ANTI-TUBERCULAR ACTIVITY OF SILVER NANOPARTICLE SYNTHESIZED FROM THE FRUITS OF CORIANDRUM SATIVUM LINN

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ABSTRACT
Tuberculosis has become a major global health problem mainly in developing countries of Asia and Africa including India. Due to the usage of antibiotics, challenge of multidrug resistant TB has increased drastically. So, there is a need for discovery of new anti-TB drugs which are safe, effective and affordable. Medicinal plants have known to be the potential source for the treatment of many ailments. Hence, the present study is designed to investigate the in vitro activity of silver nanoparticle synthesised from the fruits of Coriandrum sativum and methanol and aqueous extracts of the fruit have been evaluated against Mycobacterium tuberculosis H73Rv strain using Micro plate Alamar Blue Assay (MABA). The results revealed that the silver nanoparticle showed sensitivity against M. tuberculosis strain at a concentration 1.6 µg/ml, while methanol extract and aqueous extracts were resistant even at 100 µg/ml concentration. The present investigation suggests that silver nanoparticles synthesized from the fruit of Coriandrum sativum possess remarkable anti-tubercular activity.

KEYWORDS: Mycobacterium tuberculosis, silver nanoparticle, Coriandrum sativum fruit, methanol extract, aqueous extract, in vitro, MABA, H37Rv.

INTRODUCTION
Tuberculosis (TB) is one of the leading infectious disease caused by Mycobacterium tuberculosis and health burden in the world. It typically affects the lungs but can affect other sites as well. In 2014, nearby 80% of reported TB cases occurred in 22 countries and India
has the largest number of cases, 23% of the global total\textsuperscript{[1]}. In India, nearly 50% of patients is reported to be tuberculin test positive and one person dies from TB every minute\textsuperscript{[2,3]}. However, Due to the usage of antibiotics, challenge of multidrug resistant TB has increased drastically. So, there is a need for discovery of new anti-TB drugs which are safe, effective and affordable. Medicinal plants have known to be the potential source for the treatment of many ailments.\textsuperscript{[4,5]} India is one of the countries with unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for curing several diseases\textsuperscript{[6,7]}.

In recent years, researchers in the field of nanotechnology are finding that there is an expanding research in the synthesis of metal nanoparticles due to the potential applications for the development of novel technologies. Noble metal nanoparticles are extensively studied because of their wide applications\textsuperscript{[8-10]}. Among the various noble metal nanoparticles, gold and silver have several applications in sensors, detectors, and antibacterial agents\textsuperscript{[11-13]}. Though numerous chemical methods are available for metal nanoparticles synthesis, copious reactants and starting materials are used in these reactions that are toxic and potentially hazardous. Increasing environmental concerns over chemical synthesis routes have resulted in attempts to develop bio-mimetic approaches. One of them is the synthesis using plant extracts eliminating the elaborate process of maintaining the microbial culture and often found to be kinetically favorable than other bioprocesses. Bio-molecules as reducing agents are found to have a significant advantage over their counterparts as protecting agents.

Environmentally nanoparticles synthesis procedures do not use any toxic chemicals in the synthesis protocols. In these aspects synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining these nanoparticles. The spectacular success in this field has opened up the prospect of developing bio-inspired methods of synthesis of metal nanoparticles with tailor-made structural properties. Among the various bioreductants, \textit{Coriandrum sativum} leaves extract was chosen for the present study since they have minerals and vitamin contents including calcium, phosphorus, iron, carotene, thiamine, riboflavin, and niacin.

Coriander [\textit{Coriandrum sativum} Linn.] an annual of the Apiaceae family is one of valuable medicinal and seasoning plant. This species comes from the Mediterranean region and it is grown all over the world. The coriander fruit and essential oil isolated from it are used for medicinal purpose. It is used to treat menstrual disorder, secondary infertility, ovaritis and cervicitis. It is used to treat female diseases such as menoxenia, ovulation type dysfunctional
uterine bleeding\textsuperscript{[14]}. It is aphrodisiac to enhance sexual function and reproductive capacity. It is used for treating leucorrhea; spermatorrhea. Coriander fruit possess stimulant and carminative properties\textsuperscript{[15]}. Its oil is bactericidal and larvical\textsuperscript{[16]}. It is hypoglycemic and anti-inflammatory\textsuperscript{[17]}. The fruits are used as astringent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic, expectorant, anodyne, antidiabetic and dyspepsia\textsuperscript{[18,19]}. The author has already reported simple and eco-friendly biosynthesis of silver nanoparticles using the fruits of \textit{Coriandrum sativum} Linn and its determination of in \textit{vitro} antioxidant activity\textsuperscript{[20]} and antibacterial activity\textsuperscript{[21]}.

Hence, the present study is designed to investigate the \textit{in vitro} activity of silver nanoparticle synthesised from the fruits of \textit{Coriandrum sativum} and methanol and aqueous extracts of the fruit have been evaluated against \textit{Mycobacterium tuberculosis} H73Rv strain using Micro plate Alamar Blue Assay (MABA).

2. MATERIAL AND METHODS

2.1 Plant material

The \textit{Coriandrum sativum} fruits were collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by Botanist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. The fruits were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in air tight container for further use.

2.2 Preparation of fruit extract

Coarsely powdered fruits of \textit{C.sativum} 50 g, each were boiled with methanol and water [200 ml] respectively for 30 minutes and filtered and make up the volume to 100 ml with methanol and water respectively. The solution is preserved for further use.

2.4 Synthesis of nanoparticles

Synthesis for silver nanoparticles from the fruits of \textit{Coriandrum sativum} is prepared\textsuperscript{[20]}.

\textbf{Anti-tubercular activity}

The \textit{in vitro} activity of silver nanoparticle synthesised from the fruits of \textit{Coriandrum sativum} and methanol and aqueous extracts of the fruit have been evaluated against \textit{Mycobacterium tuberculosis} H73Rv strain using Micro plate Alamar Blue Assay (MABA). This methodology
is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The drug Pyrazinamide was used as positive standard for comparison[22].

3. RESULTS AND DISCUSSION

Medicinal plants provide numerous examples of interesting secondary metabolites with antimycobacterial activity, indicating that natural products could be a helpful for the discovery of new anti- TB agents. Medicinal plants produce an excessive diversity of phytochemicals that could be active in medicinal field. There is an increasing demand of medicinal plants all over the world because the allopathic medicines have more side effects[23]. A diversity of methods has been developed to measure the sensitivity of *M. tuberculosis*. It is interesting to mention that some medicinal plants may not contain phytochemicals which inhibit the growth of *M. tuberculosis*, but may have stimulant or modulatory effects on the immune system[24]. In present study the anti-tubercular activity was assessed by Micro-plate Alamar blue assay (MABA), using Alamar blue as the dye. The (MABA) Assay is a colorimetric oxidation reduction based assay in which the Alamar blue dye accepts electrons and changes from the oxidized, non-fluorescent blue state to the reduced fluorescent pink state. In addition to mitochondrial reductase, other enzymes such as the diaphoreses, dihydrolipoamine dehydrogenase, quinoneoxidoreductase and flavin reductase located in the cytoplasm and the mitochondria may be able to reduce Alamar blue reagent[25]. The anti-tubercular potential of silver nanoparticle synthesised from the fruits of *Coriandrum sativum* and methanol and aqueous extracts of the fruit which inhibited the growth of M. tuberculosis H37Rv strain at given in the Table 1. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth [Fig 1 and Fig 2].
The qualitative visible change in colour indicates on presence or absence of viable cells. Standard drug used were Pyrazinamide, Streptomycin and Ciprofloxacin. Only nanoparticle synthesised had antitubercular activity with an MIC value of 1.56 µg/ml and methanol and aqueous extract were resistant from 100 to 1.56 µg/ml. The MIC values for standard were found to be Pyrazinamide 3.125 µg/ml, Streptomycin 6.25 µg/ml and Ciprofloxacin 3.125 µg/ml respectively.

![STANDARD DRUG PHOTOGRAPH](image)

**Fig. 1 Standard drug photograph**

MIC values of Pyrazinamide- 3.125 µg/ml; Streptomycin- 6.25 µg/ml and Ciprofloxacin- 3.125 µg/ml.

![Concentrations used for anti-tubercular activity](image)

**Fig. 2: Concentrations used for anti-tubercular activity**

CS-01: Methanol extract; CS-02: Aqueous extract and CS-03: silver nanoparticle synthesized from *C.sativum*
Table 1: Result of anti-tubercular activity of silver nanoparticle and methanol and aqueous extract of *C. sativum*

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Sample</th>
<th>100 μg/ml</th>
<th>50 μg/ml</th>
<th>25 μg/ml</th>
<th>12.5 μg/ml</th>
<th>6.25 μg/ml</th>
<th>3.12 μg/ml</th>
<th>1.6 μg/ml</th>
<th>0.8 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CS-01</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CS-02</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CS-03</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
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</tr>
</tbody>
</table>

S – Sensitive; R – Resistance. Strain used: *M. tuberculosis* (H37RV strain).

CS-01: Methanol extract; CS-02: Aqueous extract and CS-03: silver nanoparticle synthesized from *C. sativum*

4. CONCLUSION

TB has become a serious infection as *Mycobacterium tuberculosis* develops resistance against both first and second-line anti-TB drugs. Due to this, there is an urgent need for novel anti-tuberculosis agents which should be sensitive. Hence, it is concluded that silver nanoparticle synthesised from the fruits of *Coriandrum sativum* only have shown antimycobacterial activity. Both the extracts were inactive or resistant. The MIC value of silver nanoparticle was better than the standard used for the treatment.

5. ACKNOWLEDGEMENT

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6. FUNDING

Nil

7. ETHICAL ISSUES

There is none to be applied.

8. CONFLICT OF INTEREST

None to be declared.
9. REFERENCES