BIOSYNTHESIS OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF BAUHINIA ACUMINATA USING VARIOUS PHYSICAL PARAMETERS AND ITS APPLICATIONS

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ABSTRACT

Bauhinia acuminata, from southwestern region of Kerala medicinally important in traditional system of medicine and are used as an effective natural drug. To synthesize silver nanoparticle from Bauhinia acuminata aqueous leaf extract obtained by changing different reaction parameters such as temperature, reaction time, varying ratio and concentration of plant extract and precursor (AgNO3). The characterization of Bauhinia acuminata silver nanoparticle using UV-Vis spec. Now days microbial resistance to antibiotics is a serious concern in medicine. The use of silver nanoparticles (AgNPs) as a potent antibacterial agent has received much attention. BAAgNPs have multiple targets and found to be effective against C. freundi and M. luteus was found to be <1000 and 750 μg using MIC assay. The molecular docking affinity of Phytol ligand binding to cellular receptors like 5DZL with -2.53 kcal/mol shows the effective nature of the aqueous leaf extract components. The BaAgNPs shows the effective antimicrobial inhibition against E.coli and S.aureus.

KEYWORDS: BaAgNPs, UV-Vis spec, MIC, Molecular Docking.

INTRODUCTION

Bioactive compounds present in the plants have been a great impact of substantial research in nanotechnology. The advancement in the bionanoparticulate system in nanotechnology is received global attention due to their extensive applications in the various fields. Biosynthesis of metal nanoparticles, using plant extracts as a nanofactory becomes an important subject of
researches in the field of bionanotechnology.[10] Plant-mediated silver nanoparticles synthesis protocols have an upsurge in recent past as a safe, ecofriendly and easily scaled up cost-effective an alternative for most popular conventional methods which are bound with various implications.[2] Owing to the rich biodiversity of plants, mediated nanoparticles synthesis has become a subject of interest across the globe with different plant species being rapidly explored and evaluated for synthesizing of nanoparticles.

The silver nanoparticles (SNPs) are harmless to humans and most efficient against bacteria, virus and other eukaryotic microorganism at low concentrations and without any side effects.[11] The presence of novel secondary metabolites in plant crude extracts contain such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticles. Metal nanoparticles produced using plant extracts are stable and can be monodispersed by controlling synthetic parameters, such as pH, temperature, incubation period and mixing ratio. A report about the exciting possibility that the size of particles that form intracellular could be controlled by altering key factors such as pH, temperature, substrate concentration and time of exposure to the substrate.[6] Many previous reports are demonstrating plants are widely using for synthesizing green nano particle from like Aloe Vera[4], Tridax procumbens[5], Catharanthus[15], Ananas comosus[1] and Terminalia arjuna[8], Origanum vulgare[16], Cocos nucifera[14], Rosmarinus officinalis[7], Emblica ofcinalis[13], Ocimum sanctum[23], fresh green leaves of Gracinia gummiguttaa.[3] Cissus quadrangularis[17], Bauhinia purpurea.[18]

Bauhinia acuminata is Fabaceae family, sub family Caesalpinioideae is a semi deciduous large shrub with white butterfly-like flowers. This species occurs widely in deciduous forests and scrub. Other common names include dwarf white Bauhinia (English), Safed Kachnar (Hindi) and Sivamalli (Sanskrit). Almost all parts of the plant has a medicinal properties Indian vaiydas is recommended the bark and leaves of bauhinia is used to treat biliousness and for liver disorders (Indian medical plant). Bark is helpful against cancer and diabetics, while in India the leaves and roots of this plant are used for treating respiratory ailments. Moreover, the leaf of Bauhinia acuminata is used to treat bladder stone, venereal diseases, leprosy, asthma and digestive diseases.[9] Phytochemical screening showed the presence of carbohydrate, phenolic compounds, saponins, flavonoids, oils and fats in leaves and stems of Bauhinia acuminata and leaves were identified with chemical compounds including palmitic acid, three phthalic acid esters, phthalic acid, gallic acid and ursolic acid.
In the current work emphasis on the green routed silver nanoparticles synthesis using *Bauhinia acuminata*, its biological activities. In this report, we explored the effectiveness of *B. acuminata* aqueous leaf extract as available source of reducing and stabilizing agent for silver nanoparticles. For evaluating the synthesis in a better way, altering the concentration, temperature, time that effectively controls the size, shape, stability and physicochemical properties is currently at the forefront of research into nanoparticles synthesis. The antimicrobial activity of BaAgNps against multidrug resistant organisms were also investigated.

**METHODS**

**Collection and Preparation of the leaf extract**

Leaves of *Bauhinia acuminata Linn* were collected in and around from Angamaly kerala and authenticated by Dr. S. Jayaraman, Director of Plant and Anatomy Research Centre, Chennai (Reg.No. PARC/2013/2189). *Bauhinia* leaves were rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles. The leaves were shade dried for 1 week and dried leaves were uniformly grinded using a mechanical grinder to make a fine powder.

**Extraction and fractionation of *B. acuminata* leaf tissue**

About 10 g of these fine powdered of plant was weighed and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 20 min followed by vigorously vortexed. The extracts were then filtered thrice through Whatman No.1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated at 4°C.

**Media and chemicals**

Silver nitrate (AgNO$_3$) was purchased from sigma Aldrich. Nutrient Broth (NB) and Mueller – Hinton broth (MHB) were purchased from Himedia laboratories Pvt. Ltd. Mumbai, India. All other reagents used were of analytical grade.

**Preparation of 1mM silver nitrate aqueous solution (AgNO$_3$)**

An accurately weighed 0.017g of silver nitrate was dissolved with 100 ml of double distilled water and stored in amber colour bottle until further use.

**Separation of silver nanoparticles**

The synthesized silver nanoparticles were separated by centrifugation using a REMI
centrifuge at 15,000 rpm for 15 min. The supernatant liquid was resuspended in the sterile double distilled water. The process was carried out thrice to get rid of any uncoordinated bio molecules. After, the desired reaction period, the supernatant liquid was discarded and the pellets were collected and stored at 4°C for further use. The nanoparticles solution obtained was purified and separated by repeated centrifugation at 15,000 rpm for 10 min. The centrifugation process was frequent 2–3 times to ensure the removal of any adsorbed substances on the surface of the silver nanoparticles.

**Lyophilization of silver nanoparticles**

Lyophilization has been considered as a good technique to improve the long-term stability of colloidal silver nanoparticles. The freshly prepared aqueous BaAgNps are lyophilized with a cryoprotective agent. Then it was rapidly cooled down to -50°C for 2 hr followed by primary drying at 1.03 m bar and secondary drying at 0.001 m bar.

**Optimisation of silver nanoparticles**

Influence of temperature.-Direct heating method 1 mM silver nitrate solution was prepared and taken in a conical flask. 10 mL plant extract of *B. acuminata* was added drop wise constantly to 10 mL of 1 mM AgNO₃ solution. Sliver containing *Bauhinia* leaf extract were heated from 25, 35, 45 and 60°C. The solution was kept on magnetic stirring at 200 rpm for 15 minutes. The reduction process was complete when the solution turned brownish-black which confirmed the presence of nanoparticles.

**Influence of reaction time**

1 mM silver nitrate solution was prepared and taken in a conical flask. 10mL plant extract of *Bauhinia acuminata* was added drop wise constantly to 10 mL of 1 mM AgNO₃ solution. To prove that leaf extract when combined with AgNO₃, tend to produce particles with increasing size with escalating reaction time. The reaction time was varied between 10 m, 30m, 1hr and 2 hr and try to check there is any notable a change in a particle size through UV-Vis spectrometer.

**Antibacterial assay**

**Test organisms**

*Citrobacter freundii* (NCIM 2488) and *Micrococcus luteus* (NCIM 2103), National Collection of Industrial Microorganisms, India. The test sample was evaluated for antibacterial activity by Minimum Inhibitory Concentration (MIC) against *C. freundii* and *M. luteus* at a different
concentration ranging from 20000 – 976 µg. The MIC value of test substance was compared with the activity of standard antibiotic.

**Preparation and standardisation of stock cultures**

A day prior to the experiment, a loopful culture of *C. freundii* and *M. luteus* were grown in NB at 37°C for 24 hr. The cultures were adjusted to 0.17 absorbance at 600 nm (corresponding 10^8 CFU/ml 0.5 McFarland standards) using a spectrophotometer. Further diluted to a concentration of approximately 10^5 CFU/ml.

**Preparation of resazurin and standard antibiotic solution**

The stock resazurin solution was prepared by dissolving 2.7 mg in 4 ml of sterile saline. Further working solution was prepared by dissolving 1ml of stock solution in 5ml of sterile saline. The standard antibiotic i.e., ciprofloxacin solution at 1% concentration was prepared in sterile distilled water.

**Preparation of test sample**

Test sample was prepared at 30 mg/1.5ml concentration by dissolving 30 mg of test sample in 1.5 ml of MHB. Sample was mixed using cyclomixer for 5min and sonicated for 5min.

**Determination of MIC**

Experiments were performed in triplicate under aseptic condition. A volume of 50 µl respective sterile MHB was added to all 96 wells except first three wells of the microtitre plate to which only 100 µl test product was added. From first three wells of the plate, 50 µl of the test product was double diluted to the wells containing test material 10 µl bacterial suspension of approximately 10^5 CFU/ml was added. A growth control (bacterial cell suspension + 50 µl broth medium) and broth control (only broth medium 50 µl) was kept in the 96 wells plate. A positive control that consists of the ciprofloxacin was also placed on the plate. The plates were incubated at 37°C for 24 hours. After incubation, 10 µl of working solution of resazurin dye was added to all wells. The plates were wrapped with aluminium film and incubated at 37°C for 1hr. The colour change was then assessed visually. Any colour change from purple to pink or colourless was recorded as positive growth. The lowest concentration at which there is no colour change occurred was taken as the MIC value.
Molecular docking
GC-MS analysis of leaf distillation extract showed the presence of α-Humulene (1.76%) and Phytol (65.90%), were the other major constituents taken for molecular docking analysis using cancer receptors.\cite{21} The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The 3D structure of cancer Biomarkers from *Homo sapiens* was retrieved and the cancer receptors are 3OQ9, 4OFB, 4WP7 and 5DZL. The molecular docking analysis was carried out for these receptors and the ligands.\cite{19,20}

**RESULTS AND DISCUSSION**

The synthesised plant extracts mediated silver nanoparticles were subjected to optical measurements by UV–Vis spectrophotometer. Absorption spectra of silver nanoparticles formed in the reaction media had shown absorbance peaks within the range 410 – 460 nm. It is widely accepted that UV–Vis spectroscopy could be used to observe the reduction in size and shape controlled nanoparticles in aqueous solution. Formation of nanoparticles by the reduction of metal ions is affected by a large number of factors; the active biomolecules present in the plant extract of different combinations and concentrations these include the reaction mixture, incubation temperature, reaction time, concentration, etc will decide the pathway of synthesis. Temperature possibly will play an indispensable role in synthesis of silver nanoparticle. With regard to *bauhinia* species nanoparticles synthesis was increased according to increase in temperature. Soon after the addition of plant extract to sliver nanoparticles in normal temperature 25°C there is no remarkable change in the absorption peak in periodic monitoring of UV spec analysis , but it shows the shift by increasing the temperature to 35, 45 and 60°C the absorption peak started to shifts from 410 nm in the temperature of 35°C; while by increasing the temperature up to 45°C, the band shifts to 420 nm and 60°C to 440 nm which may due to the localization of the surface plasmon resonance of the silver nanoparticles. Reaction rate and particle formation rate appears to become faster when reaction temperature increases. Cruz\cite{24} has put forward the experiments on the synthesis of silver nanoparticles in lemon verbena extracts (*Aloysia citrodor*) that increasing the reaction temperature is accompanied by an increase in the efficiency of the silver ion reduction.

Aqueous leaf extract of *B.acuminata* and AgNO₃ solution were combined, soon after it results into a colour change from pale yellow to yellowish brown and finally to dark brown.
colour. This is characteristic of the nanoparticles due to the excitation of surface plasmon vibrations in the silver nanoparticles synthesized and confirmed by obtaining the respective absorption spectra. The UV-Vis spectra recorded after time intervals of 10 m, 30m, 1hr and 2 hr from the initiation of the reaction. It was observed that the absorption intensity increased with increase in the reaction time. Comparing with other fellow workers it is more evident that when *Azadirachta indica* leaf extract and AgNO₃ were combined, increasing the reaction time tended to produce particles with increasing size.¹² *B.acuminata* leaves contains reducing sugars, flavonoids, carophyllene, alkaloids, phenolic compounds is the reason for the fast precipitation and reduction of silver nanoparticles from the reaction medium.²⁵ After 30m the colour of the solution becomes nearly constant, indicating that no silver salt was left for further reaction.

The antimicrobial activity of biologically synthesized silver nanoparticles using *B.acuminata* were found to be highly effective against gram positive bacteria *C.freundi* and *M.luteus* minimum inhibition concentration <1000 µg and <750 µg respectively shown in Table 1. In analogy with other researchers, the extract dose of 1 mg/ml of Bauhinia acuminate methanolic leaf extract showed excellent activity towards *Bacillus cereus*. (inhibition zone 13 mm). Subhashini¹⁶ analyzed antibacterial potency of nanosilver synthesized from the fruit filtrate of *Citrus sinensis* against *E.coli* (ATCC 25922) and *P.aeroginosa* (ATCC 27853). Paulkumar¹⁷ has put forward the well diffusion method using the *Piper nigrum* leaf and stem for examining the antibacterial activity of green synthesized silver nanoparticles under in vitro conditions against plant pathogen like *Citrobacter freundi* showed potent result. Doudi¹⁸ examined the three bacteria *Enterobacter aerogenes, Klebsiella oxytoca* and *Citrobacter freundi* showed the highest growth inhibitions against silver nanoparticles solution with the concentration of 100 ppm among the clinical sample. The results indicated that silver nanoparticles have good antimicrobial activity against different microorganisms.

The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures.²¹ The binding affinity of the four cancer receptors with the ligands was measured by kcal/mol. The docking scores for Phytol ligand binding to cellular receptors like 5DZL with -2.53 kcal/mol as a target and interacted in the regions with one bonds (bond distance) of B:GLU37:OE1 (1.9) with 13.69 Å RMSD and 13.93 Estimated Inhibition Constant, Ki (µM) respectively are shown in Table 2 and Figure 3.
Table 1: MIC of test sample against bacteria

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Concentration Range (μg)</th>
<th>MIC (μg)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>C.freundi</td>
</tr>
<tr>
<td>BaAgNPs</td>
<td>1500-0.576</td>
<td>&lt;1000</td>
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<tr>
<td>Ciproflaxin (std)</td>
<td>1000-0.488</td>
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Table 2: Molecular docking analysis of the ligands against various cancer receptors.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Parameters</th>
<th>Receptors</th>
<th>3OQ9</th>
<th>4OFB</th>
<th>4WP7</th>
<th>5DZL</th>
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<tbody>
<tr>
<td>α- Humulene</td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
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<td>Number of bonds</td>
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<td></td>
<td>Interacted Amino acid residues</td>
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<td></td>
<td>Bond distance Angstrom (Å)</td>
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<tr>
<td></td>
<td>RMSD (Å)</td>
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<tr>
<td>Phytol</td>
<td>Binding energy kcal/mol</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<td>Bond distance Angstrom (Å)</td>
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<tr>
<td></td>
<td>RMSD (Å)</td>
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<td>-</td>
<td>13.69</td>
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<tr>
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<td>Estimated Inhibition Constant, Kᵢ (µM)</td>
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<td>-</td>
<td>-</td>
<td>13.93</td>
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</table>

Figure 1: This graph depicts the time courses of silver nanoparticles formation obtained with 1ml of 1 mM AgNO₃ and 10 ml of B.acuminata plant extract obtained with different reaction temperature.
Figure 2: This graph depicts the formation of silver nanoparticles obtained with 1ml of 1 mM AgNO₃ and 10 ml of B.acuminata plant extract with different incubation time.

Figure 3: Binding orientations of 5DZL with the ligand Phytol.

CONCLUSION

Surfacing of bio nanoparticles in drug delivery systems might be the upcoming expansion in the field of pharmacy. Studies revealed that green synthesis of silver nanoparticles combining with plant compounds is a potential antimicrobial agent, will be useful in many biomedical applications. Hence, in demarcation to the microorganism–mediated synthesis of NPs, the use of plant biomasses or plant extracts is comparatively simpler and economic importance.
REFERENCES


