Article

Extracellular synthesis of nanoparticles using leaves of selected medicinal plants and their antibacterial activity

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Abstract

The synthesis of metal nano particles using biological systems is an expanding research area due to the potential application in nano medicine. Extracellular biosynthesis of silver nano particles was carried out by using medicinal plant extracts for the reduction of aqueous silver ions in short period. The present work reports the synthesis and investigation of the antibacterial activity of silver nanoparticles from four selected medicinal plant leaf extracts (Ocimum sanctum, Hibiscus surattensis, Sesamum indicum and Turnera angustifolia) using AgNo3 against Escherichia coli and Staphylococcus aureus. The silver nanoparticles formation was confirmed by the colour change of plant extracts and further confirmed with the help of UV-Vis spectroscopy. The antimicrobial property of silver nanoparticles was analyzed by measuring the zone of inhibition. They exhibited good antibacterial activity against Escherichia coli and Staphylococcus aureus. This paper demonstrates a single step extracellular formation of silver nanoparticles at room temperature which further harvested by simple heat drying evaporation.

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Introduction

Nanotechnology is rapidly expanding and potentially beneficial field with tremendous applications for society, industry and medicine. The use of nano sized particles has remarkable applications. Metal nanoparticles are an area of research because of their unique chemical, physical and optical properties [1]. Current research in bactericidal nano materials has opened a new area in pharmaceutical industries. Silver nanoparticles are the metal of choice as they hold the promise to kill the microbe's effectively [2]. The Silver nano particles act on a broad range of target sites both extracellular as well intracellular. Infact microbes generally have a harder time to develop resistance to silver then they do to antibiotics [3, 4]. Plants are the richest bioresources of drugs in traditional and modern medicines, folk medicine, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [5]. The plant mediated synthesis is rapid, low cost, eco- friendly and for safer human therapeutic uses [6]. Many reports are available on biogenesis of Silver nanoparticles using several plant extracts *Morinda citrifolia* [7], *Cajanas cajan* [8], *Boswellia* [9], *Turnera ulmifolia* [10], *Acalypha indica* [11], *Ocimum sanctum* [12], *Catharanthus roseus* [13], *Sesamum indicum* [14], plant latex [15] and *E.coli* [16]. Here we report an in expensive, versatile and

green method for the synthesis of Silver nanoparticles by reduction process using randomly selected four medicinal plants, *Ocimum sanctum* L. (Laminaceae), *Hibiscus surattensis* L. (Malvaceae), *Sesamum indicum* L. (Pedaliaceae) and *Turnera angustifolia*. Mill. (Passifloraceae). These plants are extensively used in traditional medicine.

Materials and Methods

Collection of plant material

Ocimum sanctum, *Hibiscus surattensis*, *Sesamum indicum* and *Turnera angustifolia* leaves were collected from in and around Khammam town. The plants were identified by the Department of Botany, SR&BGNR Govt. Arts & Science College, Khammam, Telangana. India and the herbarium sheets were preserved in the Department as a record.

Preparation of leaf extract

Fresh leaves were washed several times with tap water and later with deionised water. 10 grams of washed fine cut leaves along with 200 ml double distilled water were taken in 250 ml glass beaker and boiled for 5 minutes at 80^{o}c.The extract was cooled to room temperature and filtered with Whatman No 1 filter paper .The filtrate was centrifuged for 10 minutes at 10000 rpm, the supernatant was collected and stored at 4^{o} C. Similarly using *Ocimum sanctum, Hibiscus surattensis, Sesamum indicum* and *Turnera angustifolia* leaf extracts were prepared. The filtrates are used as reducing and stabilizing agents.

Preparation of 1 mM AgNo$_3$ solution

Accurate concentration of 1 mM AgNo$_3$ (Merck India Ltd) was prepared by dissolving 0.169 grams AgNo$_3$ in 1000 ml double distilled water and stored in Amber coloured bottle to avoid auto oxidation of silver.

Bio synthesis of Silver nanoparticles

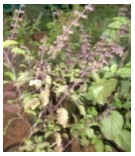
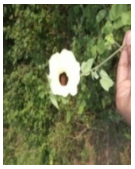
In the single step green synthesis , 5 ml of leaf extract was added to 95 ml of 1 mM aqueous Ag No$_3$ solution and heated up to 80^{o}C for 5 minutes, the colour change was observed (Table1, Figure 5, 6, 7 and 8), which stands as a preliminary conformation of the formation of Silver nanoparticles. In the same way *Ocimum sanctum* Figure1, *Hibiscus surattensis* Figure 2, *Sesamum indicum* Figure 3 and *Turnera angustifolia* Figure 4 leaf extract silver nano particles were synthesized. The silver nanoparticles solutions thus obtained were purified by repeated centrifugation at 10000 rpm for 15 minutes. The supernatant was transferred to a clean dry beaker for further settlement of particles and repeated centrifugation was carried to purify Silver nanoparticles.The sample so obtained was dried in an incubator. The particles obtained were used for further characterization. Thus the Silver nanoparticles are synthesized in a single step green approach.

Characterization

UV –Visible spectroscopy

Synthesized silver nano particles were initially characterized by sampling small aliquot of sample in to UV –Visible spectro photo meter absorption spectra at 300-700 nm using Shimadzu UV -1800 spectro photo meter.

Table 1. Selected Medicinal Plants and their Characteristic features

Plant species	Medicinal use	Color change	UV range
 **Figure 1** ***Ocimum sanctum***	Used for treating diabetes, bronchial disorders, Gastric and kidney problems.	 **Figure 5** **Pale yellow to dark red**	437 nm
 **Figure 2** ***Hibiscus surattensis***	Used for cough. paralysis, epilepsy, and jaundice	 **Figure 6 Yellow to red color**	421 nm
 **Figure 3** ***Sesamum indicum***	Used for Arthritis, skin diseases, eczema , soriasis and Scabies	 **Figure 7** **Orange to brown color**	427 nm
 **Figure4** ***Turnera angustifolia***	Used for fever, cold, Piles, diarrhea, menustrual disorders, constipation, Arthritis.	 **Figure 8** **yellow to golden yellow**	435 nm

Antibacterial activity

Antibacterial activities of plant extract mediated silver nanoparticles were assured using standard Well diffusion method. The test bacteria *Escherichia coli* and *Staphylococcus aureus* were included in this study to assess the susceptibility patterns of the nanoparticles using Streptomycin as standard. Nutrient Agar was prepared

for cultivation of the bacteria. 100 µl of fresh overnight grown cultures of the test bacteria was spread on a nutrient agar plates and punched with 1mm sterile borer 100µl of the each sample solution containing nanoparticles was added in to the holes and the plates were incubated at 37^0C for 24-48 hours and observed for the zone of inhibition. The zone of inhibition was measured in mm and compared with the standard antibiotic streptomycin. The experiments were repeated thrice and mean values of zone diameter were presented.

Results and Discussion

Synthesis of Silver nanoparticles by using Biological materials is one of the most widely used methods for the synthesis of Silver colloids .The leaf extract was added to the Silver nitrate solution which resulted in a colour change. The development of biologically inspired experimental process for the synthesis of nanoparticles is evolved as one of the important branch of nanotechnology. The present study emphasizes the use of medicinal plants for the Synthesis of Silver nanoparticles with potent anti bacterial effect.

Extracts from these plants may act as reducing and capping agents .in silver nanoparticles synthesis. Studies have indicated that biomolecules like protein, phenols, and flavonoids not only play a role in reducing the ions to the nano size, but also play an important role in the capping of the nanoparticles [17, 18]. The reduction of Ag+ ions by combinations of biomolecules found in these extracts such as vitamins, enzymes/, proteins, organic acids such as citrates, amino acids, and polysaccharides [18, 19] are environmentally benign, yet chemically complex.

The nanoparticles were preliminarily characterized by UV-Visible Spectroscopy, which is proved to be a very useful technique for the analysis of nanoparticles. Reduction of Ag+ ions in aqueous solution of silver complex during the reaction with ingredients present in the plant leaf extracts. Observations of the UV-Visible Spectroscopy revealed that silver nano particles in the solution may be correlated with UV-Visible Spectra. As the leaf extracts were mixed with the aqueous solution of the silver ion complex it was changed in to dark colour were presented in figures 5, 6, 7 and 8 in the Table 1 due to excitation of the surface plasma vibrations indicate the formation of the Silver nanoparticles [20].

UV-Visible Spectrograph of the colloid of Silver nanoparticles has been recorded as a function of time by using quartz cuvette with distilled water as the reference. The reaction between 95 ml Silver nitrate solution and 5 ml leaf extract was carried at 80 ^{o} C for one hour.

Fig10 shows the UV-Visible Spectra which are recorded after the completion of the reaction at 90 ^{o} C temperature .The UV spectrum point peck values were reported in Table 1. *Ocimum sanctum* 437 nm, *Hibiscus surattensis* 421 nm, *Sesamum indicum* 427 nm and *Turnera angustifolia* 435nm in figure 9 was confirmed that polydispersed nanoparticles were formed.

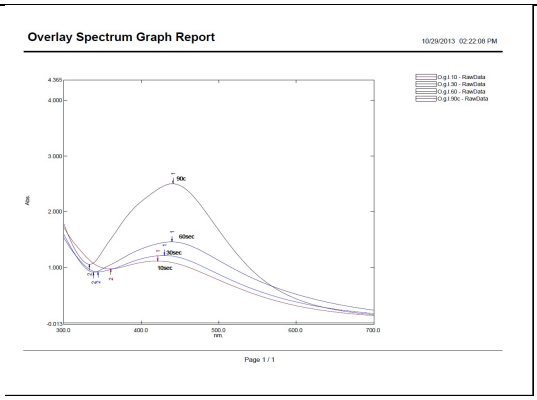


Figure 9. UV-Visible absorption spectrum

The biologically synthesized silver nanoparticles exhibited excellent antibacterial activity against the bacterial pathogens *Escherichia coli* and *Staphylococcus aureus*. It has been reported that antibacterial effect was size & dose dependent and was more pronounced against Gram-negative bacteria than Gram-positive bacteria [16, 22]. The present study also clearly indicates that the synthesized silver nanoparticles have well antibacterial action against Gram-negative bacteria than Gram-positive bacteria in Figure 10. The antimicrobial activities of colloidal silver particles are influenced by the dimensions of the particles. The smaller the particles lead to the greater antimicrobial effects [21]. The effect of antibacterial activity is higher in the case of *Ocimum sactum* followed by *Sesamum indicum*, *Hibiscus surattensis* and *Turnera angustifolia*. The tested silver nanoparticles have bactericidal effects resulting not only in inhibition of bacterial growth but also in killing bacteria [22]. The inhibition of bacterial growth reported in this study is dependent on the concentration and number of nanoparticles in the medium.

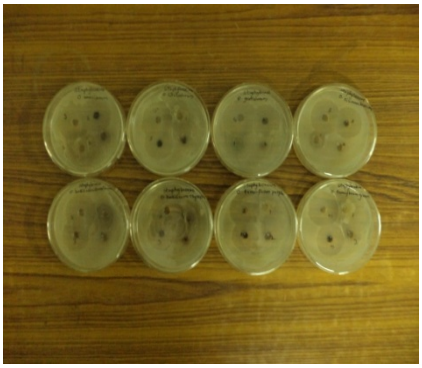


Figure 10. Zone of inhibition of biologically synthesized silver nanoparticles against bacterial pathogens.

Conclusions

The present study reveals that the four plant species are good source for synthesis of silver nanoparticles at a faster rate. The formation of silver nanoparticles was confirmed by the colour change within 30 minutes. The UV-Visible spectroscopy results reveals that the absorption with the difference in the plant species. The antibacterial efficacy against different species of bacteria confirmed that the silver nanopar-

ticles are capable of rendering antibacterial efficacy and more effective than the medicinal value of the plants. Still more experimental trials need to be conducted to support its therapeutic usage.

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