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Phytochemical investigation of the bark of *Strychnos-nux-vomica* and its antimicrobial properties

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Abstract
Strychnos-nux-vomica which belongs to the family *loganiacea* also called Kanjiram is a medium-sized tree. The bark of the plant was under investigation. The bark of the plant was collected and extracted using ethyl acetate solvent. GC-MS analysis was conducted to identify the components present in it. The major components present in this extract were strychnine and brucine. The antibacterial screening of the extract was carried out by disc diffusion method. The extract was tested against four pathogenic bacterial stains of gram positive and gram negative organism. The ethyl acetate extract of Strychnos-*nux-vomica* shows antimicrobial activity.

Keywords: *Strychnos-nux-vomica*, FT-IR, GC-MS, Disc diffusion method.

1. Introduction
Strychnos-nux-vomica which belongs to the family *loganiacea* is a medium-sized tree with a short thick trunk. Other names of *Strychnos-nux-vomica* are Kanjiram, Kuchla, Kupilu. The wood is dense, hard white, and close-grained. The branches are irregular and are covered with a smooth ashen bark [1]. The young shoots are a deep green colour with a shiny coat. The leaves have an opposite decussate arrangement, short stalked, are oval shaped, also have a shiny coat and are smooth on both sides. The leaves are about 4 inches (10 cm) long and 3 inches (7.6 cm) wide. The flowers are small with a pale green colour with a funnel shape. They bloom in the cold season and have a foul smell. The fruit are about the size of a large apple with a smooth and hard shell which when ripened is a mild shade orange colour.
There are no uses in modern medicine, although it was widely used in medicine before World War II substantially higher doses have been reported. The properties of *Nux Vomica* are those of the alkaloid strychnine. The most direct symptom caused by strychnine is violent convulsions due to a simultaneous stimulation of the motor or sensory ganglia of the spinal cord. During the convulsions there is a rise in blood pressure. Brucine closely resembles strychnine in its action, but is slightly less poisonous, as it cause paralysis of peripheral motor nerves. It is used to elevate blood pressure [2]. The seeds are first immersed in water for five days, in milk for two days followed by their boiling in milk [3]. In India, the quality/toxicity of traditional medical crude and processed *Strychnos* seeds can be controlled by examining the toxic alkaloids using established HPLC methods and/or HPLC-UV methods. *Strychnosnux-vomica* is also used in homeopathy. *Strychnos* has not been proven effective for the treatment of any illness. Since the seeds contain strychnine poison, conventional doctors do not recommend it as a medicine. It is on the Commission E list of unapproved herbs, because it is not recommended for use and has not been proven to be safe or effective. There is also no clinical trial evidence of *Strychnos* supporting it being a viable cancer treatment.
Plant material have been shown to posses potential for development as new antimicrobial agents and they may have the advantage over conventional type in terms of low mammalian toxicity, rapid degradation and local availability. Hence the chemical compounds present in them are likely to be biologically active even at lower concentrations. No work had been reported on the ethyl acetate extract of the bark of *Strychnos-nux-vomica* collected fro Pathanamthitta district, Kerala. The present work reports the components present in the ethyl acetate extract of the stem bark of *Strychnos-nux-vomica*.

2. Materials and Methods
2.1 Plant material
Bark of *Strychnos-nux-vomica* (SV) plant was collected from different areas of Pathanamthitta district. The bark of plant were collected in the middle of January 2015. Bark was collected from the plant were shade dried. After drying the plant materials were powdered.

Powders of the bark were used for extraction in ethyl acetate. Fifty gram of powder was used for extraction.

2.2 Method of extraction

Extraction: The stem bark collected were shade dried. Coarsely powdered 50g of the plant material was extracted with 500 mL of ethyl acetate. The extraction was carried out in a round bottom flask by boiling the material in the solvent with a water condenser. Refluxed the material until the solvent started to boil and the hot content was left standing overnight. Then filtered and collected the extract and added fresh solvent to residue. The process is repeated three times to complete the extraction. The combined extract collected was reduced to 20 ml.

Identification: Thin-layer chromatography is conducted for the ethyl acetate fraction.IR spectra (KBr) were taken on a JASCO FT-IR spectrometer. GC-MS analysis of this extract was conducted to identify the components present in it. GC/MS analyses were carried out using a Perkin Elmer Clarus 500 GC equipped with a Clarus 500 mass spectrometer, capillary column (0.32µm film thickness). 1µL of each sample was diluted with 300µL of Et_2O and injected (0.5µL) in the "split" mode (1:30) with a column temperature program of 40°C for 5 min, then increased to 250°C at 4°C/min and finally held at this temperature for 10min. Injector and detector were set at 150 and 270 °C, respectively, and the carrier gas was He with a head pressure of 12.0 psi. Mass spectra were acquired over 40-500amu range at 1scan/sec with ionizing electron energy 70eV, ion source 230 °C. The transfer line was set at 250 °C, while the carrier gas was He at 1.0mL/min.

2.3 Biological activity

The antibacterial screening of the extract was carried out by determining the zone of inhibition using standard method [4]. The extract was tested against four pathogenic bacterial strains of gram positive and gram negative organism by disc diffusion method [5]. The test microorganisms of gram positive bacteria: *Staphylococcus aureus, Bacillus subtilis, Streptococcus faecalis, Staphylococcus albus*. gram negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Protieus vulgaris, Klebsiella aerogenes*. Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5 °C for 1h to permit good diffusion and then transferred to incubator at 37 °C for 24h. After completion of 24h, the plates were inverted and placed in an incubator set to 37 °C for 24h.

3. Results and Discussion

3.1 Characterization of SV: Large numbers of spots were observed on TLC examination of SV, hence it was subjected to GC-MS analysis. UV spectra of SV showed characteristic peaks at 210, 232, 280 and 372 nm. In IR spectra characteristic peaks were seen at 3444 cm^{-1}, 2918.73 cm^{-1}, 2853 cm^{-1}, 1735 cm^{-1}, 1706.69 cm^{-1}, 960 cm^{-1}, 720.28 cm^{-1}. From the earlier reports *Strychnos-nux-vomica* contains flavanoids, phenols, alkaloids [6], carbohydrates, tannins, steroids, triterpenoids and glycosides. On GC-MS analysis of this fraction different compound present. The components were identified by library search spectrum. The results of GC-MS analysis of SV is given in Table 1.

Table 1: Composition of SV

Sl. No	%	Compound
1	5.61	Brucine
2	6.52	Strychnine
3	1.50	Colubrine
4	2.30	Loganin
5	1.2	Mavacurine
6	3.1	Vomicine
7	2.2	Seudobrucine
8	0.75	Pseudostrychnine
9	0.87	16-Hydroxycolubrine

The activity of each compound against the microorganism under study can be concluded from their respective zone of inhibition diameter which is given in Table 2. It was observed that all the extract exhibit biological activity, hampering the growth of one or the other organism. Table 3 shows the minimal inhibitory concentration.

GCMS analysis of *Strychnos nux vomica* from the earlier report shows that highly poisonous alkaloids strychnine and brucine are present in high percentage. The higher antimicrobial activity in ethyl acetate extract may be due to the presence of strychnine and brucine or due to the synergistic effect of the major and minor components. Usually the major components are responsible for the antimicrobial activity of plant extract, but the minor components also play major role making the whole oil more active than the combination of major components in synergism.

Table 2: Antimicrobial screening of extract

Sl. No.	Test organisms	Diameter of zone of inhibition (mm) at different concentrations			
		SV			STD
	Gram +ve bacteria	1 mg/l	2.5 mg/l	5 mg/l	2µg/disc
1	*Staphylococcus aureus*	10	12	13	20
2	*Bacillus subtilis*	10	11	14	19
3	*Streptococcus faecalis*	11	12	13	19
4	*Staphylococcus albus*	10	12	14	18
	Gram-ve bacteria	1 mg/l	2.5 mg/l	5 mg/l	2µg/disc
1	*Escherichia coli*	09	10	10	18
2	*Pseudomonas aeruginosa*	10	11	11	19
3	*Klebsiella aerogenes*	10	11	12	19
4	*Protieus vulgaris*	11	13	14	19

Standard (STD) – Ciprofloxacin 2µg/disc
Solvent – DMSO (Shows nil effect against the microorganisms under test)

Table 3: Minimal inhibitory concentration

MIC - DETERMINATION : µg/ml							
Sl. No.	**Tested organism**	**Diameter of zone of inhibition (mm) at different concentrations**					
		SV					**STD**
	Gram +ve bacteria	800	600	400	200	100	
1	*Staphylococcus aureus*	07	07	06	06	06	20
2	*Bacillus subtilis*	07	07	05	05	04	19
3	*Streptococcus faecalis*	08	07	07	06	06	19
4	*Staphylococcus albus*	07	07	06	06	06	18
	Gram –ve bacteria	800	600	400	200	100	
1	*Escherichia coli*	07	06	NI	NI	NI	18
2	*Pseudomonas aeruginosa*	07	05	03	NI	NI	19
3	*Klebsiellaaerogenes*	07	06	04	NI	NI	19
4	*Protieus vulgaris*	08	05	03	NI	NI	19

Standard (STD) – Ciprofloxacin 2µg/disc

4. Conclusion

Therefore, the bark extract of *Strychnos-nux-vomica* in ethyl acetate solvent, a potentially useful antimicrobial agent. This antimicrobial activity can be attributed to the compounds present in the extract. Hence it can be used as a potential antimicrobial agent.

5. References

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