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GREEN SYNTHESIS OF SILVER NANO PARTICLES USING FENUGREEK SEEDS EXTRACT

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ABSTRACT

In this investigation nano silver was prepared by reduction of silver nitrate using fenugreek extract. Use of natural reducing agent in the synthesis of nano silver is an environmentally friendly process in contrary of using chemical reducing agent. High resonance transmission electron microscope (HR-TEM) and X-ray diffraction (XRD) tests are used to characterize prepared nano silver. The results confirmed that, the morphology of the prepared nano silver were of spherical shape with smooth surface and average diameter of 17nm. Produced nano silver was tested as antibacterial agent and it is successful against E-coli and staphylococcus aureus due to the increase of the inhibition zone than using 1 molar silver nitrate alone i.e. the use of nano silver increased the inhibition zone of E-coli and staphylococcus aureus. So the synthesis of nano silver leads towards chemical use as antibacterial agent.

Keywords: nano silver, fenugreek, reduction, antibacterial assay.

1. INTRODUCTION

Currently, nanotechnology becomes the most promising and emerging field of research because of its potential applications in many fields such as medical sciences, marine antifouling, and agriculture (1). Nano silver is one of the most important elements due to its unique properties such as excellent electrical and thermal conductivity (2), chemical stability (3), catalytic activity (4), non-linear optical behavior and antimicrobial effects (5).

Several techniques are used for the preparation of nano silver such as wet chemical reduction, plasma evaporation, UV and IR radiation, evaporation (6) and laser ablation (7). Chemical methods may include wet conventional chemical (one- or two-phases system), photochemical (8), so no chemical (9), electrochemical (10), and radiolytic reduction (11). One of the greenest and environmentally save technology is the preparation of nano silver using plant extract (12). Silver nanoparticles are also extensively studied because works have also been carried out to synthesize nanoparticles by using microorganisms (13) and plant extracts. The process of using microorganisms for the synthesis of nanoparticles, however, involves the culture of the microorganisms which is tedious and hence the use of plant or plant extracts has advantage over the use of microorganisms. Recent researches to fabricate nanoparticles through green route use Neem leaves (14), *Aloe Vera* (15), *Bryophyllum* (16), *Alternanthera* (17), and tea extract (18) etc. as capping agents were investigates. In the present investigation nano silver is prepared using the chemical reduction method using *Fenugreek* plant extract.

1.1 Fenugreek chemical constituents

The chemical composition of Fenugreek seeds and defatted Fenugreek seeds is given in Table-1. These seeds are a rich source of fiber and protein. The fiber may be further classed as gum (gel fiber) and neutral detergent fiber. Whole Fenugreek seeds also contain 4.8% saponins. Fenugreek seed saponins are of steroidal nature (type furostanol saponins) with diosgenin as the principal steroidal saponin.

Table-1. Proximate composition % of *Fenugreek* seeds (19).

Component	Whole Seeds	Defatted Seeds
Moisture	9.0	9.0
Ash	3.0	3.5
Lipids	8.0	Negligible
Protein	26.0	28.3
Starch	6.0	6.5
Total Fiber	48.0	51.7
Gum	20.0	19.2
Neutral Detergent Fiber	28.0	32.5

1.2 Antibacterial property of fenugreek oils

Fenugreek seed oil showed its higher antibacterial property against Escherichia coli where the inhibition zones were 20 mm when the concentration of fenugreek seed oil was 100%, while at 50% and 90% concentrations the antibacterial activity was not detected (ND). However, there are some reports about the effects of clove and ginger, James *et al*. (20) reported that E.coli was sensitive to ginger and clove oil, while Krishna and Banerjee (21) also reported that ginger, clove, cinnamon, cumin, onion and garlic inhibited the growth of E.coli. They also stated that the bacterium ferments undigested carbohydrates causing flatulence. Badreldin (22) reported that ginger oil inhibited the growth of E. coli with an inhibition zone of 23 mm which was higher than the inhibition zone of fenugreek essential oil. The present study showed that fenugreek seed oil has a potent antibacterial property against Staphylococcus aureus where the inhibition zone diameters were ND, ND and 15mm at 50%, 90% and 100% oil concentrations, respectively. The bacterial species of the genus staphylococci are widely distributed in the environment; these species cause food poisoning, such as cooked meat, eggs, and fish, milk and milk products (23). Ginger oil showed antimicrobial activity against Staphylococcus aureus (22). While, ELboshra (24) reported that Staphylococcus aureus was sensitive to clove

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oil, the inhibition zone was 22mm which was lower than that reported for the oil in the present study. The fenugreek oil was also found to inhibit Salmonella typhimurium, the inhibition zone diameters were ND, ND and 15 mm when the oil concentrations were 50 %, 90% and 100%, respectively. We couldn't find reports in the literature regarding the effects of fenugreek oil against this bacterium; however, ginger oil and clove oil were reported to be effective against this bacterium (20-24).

2. MATERIALS AND METHODS

2.1 Materials

Silver nitrate and distilled water (all of high laboratory grade) were used for this study. Fenugreek seeds extract was used as reducing agent formula and also acts as the stabilizer for synthesized nano sized silver colloids.

2.2 Experimental procedure

2.2.1 Preparation of fenugreek extract

50 grams of fenugreek seeds were boiled in alkaline water (pH9) using 1molar NaOH solution. The mixture was thoroughly agitated for 2hrs using a magnetic stirrer. The mixture was then filtered to obtain the slight greenish brown liquor. The liquor was centrifuged to remove the impurities.

2.2.2 Nano sized silver particles preparation

A sample of nano silver was prepared by mixing 1 ml of fenugreek extract with 49ml distilled water then raising the temperature of the reaction medium to the desired temperature, then add silver nitrate solution drop wise. The reaction mixture was kept under constant rate of stirring (150rpm) for the experiment duration. Once the addition of silver nitrate is completed the reaction medium acquires a clear yellow color then converted to dark brown indicating the formation of silver nanoparticles. The propagation of the reaction was controlled by UV–Vis absorption; aliquots from the reaction bulk were withdrawn at different time intervals and evaluated. The color of the mixture was observed within a few minutes after heating to 60 ^{0}C t hen it was kept overnight in dark for stabilization and the change in color was noted on the next day. Glass bottles with nano silver solutions were tightly closed to prevent external contamination and evaporation.

2.3 Testing and evaluation

2.3.1 UV-VIS spectra

Ultra violet–visible spectra (UV-Vis) have been proved to be quite sensitive to the formation of nano silver particles because they exhibit an intense absorption peak due to the surface Plasmon excitation (it describes the collective excitation of conduction electrons in a metal). The UV–Vis spectra of silver nanoparticles embedded in fenugreek extract were recorded by means of a 50 ANALYTIKA JENA Spectrophotometer from 190 to 600nm. A solution containing silver nitrate dissolved in de-ionized water was used as a blank.

2.3.2 Transmission electron microscope test (TEM)

Shape and size of the obtained nano sized silver particles were characterized by means of a JEOL-JEM-1200 Transmission Electron Microscope (TEM). The TEM sample was prepared by adding a drop of the nano silver solution on a 400 mesh copper grid coated by an amorphous carbon film and let the sample for drying in open air at room temperature. The average diameter of the silver nanoparticles was determined from the diameter of 100 nanoparticles found in several chosen areas in enlarged microphotographs. Also the same apparatus is used for the examination of the morphology and X-ray diffraction test of the obtained particles.

2.3.3 XRD analysis

The structure of the as synthesized silver nanoparticle was investigated by X ray Diffraction test. The sample was casted on a glass plate and the analysis was made at the voltage of 40 kV and current of 40 mA. The source used was copper Kα line. Based on the XRD result, the average particle size has been estimated by using Debye-Scherer equation.

$$D=\frac{0.9\,\lambda}{W\,\cos\theta} \quad (1)$$

Where 'λ' is wave length of X-Ray (0.1541 nm), 'W' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter (size).

2.3.4 Antibacterial analysis

The Antibacterial activity of the extracts was carried out by inhibition zone test 14. Bacterial strains obtained from HiMedia were revived in LB broth (HiMedia) by incubating overnight at 37 ºC. Wattman filter papers of 5mm diameter were sterilized by autoclaving at 15lb/inch^2 for 15 minutes. The sterile paper were impregnated with equal volume (100µg/ml) of fenugreek extracts and silver nanoparticles, and compared with 1mM silver nitrate solution. The round paper containing each of 100µl samples were aseptically placed on plates containing Muller Hinton Agar medium (Merck, Germany) after being sprayed with each of the test pathogens. The plates were incubated at 37 ºC for 48 hours and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 8 mm were considered as low antibacterial activity. Two bacteria strains were tested using the obtained nano silver prepared by the fenugreek seeds extract. Those two strains are E-coli and staphylococcus aureus.

3. RESULTS

3.1 Synthesis of silver nanoparticles

During the preparation of the nano silver particles using the mixture of silver nitrate as metal source and fenugreek seeds extract as reducing and capping agent,

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there was change in color from yellow to brown in the synthesized sample. This indicates the reduction of Ag^+ to Ag^0. This change in color is carried out due to the excitation of surface Plasmon vibrations with the silver nanoparticles (26 & 27).

3.2 UV-Vis spectroscopy analysis

The wavelength corresponding to the surface Plasmon resonance is 430nm. UV-VIS spectroscopy could be used to examine size and shape of controlled nanoparticles in aqueous suspensions. In metal nanoparticles, like silver , the valence and conduction band lie very close to each other and as such the electrons are free to move. When the frequency of the collective oscillation of the electrons become resonant with the frequency of the incoming wave, strong absorption takes place which gives rise to the surface Plasmon resonance (SPR) absorption band. This absorption depends on the size of the nanoparticles, surrounding environment and the dielectric medium. The UV-Vis spectrum of the silver nano sample is shown in Figure1.

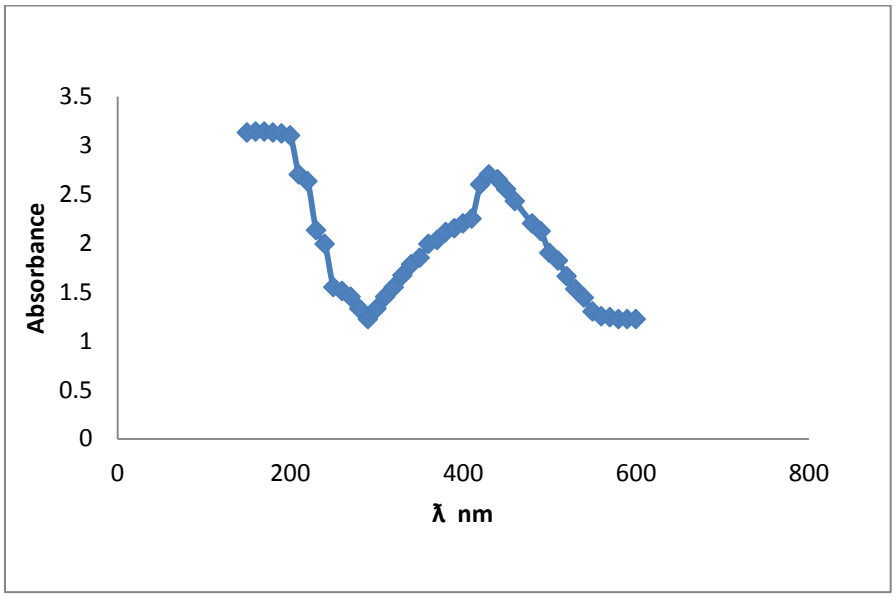

Figure-1. UV-Vis spectrum of the nano silver particles obtained by reaction of fenugreek seeds extract and silver nitrate.

3.3 XRD analysis

Results of XRD confirmed the crystalline structure of silver nanoparticles. The 2θ values of XRD pattern ranging from 30^o to 80^o and prominent peaks are found at 38.18^o, 64.06^o and 75.01^o, which correspond to the planes having Miller indices (111), (200) and (311) respectively. The average particle size is calculated to be around 17nm. Figure-2 shows the Scaning Advanced Electronic Diffraction (SAIED) pattern of the obtained nano silver particles formed by the reaction of silver nitrate and fenugreek seeds extract.

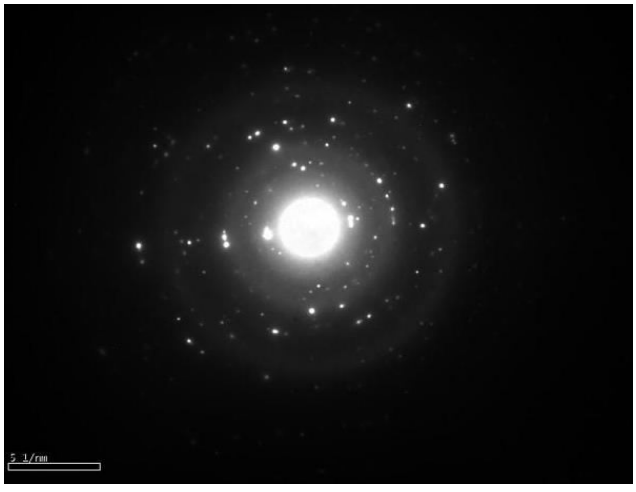

Figure-2. The SAIED pattern of the obtained nano silver particles.

3.4 HR-TEM analysis

Figure-3 depicts the HR-TEM images of the obtained nano silver particles due to the reaction of silver nitrate and fenugreek seeds extract. The morphology of the nanoparticles is depicted from this image. The image shows that most of the particles are spherical in shape with

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a smooth surface morphology with an average diameter of nearly 17nm. The image clearly shows the lattice planes of the crystal with d-spacing of 0.20 nm. The image reveals that the nanoparticles formed are more or less uniform in sizes and shapes.

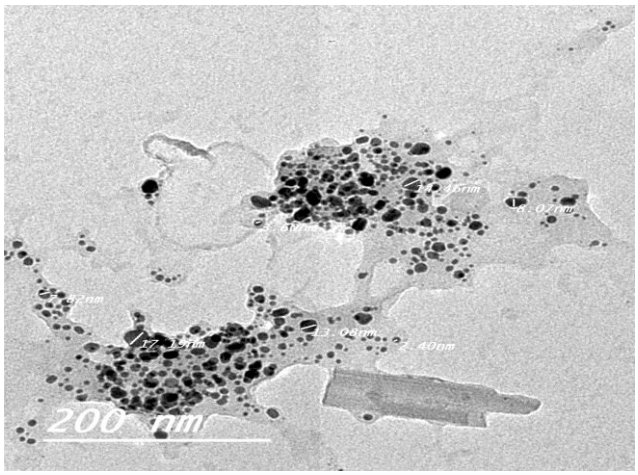

Figure-3. HR-TEM images of the obtained nano silver particles.

The histogram of the obtained nano silver particles formed by the reduction of silver nitrate with fenugreek seeds extract is illustrated in Figure-4 from which It is clear that the highest percent particle size present is 17nm (about 67%).

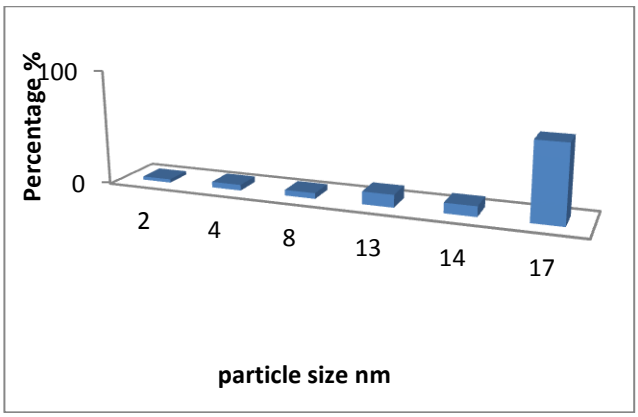

Figure-4. The histogram of the obtained nano silver particles.

3.5 Antibacterial assay

Figure-5 shows antibacterial screening assay of the silver nanoparticles. The inhibition zones against the test strains were small for 25microgram of the obtained nano silver for both tested strains, where it was 10m and 8mm for E-coli and staphylococcus aureus, respectively. As the dose of the nano silver increase to 50microgram the inhibition zone became strong which increase to 13mm and 15mm for E-coli and staphylococcus aureus, respectively. It is clear that the formation of the free radicals obtained at the surface of the silver nanoparticles were responsible for the antibacterial function18. Silver nanoparticles were attaching with the surface of the bacteria and act against the cell wall protein and control the power of bacteria, apart from this small particles attach with the larger surface area was clearly explained (28). We found the highest antibacterial activity of fenugreek seeds extract mediated silver nanoparticles and, this may explain anonymous claim on medicinal use of silver nanoparticles from fenugreek seeds extract.

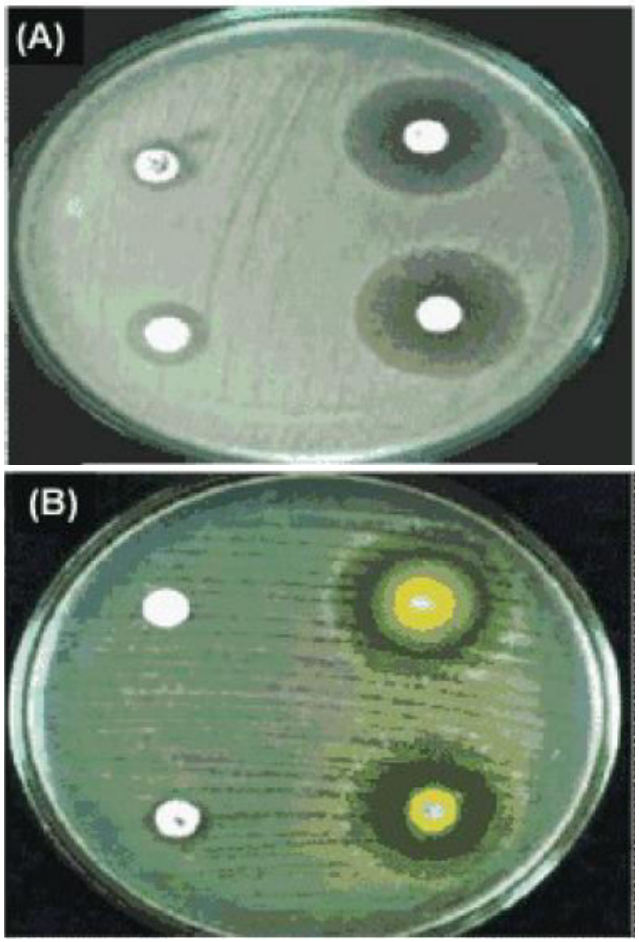

Figure-5. Antibacterial activity of nano (A) using 25mg of nano silver, and (B) using 50mg of nano silver.

4. CONCLUSIONS

The results of this study revealed that,

- Eco friendly synthetic of silver nano particles is a low cost method at ambient conditions.
- It is shown that fenugreek extract is the easy, economic and environmentally friendly process for nano particles preparation.
- Fenugreek represents a considerable improvement in the synthesis of a nano silver because it is used as reducing, capping agent and better control for size and shape.

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- Silver nano particles are effective as antibacterial agent of the E-coli and staphylococcus aureus.

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