SYNTHESIS OF SILVER NANOPARTICLES AND ANTI MICROBIAL ACTIVITY FROM 
CADABA FRUTICOSA – AN IMPORTANT ETHNOMEDICINAL PLANT TO TREAT 
VITILIGO OF KURNOOL DISTRICT, ANDHRA PRADESH, INDIA.

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<th>ARTICLE INFO</th>
<th>ABSTRACT</th>
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<td><strong>Article history</strong></td>
<td><em>Cadaba fruticosa</em> leaves 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 leaf aqueous extract of <em>Cadaba fruticosa</em>. 10 ml of leaf extract was mixed to 90 ml of 1 mM aqueous of Ag(NO$_3$)$_2$ and was heated at 60-80°C for 20 min. The color of aqueous solution changes to dark brown color. For characterization using UV-Vis spectrophotometer and AFM are used. AFM, UV-Vis spectrophotometer showed the formation of silver nanoparticles with spherical shape and average size of 35.01 nm. 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.</td>
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<td>Medicinal plant; Silver nanoparticles; Atomic Force Microscope (AFM); Inhibition zone; Secondary metabolites; Anti microbial efficacy.</td>
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INTRODUCTION

The globe endowed with a rich variety of life forms and the teeming millions of these living organisms been well-knit by the laws of nature. 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, Sidha and Unani.

WHO is encouraging the traditional drugs because of its less side effects and matter of low cost and easy availability [2]. WHO also has organized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines [3]. *Cadaba fruticosa* Known for its medicinal values in alternative systems of (Ayurveda, Unani, Sidha, Homeopathy and Chinese). It is commonly known as Indian *Cadaba* belongs to the family Capparidaceae. The leaves and roots are considered deob-struent, anthelmintic and emmenagogue and are prescribed in the form of a decoction for treating uterine obstructions. The leaves of Indian *Cadaba* are also used as a poultice to promote healing of sores. In *Sidha*, the leaf and fruit are used to treat worm infestation, swellings, eczema and constipation. The leaves are used to treat eczema [4].

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 *Cadaba fruticosa* leaves 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]. *Cadaba fruticosa* leaf powder contains a variety of important chemical compounds. It consists Cadabicine, Capparisine, Cadubicilone, α-β- Dihydroferulic acid [7,8].

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 [9]. Silver a nobel metal is known to improve the immunity since ancient times, Ag(NO₃)₂ was using for biosynthesis of nanoparticles by using leaf aqueous extract of *Cadaba fruticosa*. The possibility of using plant materials for the synthesis of nanoscale metals was initially reported by Gradea-Torresdey [10-11]. SNPs have particular properties that may perhaps have numerous applications in the field of dentistry, clothing, catalysis, mirrors, optics, photograpy, electronics and food industry [12].

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* [13].

The present work is aimed of these objectives. Ethno-medico botanical studies Qualitative analysis of phytochemical constituents by using leaf extract of *Cadaba fruticosa*. To test for the biological synthesis and Characterization of SNPs. To screen the SNPs for microbial efficacy.

MATERIAL AND METHODS

Plant material

The fresh leaves of *Cadaba fruticosa*. was collected in July 2013 from Nagarjuna Tiger Reserve Forest, Kurnool District of Andhra Pradesh, India. The leaves were cleaned, finally distilled water dried at room temperature and ground to fine powder.

Preparation of extract

25 g of leaf powder of *Cadaba fruticosa*. 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 [27] and stored in refrigerator for further use.

Phytochemical screening

10 ml leaf extract was used for preliminary phytochemical screening. The qualitative analysis of secondary metabolites was carried out by using the methods [14], for flavonoids [15], for steroids, alkaloids and phenols [16], for triterpenoids and glycosides[17], for tannins, anthraquinons, leucoanthocyanins and emodins [18], for saponins, reducing sugars and anthocyanin [19].

Preparation of 1 mM Silver nitrate solution

1 molar silver nitrate stock solution was prepared by using 1.7 g of AgNO₃ was dissolved in 10 ml distilled water. 1 mM solution was prepared by taking 1 ml of 1 M solution of AgNO₃ and 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 leaf aqueous extract of *Cadaba fruticosa*. The reduction of pure Ag²⁺ ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5th 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 (Nano surf ® 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, Pencillium 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 [27]. 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. Fresh leaves ground into paste and applied as an external application on depigmentation spots on the skin 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 *Cadaba fruticosa* leaves are extensively using in Ayurvedic medicines to treat leucoderma.

The secondary metabolites screening of *Cadaba fruticosa*. Showed that the leaf extract is rich in alkaloids, flavonoids, glycosides, steroids, phenols, reducing sugars, saponins and triterpenoids and lacking of anthocyanins, anthraquinones, coumarins, emodins, fatty acids, lignins, leucoanthocyanins, and tannins (Table-1).

### Table-1: Secondary metabolites of leaf extract of *Cadaba fruticosa*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Secondary metabolites</th>
<th>leaf 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 [20-21] and antimicrobial properties [22]. The presence of bioactive compounds indicates the medicinal values of the plant. Steroids possess ant bacterial activity [23]. Similar chemical constituents were also found in *Shorea tumbuggaia* [24], *Thespesia populnea* [25], in *Curcuma longa* [26], in *Psoralea corylifolia* [27], in *Withani somnifera* [28] and in *Plumbago zeylanica* [1].
In the present study SNPs were synthesized by using leaf aqueous extract of *Cadaba fruticosa* rapidly with in 15 min was able to be followed by color change. The fresh suspension of *Cadaba fruticosa* was cream in color. However, after adding of Ag (NO$_3$)$_2$ the sample turned to dark brown color. The color change in aqueous solution is due to the surface- Plasmon resonance (SPR) phenomena [32](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 leaf extract *Cadaba fruticosa* have the characteristic absorbance peaks at 280 and 310 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 whereas same results observed in *Boswellia ovalifoliolata* stem bark [31]. Silver nanoparticles have free electrons, which give rise to 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 35.01 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.

**Fig-1**: The color change of leaf extract of *Cadaba fruticosa* (1) plant extract without silver nitrate (2) leaf extract with 1 mM silver nitrate; b) UV-Vis spectroscopy of synthesized of silver nanoparticles, (c, e & f ) AFM of Topography of SNPs (d) Three dimensional structure of SNPs
The leaf extract of *Cadaba fruticosa* SNPs showed highest percentage of bacterial inhibition zones both gram-positive \((Staphylococcus (8.7±0.30)\) and *Bacillus (9.6±0.44)\) and gram-negative \((Salmonella (11.3±0.40)\) and *E.coli (12.2±0.43)\), (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 *Pencillium rubrum* \((9.5±0.66)\) has sensitive followed by *Paecilomyces varioti* \((9.5±0.44)\) and *Aspergillus flavus* \((7.1±0.61)\). The maximum toxicity was observed in SNPs of *Cadaba fruticosa* use 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 that of 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 leaf extract of *Cadaba fruticosa*. 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.

Table-2: Antimicrobial activity of SNPs isolated from leaf extract of *Cadaba fruticosa*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microorganisms</th>
<th>Inhibition zone in mm</th>
<th>Ag(NO(_3))(_2)</th>
<th>Plant Extract control</th>
<th>SNPs</th>
<th>Standard: Gentamycin/ Nystatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td><em>Staphylococcus aureus</em></td>
<td>7.5±0.23</td>
<td>8.2±0.27</td>
<td>8.7±0.30</td>
<td>11.8±0.58</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><em>Salmonella typhi</em></td>
<td>6.5±0.27</td>
<td>5.4±0.33</td>
<td>11.3±0.40</td>
<td>14.2±0.36</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><em>E.coli</em></td>
<td>6.8±0.37</td>
<td>6.2±0.28</td>
<td>12.2±0.43</td>
<td>12.8±0.51</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td><em>Bacillus</em></td>
<td>5.8±0.44</td>
<td>5.4±0.37</td>
<td>9.6±0.44</td>
<td>11.8±0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fungal species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><em>Paecilomyces varioti</em></td>
<td>8.8±0.55</td>
<td>7.2±0.66</td>
<td>9.5±0.44</td>
<td>12.5±0.60</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td><em>Pencillium rubrum</em></td>
<td>7.2±0.52</td>
<td>7.4±0.74</td>
<td>9.5±0.66</td>
<td>13±0.46</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td><em>Aspergillus flavus</em></td>
<td>6.5±0.47</td>
<td>5.5±0.55</td>
<td>7.1±0.61</td>
<td>11.3±0.62</td>
<td></td>
</tr>
</tbody>
</table>

[Graph-1 and Fig-3]

**Table-2: Antimicrobial activity of SNPs isolated from leaf extract of *Cadaba fruticosa***
Graph-1: Antimicrobial activity of SNPs isolated from leaf extract of *Cadaba fruticosa*

Fig-3: Antimicrobial activity of leaf extract of *Cadaba fruticosa*


e. *Paecilomyces variotii*, f. *Penicillium rubrum*, g. *Aspergillus flavus*

1. Ag(NO$_3$)$_2$, 2. plant extract, 3. SNPs, 4. Gentamycin/Nystatin

CONCLUSION

The present study includes the treatment of vitiligo using *Cadaba fruticosa* by the ethnic groups. Phytochemical screening indicates that the plant part is a good source for bioactive principle for pharmacognostic and pharmaceutical industries. The SNPs prepared by using the aqueous leaf extract of *Cadaba fruticosa*. 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 Cadaba fruticosa are capable of rendering antimicrobial efficacy and hence has a great potential in the preparation of drugs used against bacterial and fungal diseases.

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COMPETING INTEREST

The author declare no conflict interest.

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