

SYNTHESIS OF SILVER NANOPARTICLES USING *MORINGA OLEIFERA* SEEDS, *GLYCYRRHIZA GLABRA* STEMS, AND ITS ANTI-METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ACTIVITY

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Received: 03 August 2018, Revised and Accepted: 01 October 2018

ABSTRACT

Objective: The present study focused on synthesizing silver nanoparticles (AgNO_3) using *Moringa Oleifera* seeds and *Glycyrrhiza glabra* stems.

Methods: The synthesized AgNO_3 were used to evaluate the antibacterial efficacy of methicillin-resistant *Staphylococcus aureus* (MRSA) by agar well diffusion method. AgNO_3 were produced from silver nitrate using *M. oleifera* seeds and *G. glabra* stems.

Results: AgNO_3 were characterized using UV-visible spectroscopy at a wavelength of 400–460 nm. AgNO_3 from *G. glabra* stems showed high anti-MRSA activity with 32 mm zone of inhibition, and *M. oleifera seed extract* showed 30 mm inhibition zone of inhibition against MRSA.

Conclusion: The biologically synthesized AgNO_3 can be used against MRSA.

Keywords: Methicillin-resistant *Staphylococcus aureus*, Silver nanoparticles, *Glycyrrhiza glabra*, *Moringa oleifera*.

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INTRODUCTION

Nanotechnology is the study and application of tiny objects that may be used across different fields such as chemistry, biology, physics, and engineering. Nanoparticle is a major particle which performs as a whole unit in terms of transport and property. Nano means a billionth or 10^{-9} units. Its size ranges from 1 to 100 nm because it is too small in size and it occupies a position in various fields of nanoscience and nanotechnology [1]. Silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics; catalysis and microelectronics. Nowadays, there are several methods for the production of nanoparticles such as chemical and physical methods [2].

Staphylococcus aureus is an important cause of serious infections in both hospitals and community. Methicillin-resistant *S. aureus* (MRSA) include those strains that have acquired a gene giving them a resistance to methicillin antibiotics and essentially all other beta-lactam antibiotics. MRSA was first reported in 1961, soon after methicillin was introduced into the human medicine to treat penicillin-resistant *Staphylococci* [3].

Moringa oleifera tree is one of the world's most useful trees; in the tropics, it is used as the forage for the livestock; and in many countries, it is used as micronutrient powder; to treat various ailments [4]. The plant is highly valued since almost every part of the tree (leaves, roots, bark, fruits, flowers, immature pods, and seeds) is used as food with high nutritional value. In addition, the plant has been reported to possess antimicrobial properties, and this explains the reason for its wide use in the treatment of human diseases [5].

Glycyrrhiza glabra Linn., family Fabaceae, is the medicinal plant found in Asia, the Mediterranean, and parts of Southern Europe. It is commonly known as Licorice, Liquorice, sweet wood, Mulahatti, and Yastimadhu. The underground unpeeled or peeled stems or roots are used for the treatment of upper respiratory tract ailments including coughs, hoarseness, sore throat, and bronchitis. Ayurveda considers the licorice to be a "rasayana" with implication in the treatment of respiratory and

digestive disorders [6]. Silver nanoparticles (AgNO_3) can be used to treat bacterial diseases [7]. Biosynthesized AgNO_3 had several applications in pharmaceutical and large-scale commercial production [8].

The present study focused on synthesizing AgNO_3 using *M. oleifera* seeds and *G. glabra* stems. Synthesized AgNO_3 were used to evaluate the antibacterial efficacy of MRSA.

METHODS

Collection of plant materials

G. glabra stems and *M. oleifera* seeds were purchased from traditional medicine shop Ellispettai, Erode, Tamil Nadu, India. Collected plant material was washed with water and dried at room temperature. The dried sample was homogenized into a fine powder and used as a raw material and stored in airtight container for further use Fig. 1.

Cold aqueous extraction

5.0 g of dried powder of *G. glabra*, and *M. oleifera* was suspended in 50 ml cold distilled water, and the mixture was soaked for 24 h. The suspended solid was filtered through Whatman No.1 filter paper, and the dried crude extracts were stored at 4°C for further use.

Bacterial strain isolation

A total of 5 strains of wound pathogens such as *S. aureus* were isolated from patients infected with wounds. Isolates were maintained at 4°C in nutrient media.

Isolation of pathogens

An aseptically collected wound samples were inoculated with the help of standard inoculating loop on the EMB, MacConkey, and nutrient Agar. The plates were incubated for 24 h at 37°C . Following incubation, the growth of bacterial colonies was observed and the results were recorded.

Identification of pathogens

The isolated pathogens were identified on the basis of Gram's reaction and biochemical characteristics (Mac Faddin, 1980), and the results

were identified with the help of Bergey's Manual of Systematic Bacteriology.

Synthesis of AgNO₃

For the synthesis of AgNO₃, stock solution was prepared by dissolving 1 mM silver nitrate (AgNO₃; Merck, Mumbai, India) and volume made up to 150 ml with distilled water. 5 ml cold water extract of *M. oleifera* seeds and *G. glabra* stems was added to 10 ml of 1 mM AgNO₃ solution and allowed to react at room temperature.

Characterization of AgNO₃ UV-visible spectroscopy

The periodic scans of the optical absorbance between 300 and 600 nm with a UV-visible spectrophotometer (ELICO SL159) at a resolution of 1 nm were performed to investigate the reduction rate of silver ions by extract of *M. oleifera* seeds and *G. glabra* stems.

Antibacterial activity of synthesized AgNO₃ (agar well diffusion method)

The antibacterial activities of AgNO₃ were carried out against pathogenic strains of MRSA, by agar well diffusion method. Nutrient

agar medium plates were prepared, sterilized, and solidified. After solidification, the formed agar well (6 mm in diameter) was added with 100 µl of each extract solution, separately to each well. The plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zones of inhibition of AgNO₃ against the tested bacteria.

RESULTS AND DISCUSSION

Identification and biochemical characterization of bacterial isolates

From 15 bacterial strains of *S. aureus* isolated from wound samples, all the strains were identified as *S. aureus* based on the morphological and biochemical characters (Table 1)

Of these 15 strains, two strains were selected according to drug resistant and named as *S. aureus* strains 4 and 5.

Antimicrobial activity of synthesized AgNO₃ against MRSA

Addition of AgNO₃ to the cold water extracts of *M. oleifera* seeds and *G. glabra* stems extract resulted in the change of the plant extracts from colorless to pale-purple and brown, respectively Figs. 2 and 3.

The formation of nanoparticles in the *G. glabra* stems extract was indicated by the appearance of brown colour which exhibits surface



Fig. 1: (a) *Glycyrrhiza glabra* stems, (b) *Moringa oleifera* seeds



Fig. 2: Synthesized silver nanoparticles using *Moringa oleifera* seed extract and *Glycyrrhiza glabra* stem extract

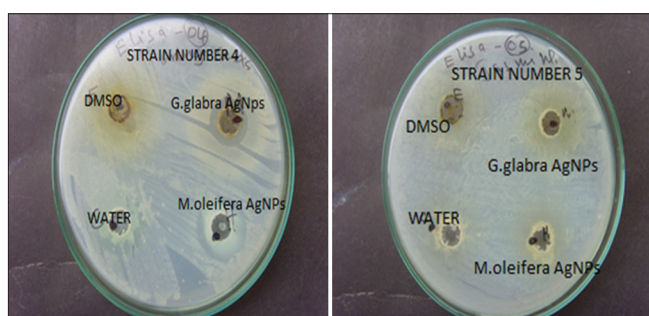


Fig. 3: Antimicrobial activities of synthesized silver nanoparticles from *Moringa oleifera* seeds and *Glycyrrhiza glabra* stems

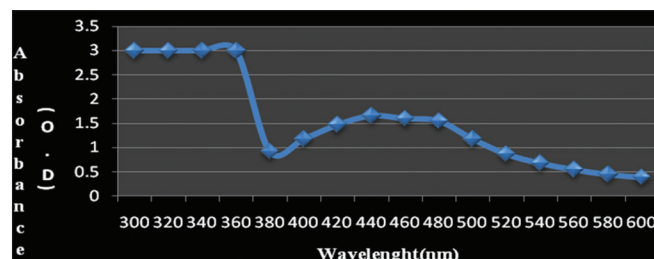


Fig. 4: Synthesized silver nanoparticles from *Glycyrrhiza glabra* stem extract.

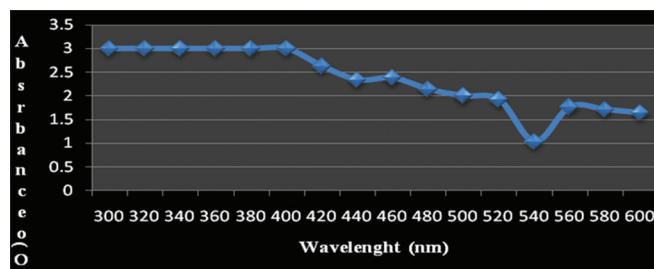


Fig. 5: The synthesized silver nanoparticles from *Moringa oleifera* seeds extract

Table 1: Biochemical characterization of *Staphylococcus aureus* isolated from clinical wound samples

Name of the test	Result
Gram stain	Gram positive
Indole test	-
Methyl red test	+
Voges-Proskauer test	-
Citrate test	+
Catalase test	+
Nitrate reduction test	+
TSI-Glucose	+
TSI-Mannitol	+
TSI-Lactose	+
TSI-Sucrose	+

+: Indicates presence and -: Indicates absence

Table 2: Antibacterial activity of synthesized AgNO₃ against MRSA

Strain	Plant extract	Zone of inhibition
<i>Staphylococcus aureus</i> -4 strain	<i>Moringa oleifera</i> seed cold extract	5
<i>Staphylococcus aureus</i> -4 strain	<i>Glycyrrhiza glabra</i> stem cold extract	11
<i>Staphylococcus aureus</i> -5 strain	<i>Moringa oleifera</i> cold extract	30
<i>Staphylococcus aureus</i> -5 strain	<i>Glycyrrhiza glabra</i> cold extract	32

MRSA: Methicillin-resistant *Staphylococcus aureus*, AgNO₃: Silver nitrate

Table 3: UV reading for AgNO₃ nanoparticles

S/NO	Nanometer	O.D of <i>Glycyrrhiza glabra</i> AgNO ₃ nanoparticles	O.D of <i>Moringa oleifera</i> AgNO ₃ nanoparticles
1	300	3	3
2	320	3	3
3	340	3	3
4	360	3	3
5	380	0.925	3
6	400	1.184	3
7	420	1.472	2.634
8	440	1.666	2.347
9	460	1.603	2.389
10	480	1.554	2.144
11	500	1.180	2.006
12	520	0.874	1.916
13	540	0.678	1.031
14	560	0.550	1.774
15	580	0.459	1.709
16	600	0.391	1.639

AgNO₃: Silver nitrate

plasmon vibrations of AgNO₃, *M. oleifera* seed cold water extract was added to the AgNO₃ solution and incubated; the mixture color was changed rapidly from the transparent color to pale-purple color due to the formation of AgNPs. The appearance of pale-purple color was due to the excitation of surface plasmon vibrations of AgNO₃ and supports that plants have strong reducing properties.

Synthesized AgNPs of methanol extract from *G. glabra* stems shown high antimicrobial activities in *S. aureus* 5 strains with inhibition zone of 32 mm, followed by 30 mm inhibition zone displayed by synthesized AgNPs from cold *M. oleifera* seeds extract on *S. aureus* 5 strains. *S. aureus* 4 strains showed very poor sensitivity reaction toward synthesized AgNPs from cold *M. oleifera* extract and *G. glabra* methanol extract with inhibition zones of 5 mm and 11 mm, respectively (Table 2).

Characterization of AgNO₃ UV-visible spectroscopy

The characterization of synthesized AgNPs was done by UV-visible spectrophotometer, and the recorded UV-absorption spectra exhibited an intense peak at 400 nm for *G. glabra* stem extracts and 460 for *M. oleifera* seeds Figs. 4 and 5. The results are given in Table 3.

CONCLUSION

The biologically synthesized AgNO₃ could be of immense use in the medical field for their efficient antimicrobial function. These findings suggested synthesized AgNO₃ from *G. glabra* extract as a potential antibacterial candidate for the superficial MRSA infection and other bacterial infections.

AUTHORS' CONTRIBUTIONS

All the authors contributed equally.

CONFLICTS OF INTEREST

There are no conflicts of interest among the authors.

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