Formulation and evaluation of antimicrobial gel embedded with plant derived silver nanoparticles

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ABSTRACT: In this article, a formulation of antimicrobial gel by using the biosynthesized silver nanoparticle (AgNPs) from Piper bettle L leaf extract. Most of the researchers have reported about the nontoxic biosynthesis of silver nanoparticles using several microorganism and plant extracts. These nanoparticles exhibit completely improved properties based on their characteristics - size, distribution, morphology and many applications. In this research, the synthesis of silver nanoparticles using Piper bettle L. Has investigated. We have synthesized silver Nano particles using silver nitrate solution with piper bettle L. Extract and characterized. Further, the synthesized nano particles were characterized by UV Vis spectroscopy, Dynamic light scattering, Particle size analysis, Zeta potential analysis, Fourier Transform Infrared, and Scanning Electron Microscopy analysis. The antimicrobial efficacy was also determined by disc diffusion method with Escherichia coli and Pseudomonas aeruginosa the silver nanoparticles showed high level of inhibition. The outcome of this research shows that biologically synthesized nanoparticle has more effect against various disease causing pathogens.

Keywords: Piper bettle L leaf; Biosynthesis; Silver Nanoparticles; Antimicrobial activity; and Antimicrobial gel

1 Introduction

The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticle show completely improved properties such as size, distribution and morphology of the particle etc., Novel application of nanoparticles and nanomaterial are emerging rapidly on various field.

Metal nanoparticles have a high specific surface area and possess unique characteristics like catalytic activity, optical, electronic, antibacterial and magnetic properties. They are gaining the interest of scientist for their novel methods of synthesis. Over the past few years, the synthesis of metal nanoparticles is an important topic of research in modern material science. Nano-crystalline silver particles find tremendous applications in the field of high sensitivity bimolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and microelectronics. However, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesis the silver nanoparticles. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganism. In medicines silver and silver nanoparticles have sample application including skin ointments, cream and gel containing silver to prevent infection of burns and open wounds, medical devices and implants prepared with silver-impregnated polymers. Nanoparticles can be synthesized using various approaches including chemical, physical and biological. Although chemical method of synthesis required short period of time for synthesis of large quantity of nanoparticles. This method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis are non-eco-friendly. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals and by-products. Many biological approaches
for both extracellular and intercellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi, and plants.

The synthesis of nanoparticles using various plants extract can be advantages over other biological synthetic process which involves the very complex procedures of maintaining microbial cultures. Many such experiments have already been started such as the synthesis of various metal nanoparticles using fungi like Fusarium oxysporum, Penicillium sp. and using some bacteria such as Bacillus subtilis etc. But, synthesis of nanoparticles using plant extract is the most adopted method of eco-friendly production and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites. There have also been several experiments performed on the synthesis of silver nanoparticles using medicinal plants such as Oryza sativa, Helianthus annus, Saccharum officinarum, Sorghum bicolour, Zea mays, Basella alba, Aloe vera, Capsicum annum, Magnolia kobus, Medicago sativa (Alfalfa), Cinamonum camphora and Geranium sp. In the field of pharmaceutical.

In the recent days, silver nanoparticles have been synthesized from the naturally occurring source like Tea (Camelia sinensis), Neem (Azadirachta indica), leguminous shrub (Sesbania drummondii) and from various natural rubbers, starch, Aloe Vera plant extract, lemongrass leaves extract, etc. The silver nanoparticles get attached to the microbial cell wall and thereby disturbing the permeability of cell wall, cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damaged by interacting with phosphorus and sulphur containing compounds, such as DNA, protein are present inside the cell. The bactericidal properties of silver nanoparticles are due to the release of silver ions which confers the antimicrobial activity. Besides the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content. They are more biocompatible.

2 Materials and methods

Materials

Silver nitrate was purchased from Qualigens fine chemical (Mumbai, India). Carbopol was obtained from SD fine chem. Limited. Glycerol, peptone, sodium chloride, agar agar, beef extract powder and yeast extract powder were purchased from Hl media. Potassium bromide was purchased from Sigma Aldrich.

Methods

Anti-microbial gel preparation by cold mechanical method

Gels were prepared by cold mechanical method. Required quantity of polymer was weighed and sprinkled slowly on surface of purified water for 2 hrs. After that it was continuously stirred by magnetic stirrer, till the polymer soaked in the water. With continuous stirring, other ingredients like glycerol were added. Finally, the drug silver nanoparticles were added to the gel with continuous stirring till drug get dispersed in gel completely. The composition of each gel was shown in Table.

<table>
<thead>
<tr>
<th>SLNO</th>
<th>COMPOSITION</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbopol-940</td>
<td>2g</td>
</tr>
<tr>
<td>2</td>
<td>Glycerol</td>
<td>2ml</td>
</tr>
<tr>
<td>3</td>
<td>AgNPs</td>
<td>2ml</td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>Up to 100ml</td>
</tr>
</tbody>
</table>

The prepared gels were packed in wide mouthed glass jar covered with screw capped plastic lid after covering the mouth with an aluminium foil and were stored in dark and cool place.

Preparation of plant extract from piper betle L. Leaves

Fresh Piper Betel L.(karpoomar) leaves were collected from Gandhi market, Tiruchirappalli and washed several times with double distilled water. Washed pieces dried for one hour in room temperature. 20 g of finely cut Piper betle L. leaves were taken and boiled in 100 ml of double distilled water for one hour in 70 °C and filtered through Whatman No 1 filter Paper. The filtrate was collected and stored at 4 °C for further use.
**Synthesis of silver nanoparticles**

Piper bettle L. extract is used to produce silver nanoparticles in this experiment Ag+ ions were reduced to Ag nanoparticles when plant extract is mixed with AgNO3 Solution in 1:8 ratios. Reduction is occurred by immediate color change from yellowish to brown color in the aqueous solution of the plant extract due to excitation of surface Plasmon Resonance in silver nanoparticle. Further formation of AgNPs in aqueous extract can be monitored by color change. Shows the color changes when the aqueous extract of Piper bettle L plant was mixed with a AgNO3 solution. The Mixture was kept at room temperature for 24 hours. The appearance of a yellowish-brown color in the reaction vessel indicates the formation of AgNPs.

**UV-Vis spectra analysis**

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum. After diluting 1 ml of sample with 4 ml of distilled water and the medium was kept for 3 hours.

**DLS analysis**

Dynamic light scattering (DLS) analysis was performed (Malvern V 6.20 version) to measure the average particle size of the NPs. Before analyzing, the sample was sonicated for 5 min to obtain a uniform dispersion of NPs. Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques were employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultrasonication. Then, the solution was filtered and centrifuged for 15 min at 25º c with 5000 rpm and the supernatant was collected. The supernatant has diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyser.

**Zeta potential analysis**

Zeta potential analysis was performed (zeta sizer Nano series) to measure the stability of the nanoparticles. The zeta potential of Ag nanoparticles acts as a function of biomaterial dosage. The absolute value of the zeta potential increased with increasing dosage due to the numerous nanoparticles produced at higher dosages. Leaves extract mediated synthesized AgNPs have high negative zeta potential values thus they are stable.

**FTIR analysis**

FTIR spectrum of the synthesized silver nanoparticles was recorded with a Shimadzu spectrometer (Model FTIR- 8400S). The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer. The solutions were dried at 75º C and dried powders were characterized in the range of 4000-400 cm-1 using KBr pellet method.

**Morphological study by Field Emission Scanning Electron Microscopy**

Morphology of synthesized AgNPs was determined by Field Emission Scanning Electron Microscopy (JEOL JSM-7800 F). FE SEM analysis was performed after drop coating of sample over the thin sheet of the aluminium. SEM images confirmed the synthesis and discrete distribution of spherical shaped AgNPs. The sample containing silver nanoparticles was centrifuged at 17000 rpm. After centrifugation the supernatant was discarded and the pellet deposited at the bottom was dried and SEM analysis was performed. FE SEM Morphology of synthesized AgNPs was determined by Field Emission Scanning Electron Microscopy (JEOL JSM-7800 F). FE SEM analysis was performed after drop coating of sample over the thin sheet of the aluminium. SEM images confirmed the synthesis and discrete distribution of spherical shaped AgNPs. The sample containing silver nanoparticles was centrifuged at 17000 rpm. After centrifugation the supernatant was discarded and the pellet deposited at the bottom was dried and SEM analysis was performed.

**3 Results and discussion**

**Conformation of silver nanoparticles by visual inspection**

The primary conformation of synthesis of silver nanoparticles from Piper bettle L. leaf extract was monitored visually by the colour change from light-yellow to brown. The samples showed colour change and colour formation is due to the reduction of Ag+ to Ag.

**UV-Vis spectroscopy analysis**

The graph shows that absorption spectra of silver nanoparticles formed in the reaction media has
absorbance peak at 415 nm. Broadening of peak indicated that the particles are poly dispersed.

**DYNAMIC LIGHT SCATTERING (DLS)**

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer.

**Zeta potential**

Leaves extract mediated synthesized AgNPs have high negative zeta potential values thus they are stable. The zeta potential value was found to be -24.1 mV.

FTIR analysis

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer.

Due to the interaction of silver nanoparticles with the extract, the vibrations are lesser in Ag – Piper bettle L extract FTIR spectrum when compared to Piper bettle L extract FTIR spectrum. It showed the presence of groups/bonds due to free O-H stretching (around 3427.85,
3443.28 cm\(^{-1}\), (polyols) C≡N stretching vibrations indicative of terpenoids group of compounds present in
(around 2924.52, 2923.56 cm\(^{-1}\)), C=O stretching vibrations (around 1626.66, 1629.55 cm\(^{-1}\)). Their presence is
responsible for the reduction of silver nitrate into silver nanoparticles.

FTIR spectra of aqueous extract of fresh piper bettle L. leaves

FTIR spectra of bio synthesized silver nano particles
FTIR spectra of AgNO3

Scanning electron microscopy

FE SEM spherical shaped AgNPs

Antimicrobial activity evaluation of synthesized silver nanoparticles

The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Sterile 6 mm diameter cork borers were pierced in the agar at equidistant spots. 20 μl of the diluted solution (16 μg/ml) was deposited on the inoculated well and left for 10 min at room temperature for the compound diffusion. The plates inoculated with bacteria were incubated at 37°C for 24 hr.
The experiment was repeated thrice and the average results were recorded. The antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) around the well. Silver nanoparticles exhibit antimicrobial properties against various types of bacteria. The antimicrobial activity of synthesized nanoparticles has been investigated against Escherichia coli and Pseudomonas aeruginosa. Synthesized and microbial silver nanoparticles of Piper bettle leaf showed more inhibitory effect against E. coli (6mm zone of inhibition) and P. aeruginosa (3 mm zone of inhibition).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Bacterial culture</th>
<th>Zone of inhibition( in mm)</th>
<th>Extract</th>
<th>AgNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E.coli</td>
<td>6mm</td>
<td>8mm</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>P.aeruginosa</td>
<td>3mm</td>
<td>12mm</td>
<td></td>
</tr>
</tbody>
</table>

4 Conclusion

Thus an eco-friendly, nontoxic and cost effective method have been developed for the biosynthesis of AgNPs using Piper bettle leaf extract. The phytochemicals such as terpenoids, tannin, flavonoids, proteins, phenols, and saponins present in the Piper bettle leaf extract play an important role as reducing agent and as well as capping agent at room temperature. So that this method is found to be a best alternative when compared with the chemical synthesis of AgNPs and it can be also adopted for the biosynthesis of AgNPs at a large scale. The biosynthesized AgNPs has antimicrobial activity against various clinical pathogens such as E. coli, and P. aeruginosa. A microbial gel was formulated using biosynthesized AgNPs, further invivo studies and to be performed in future. To bring the formulated Antimicrobial Gel as a cost-effective, patient-affordable Gel in the market against bacterial infections.

References


Competing Interests:
The authors declare that they have no competing interests.

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