VALIDATION AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM
STRYCHNOS NUX-VOMICA—AN IMPORTANT ETHNOMEDICINAL PLANT
OF KURNOOL DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT
Strychnos nux-vomica seed are extensively used by the ethnic groups of Kurnool district, Andhra Pradesh, India to
cure vitiligo. Biological synthesis of silver nanoparticles was carried out from seed aqueous extract of Strychnos
nux-vomica 10 ml seed extract was mixed to 90 ml of 1 mM aqueous of Ag(NO3)2 and was heated at 60-80°C for
20 min. The color change of aqueous solution into dark brown color. For characterization using UV-Vis
spectrophotometer and AFM. AFM and UV-Vis spectrophotometer showed the formation of silver nanoparticles
with spherical shape and average size 41.72 nm. SNPs have good antimicrobial activity against different
microorganisms.

KEY WORDS
Medicinal plant; Silver nanoparticles; Atomic Force Microscope (AFM); Inhibition zone; Secondary metabolites;
Antimicrobial efficacy.

INTRODUCTION
India is one of the twelfth mega biodiversity hotspot
in the world. Eastern Ghats are one among them,
which are characterized by different wild medicinal
flora. Nallamalai hills as a part of Eastern Ghats in
Kurnool District of Andhra Pradesh, India. Mainly four
ethnic groups (Chenchu, Sugali, Yerukala and Yanadi)
are inhabited in this region [1]. In this connection a
plethora of knowledge, information and benefits of
herbal drugs in our ancient literature of Ayurvedic,
Siddha and Unani [1]. WHO is encouraging the traditional drugs because of
its less side effects and matter of low cost and easy
availability [3]. WHO also has organized the
importance of traditional medicine and has been
active in creating strategies, guidelines and standards
for botanical medicines [4].

The medicinal herb Strychnos nux-vomica known for
its medicinal values in alternative systems of
(Ayurveda, Unani, Siddha, Homeopathy and Chinese)
holistic health and herbal medicine. It is belongs to
the family Strychnaceae. The paste prepared by
mincing fresh plants has got an anti-inflammatory
effect and may be applied to insect bites, stings,
swellings and other skin diseases.

Skin diseases are commonly occurring among the
rural masses due to poor hygienic conditions, poor
sanitation facilities and contaminated water etc., the
traditional healers of these ethnic groups are
extensively using Strychnos nux-vomica seed to treat
leucoderma. Leucoderma is a skin disorder in human
for a number of reasons depigmentation occurs due
to auto immune disorder [5] or lacking of pigments
due to absence of melanocytes [6].

Strychnos nux-vomica seed powder contains a variety
of important chemical compounds. It consis
Strychnine, Brucine, Pseudostrychnine,
Pseudobrucine, Secoxyloganin, Caffeic acid, P-
Hydroxybenzoic acid, P-Hydroxyphenylacetic acid, Uvaol, Lupeol, 11-Oxo-α-Amyrin palmitate, Maltol [7]. Nanoparticles are gaining much importance especially in medicinal field. Synthesis of metal nanoparticles receives great attention due to their unusual optical, chemical, phytochemical and electronic properties [8]. Silver a noble metal is known to improve the immunity since ancient times, Ag(NO₃)₂ was using for biosynthesis of nanoparticles by using seed aqueous extract of Strychnos nux-vomica. The possibility of using plant materials for the synthesis of nanoscale metals was initially reported by [9, 10]. SNPs have particular properties that may perhaps have numerous applications in the field of dentistry, clothing, catalysis, mirrors, optics, phytography, electronics and food industry [11].

At present extensive work has been done to develop new drug from natural products because of the resistance of microorganisms to the existing drug. The pathogens like E. coli, Bacillus, Salmonella typhi and Staphylococcus aureus ([12]. In this study, ethno-medico botanical studies, qualitative analysis of phytochemical constituents by using seed extract of Strychnos nux-vomica and biological synthesis of SNPs and screening of SNPs for microbial efficacy.

**MATERIAL AND METHODS**

**Plant material**
The fresh seed of Strychnos nux-vomica was collected in June 2013 from Nagaloty reserve Forest, Kurnool District of Andhra Pradesh, India. The seed were cleaned, finally distilled water dried at room temperature and ground to fine powder.

**Preparation of extract**
25 g of seed powder of Strychnos nux-vomica was taken into 250 ml conical flask and added 100 ml of sterile distilled water and boiled for 10 min at 100°C on water bath. Then plant material extracts were collected in separate conical flask by standard filtration method and stored in refrigerator for further use.

**Phytochemical screening**

**Preparation of 1 mM Silver nitrate solution**
1 molar silver nitrate stock solution was prepared by 1.7 g of AgNO₃ was dissolved in 10 ml distilled water. 1 mM solution was prepared by 1 ml of 1 M solution was made up to 100 ml with 99 ml of distilled water. This solution was stored in amber colored bottle for further use.

**Synthesis of silver nanoparticles**
SNPs were synthesized by using seed aqueous extract of Strychnos nux-vomica. The reduction of pure Ag²⁺ ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5⁰ h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Vis Spectrophotometer. The size and shape of SNPs were confirmed with AFM (Nanosurf ®, AG, Switzerland; Product: BTO2089, VI, 3RO).

**UV-Vis spectra analysis**
The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3 hrs. after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was carried out by using UV-Vis spectrophotometer (Systronics type 118).

**AFM analysis**
The silver nanoparticles extracted by the above protocol were visualized with an Atomic Force Microscope (AFM). A thin film of the sample was prepared on a glass slide by dropping 100 μl of the sample on the slide and was allowed to dry for 5 min, the slides were then scanned with the AFM (Nanosurf ® AG, Switzerland, Product: BTO2089, 3RO). Nanosurf ® Easyscan-2 software was used for the AFM Analysis (VIT, Vellore, Tamil Nadu).

**Microorganisms**
Pure cultures of Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi species of bacteria and Paecilomyces variotii, Penicillium rubrum and Aspergillus flavus species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India.
Antimicrobial activity
The antimicrobial activities of SNPs were carried out with paper disc diffusion method using nutrient agar medium and potato dextrose agar medium for bacterial and fungal cultures respectively. Zones of inhibition for control, SNPs and silver nitrate were measured after 24 h and 7 days and compared with standard drugs Gentamycin and Nystatin for bacterial and fungal growth respectively. The experiments were repeated thrice and mean values of inhibition zone diameter were presented.

RESULTS AND DISCUSSION
The ethnic groups (Chenchu, Sugali, Yerukala and Yanadi) of Kurnool District, Andhra Pradesh, India. Traditional healers of these groups have staunch confidence to treat leucoderma skin disease. Dry seeds ground, made into ash, mixed with neem oil and applied as an external application to treat leucoderma. This ethnomedicinal information was cross checked with Ayurvedic physicians, Sri Venkateswara Ayurvedic College, Tirupati, Andhra Pradesh, India for authentication. In this regard Strychnos nux-vomica seeds are extensively using in Ayurvedic medicines to treat leucoderma[2].

The secondary metabolites screening of Strychnos nux-vomica showed that the seed extract is rich in alkaloids, flavonoids, tannins and triterpenoids, glycosides, lignins, steroids and lacking of anthocyanins, anthraquinones, coumarins, emodins, fatty acids, leucoanthocyanins, phenols and reducing sugars and Saponins, (Table-1).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Secondary metabolites</th>
<th>Seed extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Anthocyanins</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Anthraquinones</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Emodins</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Fatty acids</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Lignins</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Leuco anthocyanins</td>
<td>-</td>
</tr>
<tr>
<td>11.</td>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>Reducing sugars</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>16.</td>
<td>Triterpenoids</td>
<td>++</td>
</tr>
</tbody>
</table>

Note: ‘+’ indicates presence, ‘++’ indicates presence of more amounts, ‘−’ indicates absence

Flavonoids, are medically used as antistomatic, diarrhoea and anti-inflammatory, anti cancer and anti oxidative. It also known to possess antiviral and anti fungal [19, 20] and antimicrobial properties[21]. The presence of bioactive compounds indicates the medicinal values of the plant. Steroids and triterpenoids possess anti bacterial activity [22]. Tannins possess astringent, anti-inflammatory, anti diarrhoeal, antioxidant and antimicrobial activities [23]. Similar chemical constituents were also found in Shorea tumbuggaia [24], Thespesia populnea [25], in Curcuma longa [26] and in Psoralea corylifolia [27, 28].
In the present study SNPs were synthesized by using seed aqueous extract of *Strychnos nux-vomica* rapidly with in 15 min was able to be followed by color change. The fresh suspension of *Strychnos nux-vomica* was cream in color. However, after adding of Ag (NO$_3$)$_2$ the sample turned to dark brown color. The colour change in aqueous solution is due to the surface- Plasmon resonance (SPR) phenomenon (Fig-1). The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrums of colloidal solutions of SNPs synthesized from the seed extract *Strychnos nux-vomica* have the characteristic absorbance peaks at 260 and 430 nm respectively (Fig-1(b)). This illustrated the presence of homogenous distribution of hydrosol SNPs after 15 min of stirring [29].

It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape of nanoparticles in aqueous suspension [30]. The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions same results observed in *Boswellia ovalifoliolata* stem seed [31]. Silver nanoparticles have free electrons, which give rise to an SPR absorption bonds [32], due to the combined vibration of electrons of metal nanoparticles in resonance with the light waves [33, 34]. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles.

The size and shape of SNPs was detected by using AFM (Atomic Force Microscope). Size of SNPs was 41.72 nm, spherical in shape (Fig-1(d)). The result obtained in this study is interesting because it can serve as a foundation in terms of identification of potential forest plants for synthesizing SNPs.

The seed extract of *Strychnos nux-vomica* SNPs showed highest percentage of bacterial inhibition zones both gram-positive (*Staphylococcus* (10.4±0.28), *E.coli* (9.8±0.23) and *Bacillus* (9.5±0.52) and gram-negative (*Salmonella* (8.9±0.22) and (Table-2), (Graph-1) and (Fig-3). The results were compared to that of standard antibacterial antibiotic Gentamycin. The antifungal activity was studied and the results were compared to that of antifungal antibiotic Nystatin. The result showed that *Penicillium rubrum* (9.3±0.55) has sensitive followed by *Paecilomyces variotii* (8.3±0.40) and *Aspergillus flavus* (8.1±0.42). The maximum toxicity was observed in SNPs of *Strychnos nux-vomica* that Ag(NO$_3$)$_2$. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction.

The results were compared to that of standard antibiotics Gentamycin / Nystatin anti bacterial and antifungal respectively. Standard drugs (Gentamycin / Nystatin), showed higher inhibition zones, because these are highly purified forms which may be cost and leads to side effects in high dosage, whereas the SNPs are biologically synthesized form with less in cost, eco-friendly, safe and pollutant free with less or no side effects.

In general, gram-positive bacteria appeared to be more tolerant to silver than gram-negative cells. The cell wall of gram-positive bacteria contains multiple layers of peptidoglycon compared to the cell wall of gram-negative bacteria. Thus, gram-positive bacteria may allow less Ag$^+$ to reach the cytoplasmic membrane than gram-negative bacteria [35]. The SNPs are also reported to be nontoxic to human and most effective against bacteria, viruses and other eukaryotic micro-organisms at very low concentrations and without any side effects [36].

The results showed seed extract of *Strychnos nux-vomica* SNPs could be used as an eco-friendly antimicrobial agent in the control of bacterial and fungal diseases. Biologically synthesized SNPs are less in cost, eco-friendly, safe and pollutant free with less or no side effects.

**CONCLUSION**

The present study includes the treatment of leucoderma using *Strychnos nux-vomica* by the ethnic groups. Phytochemical screening indicates that the plant part is a good source for bio active principle for pharmacognostic and pharmaceutical industries. The SNPs prepared by using the aqueous seed extract of *Strychnos nux-vomica*. The aqueous silver ions exposed to the extracts, the synthesis of SNPs were confirmed by the change of color of plant extracts. These environmentally benign SNPs were further confirmed by using UV-Vis spectroscopy finally the size and shape of the SNPs was characterized by AFM.
analysis. The results indicated that SNPs have good antimicrobial activity against different microorganisms due to the cumulative effect of secondary metabolites or active molecules present in the plant extract of selected medicinal plant used by ethnic groups of Kurnool district of Andhra Pradesh, India to cure skin diseases. It is confirmed that SNPs of *Strychnos nux-vomica* are capable of rendering antimicrobial efficacy and hence has a great potential in the preparation of drugs used against bacterial and fungal diseases.

**ACKNOWLEDGEMENTS**

Authors are highly thankful to Ethnic groups of Kurnool district for providing valuable ethnic information and to VIT University, Tamil Nadu for AFM studies. The authors are highly thankful to DST for financial assistance.

### Table-2: Antimicrobial activity of SNPs isolated from seed extract of *Strychnos nux-vomica*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microorganisms</th>
<th>Inhibition zone in mm</th>
<th></th>
<th></th>
<th>Standard: Gentamycin/Nystatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ag(NO₃)₂</td>
<td>Plant extract control</td>
<td>SNPs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.6±0.26</td>
<td>5.4±0.41</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td></td>
<td>5.5±0.22</td>
<td>4.3±0.16</td>
</tr>
<tr>
<td>2</td>
<td><em>Salmonella typhi</em></td>
<td></td>
<td></td>
<td>6±0.16</td>
<td>5.6±0.53</td>
</tr>
<tr>
<td>3</td>
<td><em>E.coli</em></td>
<td></td>
<td></td>
<td>6.4±0.64</td>
<td>6.8±0.26</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacillus</em></td>
<td></td>
<td></td>
<td>6±0.52</td>
<td>5.3±0.80</td>
</tr>
<tr>
<td>5</td>
<td><em>Paecilomyces varioti</em></td>
<td></td>
<td></td>
<td>6.6±0.52</td>
<td>6±1.34</td>
</tr>
<tr>
<td>6</td>
<td><em>Pencillium rubrum</em></td>
<td></td>
<td></td>
<td>6.5±0.44</td>
<td>6.1±0.61</td>
</tr>
<tr>
<td>7</td>
<td><em>Aspergillus flavus</em></td>
<td></td>
<td></td>
<td>6.4±0.64</td>
<td>6.8±0.26</td>
</tr>
</tbody>
</table>

**Graph-1: Antimicrobial activity of SNPs isolated from seed extract of *Strychnos nux-vomica***
Fig-1: The color change of seed extract of Strychnos-nux-vomica
(1) Plant extract without silver nitrate (2) leaf extract with 1 mM silver nitrate; b) UV-Vis spectroscopy of synthesized silver nanoparticles, (c, e & f) AFM of Topography of SNPs (d) Three dimensional structure of SNPs
Fig-3: Antimicrobial activity of seed extract of Strychnos-nux-vomica

a. Staphylococcus aureus, b. Salmonella typhi, c. Bacillus, d. Escherichia coli

e. Paecilomyces variotii, f. Pencilium rubrum, g. Aspergillus flavus

1-Ag(NO$_3$)$_2$, 2-plant extract, 3-SNPs, S-Gentamycin/ Nystatin
REFERENCES


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