SYNTHESIS OF PLANT-MEDIATED SILVER NANOPARTICLES USING COMMIPHORA WIGHTII (GUGGUL) EXTRACT AND STUDY THEIR ANTIBACTERIAL ACTIVITIES AGAINST FEW SELECTED ORGANISMS

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ABSTRACT

The primary objective of this investigation is to green synthesis of silver nanoparticles by using Commiphora wightii leaves extracts and to evaluate their antimicrobial activity against few selected organisms. The original suspension of plant extracts was yellowish-green in colour. However, after addition of AgNO3 within 20mins, the suspension showed colour change and turned dark brown after 5 hrs of incubation at room temperature. UV-Vis spectral analysis in the range of 200-600 nm, confirmed the formation of silver nanoparticles. It is a well-known, that silver ions and nanoparticles are very much potential antimicrobial molecule as they possess many inhibitory and bactericidal effects and so it can be used as an antibacterial agent. The antibacterial activity of silver nanoparticles is proved by the zone of inhibition in nutrient agar media. The silver nanoparticles biosynthesized by Commiphora wightii plant leaves extracts showed antibacterial activity against three selected Gram Negative organisms and one Gram Positive organisms such as Escherichia coli, Pseudomonas aeruginosa, Baccillus subtilis and Staphylococcus aureus. However, the antimicrobial effect varied with the variation in salt concentration. Additionally, the silver nanoparticles by Commiphora wightii leaves extracts showed good inhibition activity towards Escherichia coli and Pseudomonas aeruginosa. The use of silver nanoparticles in drug delivery systems might have good future.
KEYWORDS: Antimicrobial, Green-synthesis, Bactericidal effect, Leaf extract, Silver Nanoparticles.

INTRODUCTION
Nanotechnology is a field of research and innovation, which concerned with mainly materials and devices not only on the scale of atoms but also in molecules level. The promise of nanotechnology is enormous. It is a broad multi interdisciplinary area of research, development and industrial activity which has been growing rapidly worldwide for the last few decade (Ahmed et al., 2015). Green-synthesis of nanoparticles is now established as an emerging area of nanoscience research. Many plants like Carica papaya, Capsicum annum, Emblica officinalis, Hibiscus rosasinesis, Coriandrum sativum, Tamarindus indica etc. have shown the potential of reducing silver nitrate to form silver nanoparticles (Bhakya et al., 2015). Green synthesis has many advantages over chemical and physical method such as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Chandran et al., 2006). Silver is having inhibitory effect on microbes present in medical and industrial process, as reported by many researchers. The most important application of silver and silver nanoparticles is in the field of medical industry where it is used as topical ointments to prevent infection against burn and open wounds. Apart from these, biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens (Diptendu et al., 2017).

Commiphora wightii (Guggul) is a flowering plant in the family of Burseraceae. In present study, a cost effective and eco-friendly process for green synthesis of silver nanoparticles from AgNO₃ solution with the aqueous leaf extracts of Commiphora wightii (Guggul) is reported. The experimental parameter such as concentration of salt solution was varied, for optimizing the synthesis of the nanoparticles. Further these plant synthesized nanoparticles were evaluated for their enhanced antimicrobial activities against some pathogenic strains of bacteria.

MATERIALS AND METHODS
Preparation of plant leaf extracts
Twenty gram of fresh leaves of Guggul were taken and washed with distilled water separately. Leaves were cut into fine pieces and crushed with 100 ml sterile distilled water using motor and pistal. Contents were boiled with constant stirring for ten minutes. After
cooling contents were filtered with Whatman No.1 filter paper (pore size 25 μm). Dark yellow coloured extracts were obtained, which were used as reducing agent and stabilizer (Diptendu et al., 2017).

**Synthesis of Silver Nanoparticles**

10ml of Guggul leaves extract were added into 90 ml of aqueous solution of Silver nitrate for reduction into Ag\(^+\) ions separately and kept at room temperature for 5 hours. Different concentrations of silver nitrate were used to standardize the optimum concentration of silver nitrate for synthesis of silver nanoparticles. The concentrations ranged from 1mM, 2mM, 3mM and 4mM of silver nitrate. The colloidal suspension thus obtained was centrifuged at 3000 rpm for 20 min and the pellet obtained after discarding the supernatant was re-dispersed in sterile distilled water. The centrifugation process was repeated 2 to 3 times for the removal of any adsorbed substances on the surface of silver nanoparticles (AgNps). The synthesized nanoparticles were then used for the evaluation of their antimicrobial activity on selected bacterial species (Diptendu et al., 2017).

**Characterization of the synthesized silver nanoparticles**

The reduction of metallic Ag\(^+\) ions by plant extracts was monitored by measuring the UV-Vis spectrum after about 16 hours of reaction. A small aliquot was taken from the main reaction mixtures and it was diluted with sterile distilled water (1:5). Spectrum was then taken on a wavelength from 200nm to 800nm on UV-Vis spectrophotometer (Systronis Double beam spectrophotometer 2202) (Diptendu et al., 2017).

**Antibacterial assay**

The antibacterial activity of Guggul aqueous extracts and the Ag NPs developed at different AgNO\(_3\) concentrations (1, 2, 3, and 4mM) were evaluated against four types of bacteria. The antibacterial activity was performed using agar well diffusion method against different pathogenic microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* (Gram Negative) and *Staphylococcus aureus* (Gram Positive) and the inhibition zones (cm) were measured. The pure cultures of bacteria were subcultured on Mueller-Hinton agar (MHA). Each strain was inoculated uniformly onto the individual plates using sterile method. Wells of 5 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 25 μL of nanoparticle solution was filled onto each well on all plates. After incubation at 37°C for 24 hours, the diameter of zone of inhibition was measured in cm (Diptendu et al., 2017).
RESULTS AND DISCUSSION

Formation of silver nanoparticle is indicated by change in colour of solution. This colour change was observed within 20 min from addition of extract in the solution, which is indicating the formation of silver nanoparticles. The colour of the blank (pure AgNO₃) remains unchanged during the entire period which can be easily understand from the photographs.

Fig.1. Photograph showing color change of the Guggul extract upon the formation of Ag NPs from different concentrations of AgNO₃: (a) 1 mM, (b) 2 mM, (c) 3 mM, and (d) 4 mM.

The optical properties of AgNPs were evaluated by UV-Vis absorption spectroscopy to confirm the formation stability of metal nanoparticles. Due to surface plasmon resonance (SPR), a strong absorption of electromagnetic waves is exhibited by metal nanoparticles in the visible range. Because of the collective oscillations of the conduction electrons, the SPR phenomenon arises when nanoparticles are irradiated with visible light. It is well understood
that AgNps exhibit a yellowish-brown color in aqueous solution due to the excitation in UV-visible spectrum depending upon the particle size.

![UV-Vis spectra of the formed AgNPs by Guggul extract recorded as a function of AgNO₃ concentration.](image)

Fig.2. UV-Vis spectra of the formed AgNPs by Guggul extract recorded as a function of AgNO₃ concentration.

The presence of nanoparticles was confirmed by obtaining a spectrum in visible range of 200nm to 600nm. Optimum concentration for synthesis of nanoparticles was standardized using different concentrations of silver nitrate (1, 2, 3, and 4mM). The optimum concentration suitable for nanoparticles synthesis by Guggul extract found to be 3mM from the above picture (Fig. 2).

Green synthesized silver nanoparticles by this method were studied for antimicrobial activity against pathogenic bacteria by well diffusion method. It was found that silver nanoparticles have antibacterial activities at concentration of 25μl/well. AgNO₃ was used as a control. The silver nanoparticles biosynthesized by plant extracts showed inhibition zone against microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Baccillus subtilis* (Gram Negative) and *Staphylococcus aureus* (Gram Positive). Maximum zone of inhibition (MZI) are listed in Table I and Figure 3. From the tables, it is proved that the nanoparticles synthesized are good candidates for their usage as an antibacterial drugs in near future.
Fig. 3. Antibacterial activity of Guggul extract formed AgNPs against selected pathogens: A-AgNO₃; E-Guggul Extract; 1-1mM AgNP’s; 2-2mM AgNP’s; 3-3mM AgNP’s and 4-4mM AgNP’s.

Table No.I. Showing Maximum zone of inhibition (MZI) by AgNPs formed by Guggul leaves extract on selected pathogens.

<table>
<thead>
<tr>
<th>Organism</th>
<th>AgNO₃ (cm)</th>
<th>Extract (cm)</th>
<th>AgNP’s from different concentrations of AgNO₃ (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1mM</td>
</tr>
<tr>
<td>E.coli</td>
<td>-</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>S.aureus</td>
<td>-</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td>B.subtillis</td>
<td>-</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>-</td>
<td>0.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

DISCUSSION
Silver ion get reduced into silver nanoparticles upon exposure to the Guggul leaves extracts which intern could be followed by colour change. In our study, silver nanoparticles produced dark brown colour in aqueous solution. Reason of these characteristic colour variations is because of the excitation of the surface plasmon resonance present in the metal nanoparticles (Dheeban Shankar et al., 2014). The frequency and width of the surface plasmon absorption depends on the certain factors like size and shape of the metal nanoparticles, on the dielectric constant of the metal itself, and the surrounding medium influence (Karthick et al., 2011). Generally the UV–VIS spectroscopy could be applicable to findout the size and shape-controlling activities of nanoparticles in aqueous suspensions (Krishnaraj et al., 2010 ; Mock et al., 2002 and Shukla et al., 2012). We used the different concentrations of silver nitrate (1, 2, 3, and 4mM) for optimizing the concentration for synthesis of nanoparticles. The presence of nanoparticles was confirmed by obtaining a
spectrum in visible range of 200nm to 600nm. The optimum concentration suitable for nanoparticles synthesis by Guggul leaves extract was found to be 3mM (Fig.2).

We have studied here, the application of silver nanoparticles as an antimicrobial agent against four selected human pathogens. However, the antimicrobial effect varied with the variation in salt concentration. Additionally, the silver nanoparticles by Guggul leaves extracts showed good inhibition activity towards *E.coli* and *P. aeruginosa*.

The mechanism of inhibitory action of silver nanoparticles on microorganisms are of various reasons such as by their adhesion to the cell membrane and further penetration inside, or by interaction with phosphorus containing compounds like DNA disturbing the replication process, or preferably by their attack on the respiratory chain (Parashar *et al*., 2012 and Parial *et al*., 2012). It has also been expected that a strong interaction takes place between the silver ions and thiol groups of various important enzymes and made them inactive (Ramalingam *et al*., 2014 and Sable *et al*., 2012). Some studies reported that the permeability and respiration functions of the cell got altered due to the attachment of the nanoparticles on to the surface of the cell membrane (Srivastava *et al*., 2011 and Vanmathi Selvi *et al*., 2012). Experimental evidence strongly suggested that when organism treated with silver ions, DNA lost its replication ability, which indicates the loss of cell viability and finally cell death occur.

**CONCLUSION**

In our conclusion, we must delivered this message that the rapid biological synthesis of silver nanoparticles using Guggul extracts provides a stable, eco-friendly and efficient cost-effective route for drug delivery systems and so further research to study in near future.

**REFERENCES**


