



Antibacterial and antioxidant activity of *Strychnos nux vomica* flower extract

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ABSTRACT

This study was aimed to investigate the antibacterial and antioxidant activity of the methanolic extract of *Strychnos nux vomica* flowers. Results showed that the plant was able to inhibit all the microorganisms chosen however its effect was higher with *Candida albicans* and *Klebsiella pneumoniae* with Chloramphenicol (10ug/ml) as the standard drug of choice. The samples were evaluated for their radical scavenging activity against the synthetic DPPH(2,2-diphenyl-1-picryl-hidrazila), results showed a high antioxidant activity. Further, this study showed that the flowers of this plant can be used to treat against infections caused by these tested microorganisms. More research is needed to isolate the active compounds, determine the structural composition and their mode of action in inhibiting these bacteria.

Keywords: *Strychnos nux vomica* flowers, antibacterial activity, antioxidant activity.

INTRODUCTION

World Health Organization has estimated that 80% of the population in developing countries rely on traditional medicines for their disease remedy[1]. Traditional medicine in rural India has taken a front seat with the use of the available herbal resources from the nature in healing diseases. Even though the western allopathic medicine has succeeded in eliminating certain diseases, alleviating pain, and vaccinating people as a preventive measure, it has also contributed to the recent emerging problems like multi drug resistant bacterial strains which are a real threat to the population. With lot of reported side-effects of the modern drug therapy, there is always a search for disease remedy in alternative medicines like Yoga, Naturopathy, Ayurvedha and Siddha. Wisdom of the Indian Ancestors have become the need of the hour and is found being honored by the rest of the World, as there is a quench to follow the practices of Indian indigenous medicine. Based on the indigenous practice of the products from the toxic tree *Strychnos nux vomica* in these area and the urge World faces to discover new drugs to tackle the evolving bacterial strains, we went on to document the antibacterial and antioxidant property of the methanolic extract from the flowers of the above said tree.

Strychnine is one of the oldest poisons from the tree *Strychnos nux vomica* which contains the alkaloids brucine and strychnine in greater quantity[2]. Any part of the tree if consumed will bring effects that can be fatal. Modern medicine has not identified any potential therapeutic usage of this plant, however traditional Ayurvedha medicine recognizes it as a therapeutic agent after a special detoxification procedure called as Sodhan karma[3]. Literature evidence is available for its anticonvulsant[4], anti tumor[5], antiamnesic[6], analgesic[7], anti-inflammatory[7], immune-modulatory [8], anti-snake venom[9], hepatoprotective[10], antidiarrhoeal[11] and anticholestatic[10] activity. Most of the scientific evidences on the above said medicinal benefits are documented with the extracts of either leaves, seeds or bark only. Research findings on the medicinal capability of the flowers from the said tree is

very less. Flowers of a plant attracts the pollinating agents like bees, wasps, birds etc. Flowers with bright colors, fragrance and honey load is the rule of the nature to enhance pollination. Here in this tree that is loaded with poisonous phytochemicals we believed in the existence of positive components that would benefit the animals that could approach the flower for some reasons. Hence our objective in this study included the antibacterial and antioxidant property of the *Strychnos nux vomica* flowers.

EXPERIMENTAL SECTION

i) Sample collection and preparation

The flowers from the tree *Strychnos nux vomica* was collected during the month of April, 2015 from the region of Ammapettai, near Tiruporur Kancheepuram District of Tamilnadu. Botanical identification of the tree was done by Professor.P.Jayaraman, Director, Plant anatomy Research Center, Medicinal plant research unit, West Tambaram, Chennai{PARC/2013/2147}.

ii) Extraction procedure

Hundred grams of the fresh flowers collected was shade dried for a period of 4days.These dried flowers weighing around 60gms, were homogenized with 200 ml of methanol in a glass beaker for 3days.The product was filtered and the filtrate was collected in a glass beaker and allowed to air dry. The collected crude methanolic extract(1.5ml) for further study.

iii) Bioassay study

a)Preparation of the bacterial suspension.

The bacterial suspension was prepared to match the McFarland standard which was prepared by dissolving 0.5gm of BaCl₂ in 50 ml of dissolved water to obtain a 1% solution of Barium chloride. This was mixed with 99.5ml of 1% Sulphuric acid solution. Three to five identical colonies of each test bacteria was taken from the stock and dropped in Mueller Hinton broth (Himedia, Mumbai). The broth culture was incubated at 37°C for 2 to 6 hours until it reached turbidity similar to the Mc Farland standards.

b)Preparation of the extract concentrations and antibiotic.

Stock solutions for the extracts were prepared by dissolving 100 mg in 1 ml of methanol. An antibiotic control was made by dissolving 10 µg of Chloramphenicol in 1ml of sterile water.

c)Determination of the bioactivity of the extract.

Test pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Bacillus subtilis* and *Klebsiella* were used for in vitro antibacterial activity. These selected pathogenic strains were obtained from Microbiological Division (Jayagen Biologics Analytical Laboratory, Jayagen Biologics, Chennai).

In vitro antibacterial activity

The antibacterial activity was determined by disc diffusion method (Kirby et al., 1966)[12]. About 25 mL of molten nutrient agar was poured into a sterile petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted 0.6) 100 µl of above said pathogenic bacteria cultures were transferred onto plate and made culture lawn by using sterile L-rod spreader. After five minutes setting of the bacteria, the test samples were dissolved in sterile water and impregnated onto the sterile disc (40µL) with various concentrations such as 20mg/100µL, 40mg/100µL, 60mg/100µL and 80mg/100µL (Himedia, Mumbai, India). The drug loaded discs were deposited onto the plate between 24 mm diameter distance. The solvent water loaded disc served as control. The plates were incubated at 37°C in a 40 W fluorescent light source (~ 400 nm) for 24 h. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

Free radical scavenging activity

The effect of crude extract of *Strychnos nux vomica* flowers on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997)[13]. Two mL of 6 ×10⁻⁵ M methanolic solution of DPPH were added to 50 µl of a methanolic solution (5 mg/1 ml) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound [quercetin] were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was noted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration as follows,

All determinations were performed in duplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994)[14].

RESULTS

Our results described well the antibacterial and anti oxidant property of the methanolic extract of the *Strychnos nux vomica* flowers.

Table 01. Zone of inhibition created by the crude extract on different bacteria

Organism	10µl/disc	20µl/disc	30µl/disc	40µl/disc
<i>Staphylococcus aureus</i>	10mm	12mm	15mm	16mm
<i>Pseudomonas aeruginosa</i>	5mm	10mm	11mm	13mm
<i>Candida albicans</i>	10mm	13mm	16mm	18mm
<i>Bacillus subtilis</i>	10mm	11mm	13mm	14mm
<i>Klebsiella pneumoniae</i>	11mm	13mm	15mm	18mm

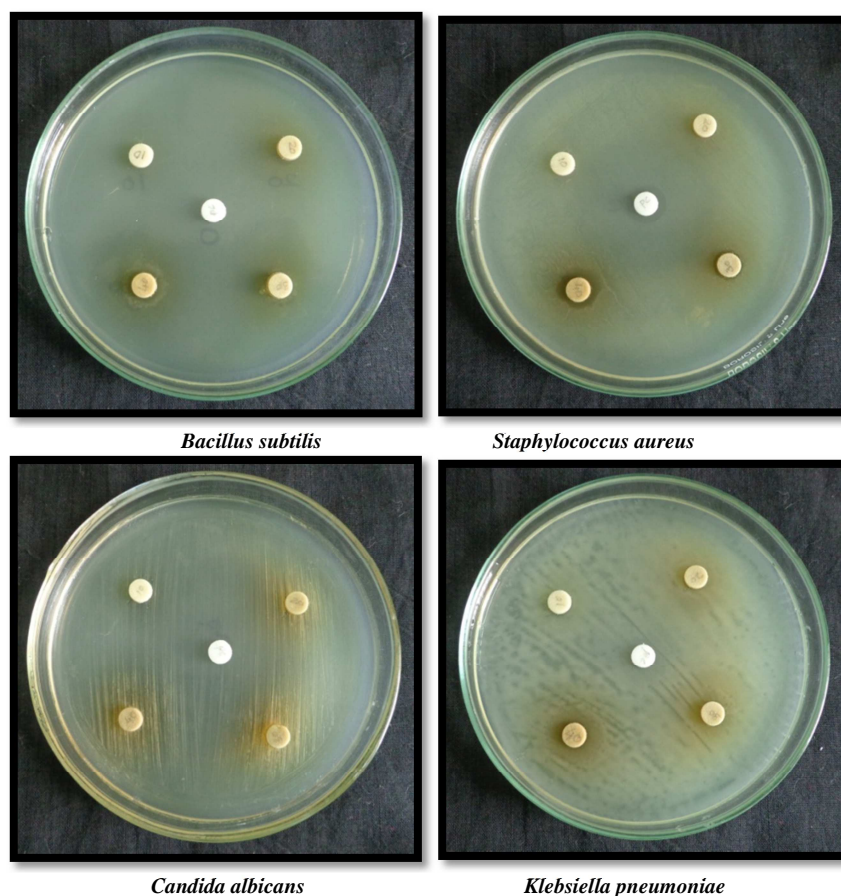
$$\text{Percentage of inhibition (PI)} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

Where $A_{C(0)}$ is the absorbance of the control (DPPH) at $t = 0$ min; and $A_{A(t)}$ is the absorbance of the plant extract at $t = 16$ min.

$$\text{Percentage of inhibition (IP)} = 78.49\%$$

The antioxidant property of the methanolic extract of the flowers is good and hence its proved that the plant has natural antioxidants in greater quantity.

Figure 1. Zone of inhibition as seen in the discs



*Pseudomonas aeruginosa*

DISCUSSION

Antibacterial activity of flower extract

It was observed that, *Strychnos nux vomica* flower heads not only had antibacterial activity but also strong antioxidant activity. It is evident from the literature review that *Strychnos nux vomica* is known for its therapeutic role from the extracts of seeds, bark and leaves[4,15,16]. Phytochemically the plant contains alkaloids like Strychnine, Brucine, Strychnicine, Loganin, Cafeotannic acid and Copper[17,18].

Radical scavenging activity of flower extract

DPPH radical scavenging assay is the most popular and simple invitro radical scavenging assay method. DPPH is a stable, nitrogen centered, commercially available, organic free radical and has an absorption maxima at 515-517nm in methanol. On accepting hydrogen from donor (antioxidant), the solution of DPPH loses the characteristic deep purple colour and becomes yellow coloured diphenylpicryl hydrazine[19]. Our results on antioxidant property is similar to that established with the antioxidant property of the seeds of this plant[20]. The methanolic extract of the flowers exhibited 78.49% of marked scavenging activity. This confirms the presence of antioxidant property in the flower heads of the plant.

CONCLUSION

Literature on the medicinal benefits of the leaves, bark and seed of this plant is well documented. However this report on the activity of the methanolic extract from the flower heads of the plant *Strychnos nux vomica* is the first one and it is proved that these flowers are loaded with antibacterial phytochemicals and hence it might be useful in the antiseptic and disinfectant formulations. Further studies involving different animal disease models will help us to explore its real efficiency against microbes and its role in curing many diseases.

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