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RESEARCH ARTICLE

TO ANALYSIS THE ANTIOXIDANT POTENTIAL OF *STRYCHNOS NUX-VOMICA*

***Kandhavelu, M. and Senthilkumar, P. K.**

Division of Microbiology, Faculty of Science, Annamalai University, Chidambaram, Tamil Nadu, India

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ABSTRACT

The present study provides information on antioxidant analysis of different extracts of *Strychnos nux-vomica* leaf. The various parts of *Strychnos nux-vomica* are recommended for upset stomach, vomiting, abdominal pain, constipation, intestinal irritation, heartburn, insomnia, certain heart diseases, eye diseases, depression, migraine headaches, nervous disorders, problems related to menopause in women and respiratory diseases in the elderly. Different extracts of *Strychnos nux-vomica* were prepared based on the polarity in solvents of hexane, chloroform, ethyl acetate, acetone and methanol. Among all the solvents used methanol extract of *Strychnos nux-vomica* showed potential antioxidant properties as tested by the methods of radical scavenging activity. Hence the present exploration of antioxidants studies of *Strychnos nux-vomica* will be useful in the synthesis and preparation of new drugs of pharmaceutical importance.

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INTRODUCTION

Strychnos nux vomica is a poisonous deciduous tree of Loganiaceae family which is abundantly found in India. This tree known as Kupilu of Kuchila in ancient Ayurvedic texts has been described as an Akshepajanana drug (convulsant) and is included under upavisha varga (poisonous plants). Though it is highly poisonous but, can be used as a therapeutic agent after appropriate sodhan (purification) procedures as described in ancient texts of Ayurveda. This is an evergreen tree usually 30 meters high and 1-1.8 meter in girth. The leaves are 8 – 15cm long, broadly elliptical with prominent central nerves. Flowers are greenish white in colour and the berries are round and 2.5 – 5.0cm in diameter. The ripe fruits contain seeds which are poisonous, flat, circular discs, 2.5 x 0.6cm, slightly concave on one side and convex on the other, ah grey in colour, have a shiny surface and are covered with silky hairs. Unbroken seed when ingested are not poisonous, as the hard pericarp is not soluble in digestive juices (Kirtikar and Basu, 1935).

The main active principles of seeds are alkaloid such as strychnine, brucine and loganine. They also contain vamicine, colubrine, logamine glycoside and fatty substances upto 3% alkaloids. Total alkaloids ranges from 2.6% to 5.3% of which approximately half proportion is of strychnine, but bark yields only brucine. Recently, the reinvestigation of *strychnos nux - vomica* resulted in the isolation of two colourless monoquaternary bisindole alkaloids from the seeds named as 4-N- hydroxymethyl strchnidin – 17 – acetic acid and 10, 11 – dimethoxy -4-N-hydroxymethyl strchnidin-17-acetic acid. In another study, isolation of a coloured monoquaternary bisindole alkaloid (strychno chrysine) from the roots was done. Strychnine is colourless, bitter, odourless, rhombic, prism shaped crystals. It is very stable and does not change during putrefication of the dead body. Strychnine competitively antagonizes the inhibitory neurotransmitter glycine by blocking its post-synaptic uptake by brainstem and spinal cord receptors. Its action is particularly in the anterior horn cells (Mahapatra Arun Kumar *et al*., 2012). The fatal dose of strychnine is 15 – 50mg and fatal period ranges from 1 – 2 hrs. This present paper aims to evaluate the role of detoxification process (Sodhan Karma) of *Strychnos nux-vomica* as mentioned in ancient texts of Ayurveda backed with recent research evidences and to review the therapeutic potential of *Strychnos nux-vomica.*

The purified *Strychnos nux vomica* seeds were for the treatment of rheumatoid arthritis, gout, sciatica, backache, lumbago and all types of muscular pain. It is traditionally used as a nervine tonic, digestive stimulant and aphrodisiac traditionally. Various Ayurvedic medicines like Agnitundi vati, Vishatinduka vati, Vishagarbha taila contains detoxified *Strychnos nux-vomica* seeds. Ayurveda advocate purification process (Sodhan Karma) for the detoxification of poisonous drugs before they can be utilized as therapeutic agent. The seeds of *strychnos nux-vomica* are considered highly poisonous and are considered inappropriate to be used in therapeutics (Mahapatra Arun Kumar *et al*., 2012).

****Corresponding author: Kandhavelu, M.***
Division of Microbiology, Faculty of Science, Annamalai University, Chidambaram, Tamil Nadu, India.

MATERIALS AND METHODS

Collection of plants

Healthy and well grown leaves of *Strychnos nux vomica* were collected from Cuddalore district, Tamil Nadu, India. The leaves were immediately brought to the laboratory using separate sterile polythene bags. First they were washed with tap water, then surface sterilized in 10 per cent sodium hypochlorite solution to prevent the contamination of microbes (Krishnan kannathasan *et al*., 2011), then rinsed with sterile distilled water and air dried in shade at room temperature. Voucher specimen (286, dt. 28.04.2015) deposited as herbarium and authenticated in Department of Botany, Annamalai University, Tamilnadu, India.

Reagents

1. Sulfuric acid: 0.6M
2. Sodium phosphate :28mM
3. Ammonium molybdate:4mM

The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank.

Table 1. Antioxidant activity of *Strychnos nux vomica*

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
DPPH	17.28 ± 1.20	32.28 ± 2.25	62.73 ± 4.39	83.64 ± 5.85	34.91961
Standard (Ascorbic acid)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	51.22807
Total antioxidant	14.68 ± 1.02	29.06 ± 2.03	62.18 ± 4.35	84.68 ± 5.92	42.41088
Standard (Ascorbic acid)	22.35± 1.80	51.23± 4.09	72.54± 5.80	86.35± 6.91	51.95885
Superoxide	16.42 ± 1.14	35.71 ± 2.49	66.07 ± 4.62	82.14 ± 5.24	31.62115
Standard (Ascorbic acid)	31.25 ± 2.50	64.23 ± 5.13	89.54 ± 7.16	98.51 ± 7.88	49.94723
Fe2 +chelating activity	18.46 ± 1.29	34.61 ± 2.42	67.30 ± 4.71	85.23 ± 5.96	30.96362
Standard (Ascorbic acid)	35.23 ± 2.81	65.21 ± 5.28	78.51± 6.28	98.65 ± 7.89	48.79828
Hydroxyl radical	15.41 ± 1.02	29.58 ± 2.07	65.00 ± 4.55	87.08 ± 6.09	35.26987
Standard (Ascorbic acid)	32.21± 2.51	56.45± 4.40	78.65±6.13	92.75±7.2	50.63149

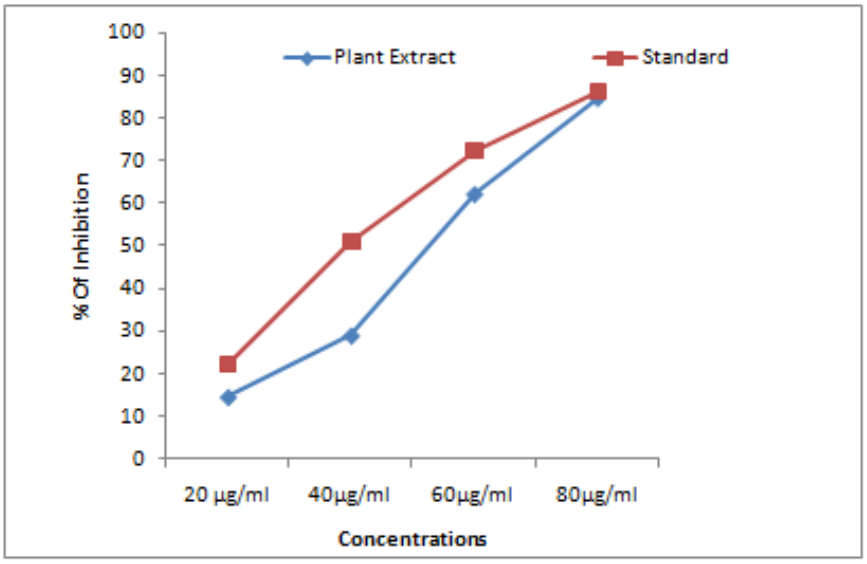

Fig. 1. Total Antioxidant activity of *Strychnos nux vomica*

Preparation of plant extracts

Forty grams of the powdered leaves were loaded in soxhlet apparatus and extracted in 125 mL of different solvents *viz.,* Hexane, chloroform, ethyl acetate, acetone and methanol. The extractions were evaporated at rotary evaporator at 40°C (Vogel, 1978).

Determination of Total Antioxidant Capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999).

The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The scavenging activity was calculated according to the following equation: % Inhibition

$$\% \text{ of Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

RESULTS

DPPH Assay

The half inhibition concentration (IC_{50}) of ascorbic acid and plant extract were 51.22µg ml^{-1} and 34.91 µg ml^{-1} respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid. DPPH radical scavenging activity of plant extract of plant and standard as ascorbic acid are presented in Table 1.

Total antioxidant activity

The yield of the methanol extract of the plant extract and its total antioxidant capacity are given in Table 1 and fig 1. Total antioxidant capacity of plant is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. The half inhibition concentration (IC_{50}) of ascorbic acid and plant extract were 51.95µg ml^{-1} and 42.41 µg ml^{-1} respectively**.**

Superoxide anion radical scavenging activity

The superoxide anion radical scavenging activities of the extract from plant assayed by the PMS-NADH system were shown in Table 1. The superoxide scavenging activity of plant was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of plant was 31.62 µg ml^{-1} and ascorbic acid were 49.94µg ml^{-1} respectively**.** These results suggested that plant had notably superior superoxide radical scavenging effects.

The ferrous ion chelating activity

The formation of the ferrous ion– Fe^{2+} complex is interrupted in the presence of aqueous extract of Plant, indicating that have chelating activity with an IC_{50} of 30.96 µg ml^{-1} and ascorbic acid was 48.79µg ml^{-1} respectively (Table 1).

Hydroxyl radical scavenging activity

Table 1 showed the plant exhibited concentration dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction system. Hydroxyl radical is very reactive and can be generated in biological cells through the Fenton reaction. The IC_{50} of plant and standard was 35.26 and 50.63µg/ml respectively. The hydroxyl radical scavenging activity of hesperidin and both standards decreased in the order of plant > ascorbic acid.

Conclusion

On the basis of the results of this study, it clearly indicates that Plant extract had powerful *in vitro* antioxidant capacity against various antioxidant systems as DPPH, superoxide anion scavenging, Hydroxyl radical scavenging and ferrous ion chelating. From our results, the antioxidant activity of Plant extract was concentration dependent. From the above assays, the possible mechanism of antioxidant activity of Plant extract includes reductive ability, metal chelator, hydrogen donating ability and scavengers of superoxide and free radicals.

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