitory concentration (MIC) assay methods, the ethyl acetate extract of nux vomica bark was found to exhibit potent antimicrobial activity against both, gram positive (*Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis* and *Staphylococcus albus*) and gram negative (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes* and *Protieus vulgaris*), pathogenic bacterial strains were reported by Thambi and Cherian (2015).

Similarly, different researchers were reported that various polarity of the extracts such as hexane, chloroform, ethyl acetate and ethanol of the leaves possess different degree of growth inhibitory prospective against *Shigella flexneri, P. mirabilis, P. vulgaris, Vibrio cholera, E. coli, P. aeruginosa, S. aureus, Salmonella typhimurium, K. pneumoniae* and *Enterobacter faecalis*. Among these the methanol extract was found to be most active against the pathogenic bacterial strains tested (Kalaivanan et al., 2014; Prabha et al., 2014 & Magdalin and Reginald 2014).

Gnanavel et al., (2012) reported that the n-butanol extract of leaves showed strong inhibitory potential against some pathogenic bacterial (*S. aureus, K. pneumonia, B. subtilis*) and fungal (*Aspergillus terreus, A. flavus* and *A. niger*) strains. Our current findings also in accordance with these study and shown maximum inhibitory of 9 mm in petroleum ether extract followed by methanol extract 8mm against *E.coli* bacterial strain. Also the overall activity was decentin methanol extract compared to other extracts (Table 2).

Methanol extract of *nux vomica* flowers showed significant anti-microbial activity against both pathogenic bacterial strains *P. aeruginosa, S. aureus, B. subtilis, K. pneumoniae* and fungal strains viz. *Candida albicans* by disc diffusion method was reported by Mohesh et al., (2015). The present study it revealed the anti-fungal activity of the petroleum ether extract exhibited highest zone of inhibition at different concentration level 75%, 50% and 25% respectively showed 9mm, 8mm and 7mm zone of inhibition against *Penicillium notatum* followed by methanol extract. Whereas acetone extract did not showed any inhibitory action against both fungal strains *P. notatum* and *Fusarium oxyporum* tested (Table 3 & Fig.1). The overall microbial study suggests that *Strychnos nux-vomica* has the potent of microbial activity due to presence of phyto-chemical compounds.

Table 2: Antibacterial activity of different extracts of bark of *Strychnos nux-vomica* against *Serratia marcescens* and *Escherichia coli.*

Sl. No.	Name of the organisms	Solvent system used	Zone of inhibition (mm)/ Concentration of the extract (%)				
			Control	25%	50%	75%	100%
1.	*S. marcescens*	Petroleum ether	17	6	6	-	-
		Acetone	11	6	6	6	5
		Methanol	8	6	6	7	6
2.	*E. coli*	Petroleum ether	10	7	9	7	6
		Acetone	5	-	-	5	6
		Methanol	6	7	8	-	-

Table.3 Anti-fungal activity of different extracts of bark of *Strychnos nux-vomica* against *Penicillium notatum* and *Fusarium oxyporum.*

Sl. No.	Name of the organisms	Solvent system used	Zone of inhibition (mm)/ Concentration of the extract (%)				
			Control	25%	50%	75%	100%
1.	*P. notatum*	Petroleum ether	7	7	8	9	5
		Acetone	-	-	-	-	-
		Methanol	7	6	6	6	6
2.	*F. oxyporum*	Petroleum ether	6	7	6	-	6
		Acetone	-	-	-	-	-
		Methanol	7	6	6	-	6

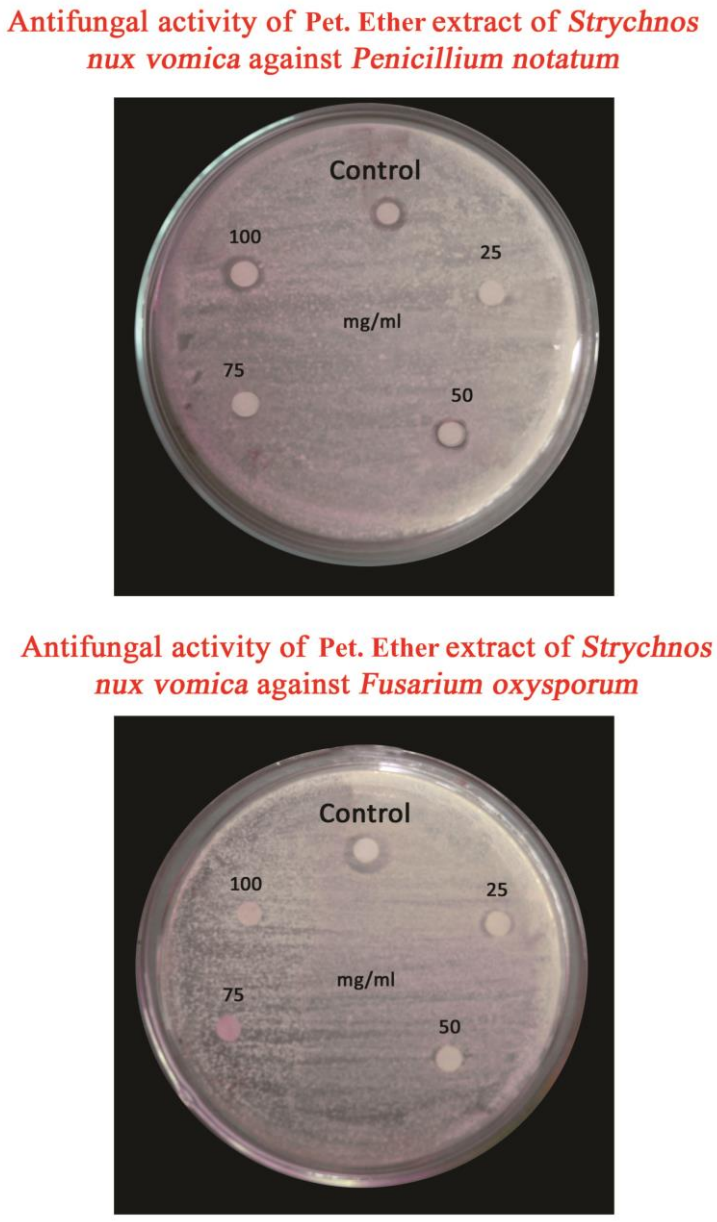

Figure 1. Shows the inhibition of crude extract of *Strychnos nux-vomica* against fungal pathogens

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