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Ocimum americanum L. leaf extract mediated synthesis of silver nano particles: A noval approach towards weed utilisation

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ABSTRACT

The synthesis of metal nanoparticles using biological systems is an expanding research area due to the potential application in nano medicine. Extracellular biosynthesis of silver nanoparticles was carried out by using Ocimum americanum L. leaf extract for the reduction of aqueous silver ions in short period. In view of the importance of silver nanoparticles, the present work reports the synthesis, characterization and investigation of the antibacterial activity of silver nanoparticles from Ocimum americanum using $AgNO_3$. The antimicrobial property of silver nanoparticles was analyzed by measuring the zone of inhibition against E.coli, pseudomonas, streptococci bacteria. The silver nanoparticles formation was identified immediately by the colour change and further confirmed with the help of SEM, EDAX, FTIR, UV-Vis spectroscopy. This paper demonstrates a single step green synthesis that the reaction of aqueous silver ions with plant extract resulted in extracellular formation of silver nanoparticles which further harvested by simple heat drying evaporation. The results indicate that the silver nanoparticles may have an important advantage over conventional antibiotics.

Key words: Silver nanoparticles, SEM, EDAX, Zone of inhibition.

INTRODUCTION

Nanotechnology is rapidly expanding and potentially beneficial field with tremendous applications for society, industry and medicine. Current research in bactericidal nano materials has opened a new area in pharmaceutical industries. The Silver nano particles act on a broad range of target sites both extracellular as well intracellular. In fact microbes generally have a harder time to develop resistance to silver then they do to antibiotics [1, 2]. Plants are the richest bio resource of drugs in traditional and modern medicine [3]. The plant mediated synthesis is rapid, low cost, eco- friendly and for safer human therapeutic uses [4]. Many reports are available on biogenesis of Silver nanoparticles using several plant extracts such as Parthinium [5], Desmodium [6], Morinda citrifolia [7], Turnera ulmifolia [8], Acalypha indica [9], Ocimum sanctum [10], catharanthus roseus [11], Ocimum canum [12], plantlatex [13] and E.coli [14] etc. Here we report an in expensive, versatile and green method for the synthesis of Silver nanoparticles by reduction process using Ocimum americanum L. the plant is extensively used in traditional medicine. The genus Ocimum belongs to Lamiaceae family has worldwide distribution and consists of 160 species with 24 species native to India [15]. The essential oils of Ocimum contain compounds such as euginole, linalool, geraniol, 1, 8-cineol, citral and camphor [16]. These essential oils are being used as pharmaceutical agents because of their anticancer, antiasthamatic, anti stress, antimicrobial activity [17], anti diabetic [18] and antioxidant activity [19]. Herein we report the Synthesis, characterization and antibacterial activity from *Ocimum americanum L.* which provide a new platform to make it a value added weed for nanotechnology based industries in future.

MATERIALS AND METHODS

2.1 Collection of plant material

India has great potential of biodiversity. The genus Ocimum belongs to Lamiaceae family. *Ocimum americanum* L.(Fig.1) syn. *Ocimum canum* sims, is a traditional medicinal plant distributed all over India mostly on waste lands, river banks and sides of paddy fields. Local name in Telugu: Kukkatulasi, Hindi: Kala tulasi, Tamil: Nayi tulasi, Malayalum: Kattu tulasi,and the trade name is Hoary basil. The plant is a much branched strongly aromatic herb, branches are grooved and pubescent. Leaves are elliptic and ovate. Flowers are in whorls, white or cream in colour as terminal racemes. Fruits are nutlets, oblong and black.

Ocimum americanum leaves were collected from in and around waste lands of Khammam, Andhra Pradesh. The plant was identified by the Plant systemic laboratory Department of Botany, Kakatiya University, Warangal, A.P.India and the herbarium sheets were preserved in the Department as a record.

Fig.1 *Ocimum americanum* L.

2.2 Preparation of leaf extract

Fresh Ocimum leaves were washed several times with tap water and later with deionised water. 10 gm of washed fine cut leaves along with 100 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. The filtrate acts as reducing and stabilizing agent.

2.3 Bio synthesis of Silver nanoparticles

Accurate concentration of 1 mM $AgNO_3$ (Merck India Ltd) was prepared by dissolving 0.169 gm $AgNO_3$ in 1000 ml double distilled water and stored in Amber coloured bottle to avoid auto oxidation of silver.

In the single step green synthesis, 5 ml of leaf extract was added to 95 ml of 1 mM aqueous $AgNO_3$ solution and heated up to 80^{o}C for 5 minutes, the colour change was observed (Fig.3), which stands as a preliminary identification of the formation of Silver nanoparticles. The silver nanoparticles solution thus obtained was 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 using cooling microfuge to get dried and purified Silver nanoparticles. The particles obtained were used for further characterization.

3. Characterization

3.1. UV –Visible spectra analysis

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

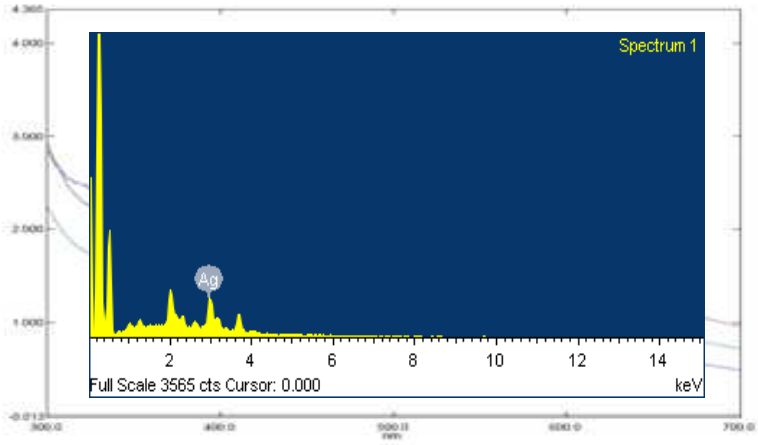

Fig .2 UV-Visible absorption spectrum of *Ocimum americanum*, Silver nanoparticles at 10, 30, 60, min. time interval at 90^{0}c temperature

Fig.3 *Ocimum americanum* leaf extract, aqueous $AgNO_3$, Silver nanoparticles at 10, 30, 60, min. time interval at 90^{0}c temperature and their color changes respectively

3.2. SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was carried using Zeiss EV-18 model. A thin film of the sample was prepared on a carbon coated copper grid by placing small amount of the sample on the grid. Then the film on the SEM grid was allowed to dry using a mercury lamp for 5 min.

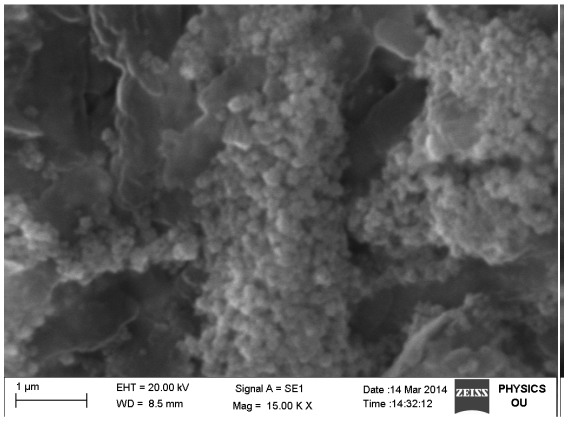

Fig .4

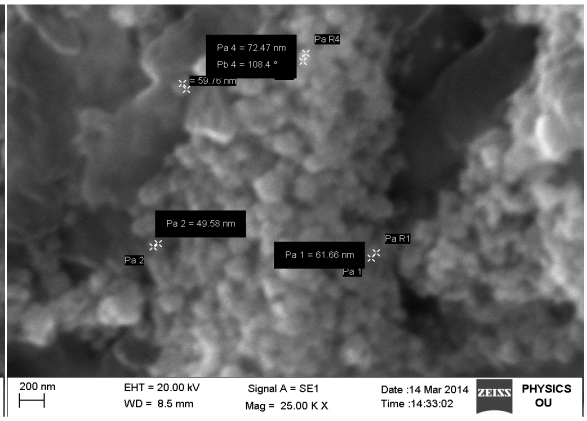

Fig .5

Fig.4 SEM image of silver nanoparticles formed by *Ocimum americanum*
Fig.5 SEM image showing silver nanoparticles size<100nm

3.3 EDX Analysis

Energy Dispersive X-ray analysis (EDX) was recorded on Zeiss EV-18 model. The peaks obtained from EDX gives the purity of the material.

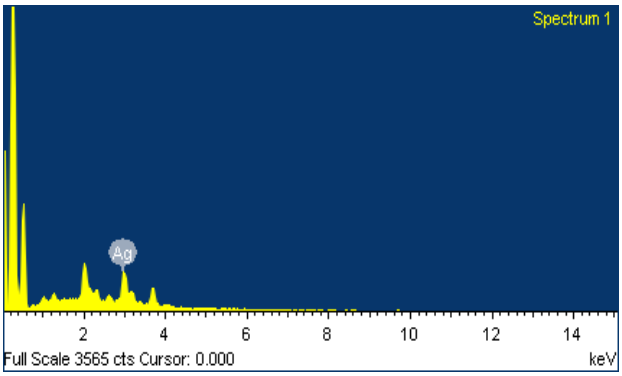

Fig .6 EDX image of silver nanoparticles produced from *Ocimum americanum*

4. FTIR- Spectroscopy

Fourier-transform spectroscopy PerkinElmer model was used for the analysis of the reduced silver. The spectrum was taken in mid-IR region of 400-4000cm^{-1}.The sample was mixed with pure KBR crystals in the ratio of 1:100 and the pellet was fixed in the sample holder for the analysis.

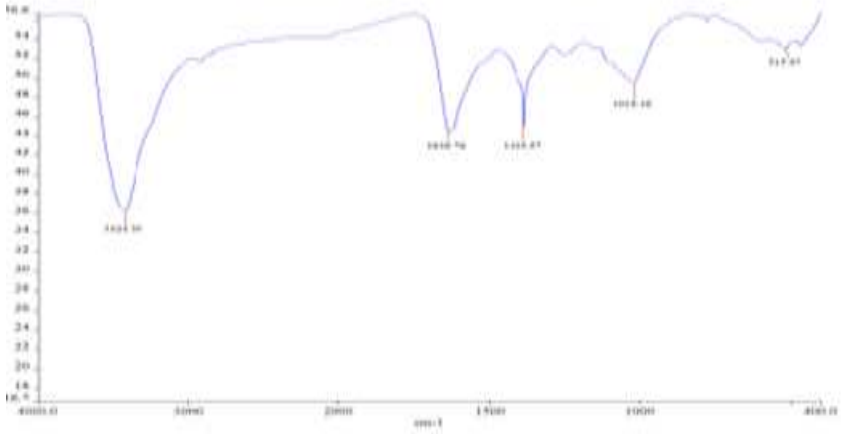

Fig .7 FTIR-spectrum of bio synthesized silver nanoparticles formed by *Ocimum americanum*

4. Antibacterial activity

The Kirby-Bauer disc diffusion method [20, 21] was used to screen the antibacterial activity of plant extract mediated silver nanoparticles on selected bacteria. For disc diffusion method, Bacterial cultures were prepared in nutrient broth. About 100ml of nutrient broth was taken in 250ml flasks and the flasks were inoculated with each of the test bacteria and incubated at 37^{0}C for 24hours. This was taken as inoculums and was swabbed onto petriplates containing 4mm thick Nutrient agar. For disc diffusion method, bacterial cultures were prepared in nutrient medium. Filter paper discs saturated with silver nanoparticles were placed onto these plates with the help of sterile forceps and incubated at 37^{0}c for 24-48 hours and observed for the zone of inhibition. The test bacteria (human pathogenic bacteria) are Escherichia coli, Streptococcus pneumoniae, Pseudomonas aeruginosa were brought from Department of Microbiology, Mamatha Medical College, Khammam. They were included in this study to assess the susceptibility patterns of the nanoparticles using Streptomycin as standard. The zone of inhibition was measured with transparent ruler in millimeter and compared with the standard antibiotic streptomycin. The experiments were repeated thrice and mean values of zone diameter were presented in Table No.1.

Table No: 1

S.No	Bacterial pathogens	Diameter of zone of inhibition in mm
1.	*Streptococcus pneumoniae*	09
2.	*Pseudomonas aeruginosa*	08
3.	*Escherichia coli*	10

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 present study emphasizes the use of *Ocimum americanum* for the Synthesis of Silver nanoparticles with potent anti bacterial effect.

Extract from this plant may act as reducing and capping agents in silver nanoparticles synthesis. Studies have indicated that biomolecules like proteins, 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 [22, 23]. 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 [23, 24] 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. As the leaf extracts were mixed with the aqueous solution of the silver ion complex it was changed in to yellow to brown colour (Fig.3) due to excitation of the surface plasma vibrations indicate the formation of the Silver nanoparticles [25]. UV-Visible Spectrograph 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 90 ^{o} C. Fig.2 shows the UV-Visible Spectra which are recorded after the completion of the reaction at different time intervals (10, 30, 60 min) at 90 ^{o} C temperature .The UV spectrum absorption is recorded at 427 nm, 429 nm, 432 nm respectively (Fig.2) was confirmed that polydispersed nanoparticles were formed.

The SEM image (Fig.4) is showing high density silver nanoparticles synthesized by the leaf extract, further confirmed the development of silver nano structure. The SEM image shows the formation of spherical nano particles. They were clearly distinguishable in 20-100nm size (Fig.5).

The EDX spectra reveal the purity of the material and the complete chemical composition of synthesized silver nanoparticles. In the present EDX analysis (Fig.6) high percentage of silver indicating the purity of the synthesized sample.

The FTIR spectrum of silver nanoparticles are shown in Fig.7. The band at 3424 cm^{-1} is assigned to the O-H stretching of H-bonded alcohols and phenols. The band at 1636 cm^{-1} corresponds to the N-H bending of primary amines. The bands at 1384 cm^{-1} are related to the C-N streching of aromatic amine group. Whereas in the region 1018 $cm^{-1}$are corresponding to the C-C streching of alcohols, carboxylic acids, ethers and esters are the binding metal with to form a silver nanoparticle is confirmed.In the present study , the peaks are more charecteristic of eugenol,linalool and flavanoids.

Fig. 8a Streptococcus pneumoniae, Fig. 8b Pseudomonas aeruginosa, Fig. 8c Escherichia coli

Fig.8 Zone of inhibition of biologically synthesized silver nanoparticles against bacterial pathogens

The biologically synthesized silver nanoparticles exhibited excellent antibacterial activity against the bacterial pathogens Streptococcus pneumoniae (Fig.8a), Pseudomonas aeruginosa, (Fig.8b) and Escherichia coli (Fig.8c). It has been reported that antibacterial effect was size & dose dependent and was more pronounced against Gram-negative bacteria than Gram-positive bacteria [14, 26]. The present study also clearly indicates the synthesized silver nanoparticles have significant antibacterial activity against Gram-negative bacteria than Gram-positive bacteria. 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 [27]. The zone of inhibition is higher in the case of E.coli followed by Streptococcus pneumoniae, Pseudomonas aeruginosa when compared with standard streptomycin (Fig.8). The

inhibition of bacterial growth reported in this study is dependent on the concentration and number of nanoparticles in the medium.

CONCLUSION

The present study reveals that the *O. americanum* is 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 bio reduced silver nanoparticles were characterized using UV-Vis, FTIR spectroscopy and SEM techniques. The antibacterial efficacy against different species of bacteria confirmed that the silver nanoparticles are capable of rendering antibacterial efficacy and provide a new platform to make it a value added weed for nano technology based industries in future.

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