RESEARCH ARTICLE

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Green method of synthesis silver nanoparticles as using fenugreek seeds extract (*Trigonella foenum-graecum*) and its application as antibacterial agent

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KEWORDS

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ABSTRACT

In the present research, spherical silver nanoparticles (AgNPs) of 33-75 nm size have been synthesized using AgNO3 solution and aqueous extract of Fenugreek plant seeds as a reducing agent. The principle is based on the reduction of AgNO3 by the extract of fenugreek seeds. The nanoparticles were characterized and investigated by X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). The size and shape of the nanoparticles were found to be sensitive to the quantity of the extract. Silver nanoparticles are broad-spectrum antibacterial agents and the internalization of nanoparticles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor mediated endocytosis. This approach is not only of a green rapid synthesis kind and considered as a better alternative to chemical synthesis, but also found to be effective for large scale synthesis of silver nanoparticles.

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INTRODUCTION

In recent years, nanotechnology plays an important role in our day to day life as a result of not only engineer form and size of metal however the essential properties such as chemical, physical, mechanical, optical and particles change, and may additionally be modified (Alqudami and Annapoorni, 2007). Nanotechnology has succeeded in numerous fields like health care, food and feed, cosmetics, energy science, electronics, mechanics, space industries, environmental health, bioscience, chemical industries, drug and sequence delivery (Korbekandi and Iravani 2012). Nanotechnology has exuberantly been used for the treatments of cancer and other diseases (Brigger *et al.*, 2012). Recently, the green chemistry that aims to cut back or eliminate substances dangerous to human health and also the surroundings within the style, development and implementation of chemical processes and merchandise is changing into additional and additional vital (Poliakoff *et al.*, 2002). The use of nanoparticles is gaining importance in the present century, as they possess definite chemical, optical and mechanical properties. Metal nano- particles are of importance because of their potential applications in chemical change, photonics, biomedicine, antimicrobial activity and optics (Govindraju *et al.*, 2009).

The employment of phytochemicals within the synthesis of nanoparticles is a vital mutualism between nanotechnology and green chemistry (Huang *et al.*, 2007). To accommodate the twelve principles of green chemistry, several researches tried to avoid or reduce the uses of dangerous chemicals and solvents, like exploitation natural materials rather than ancient cyanogenic chemicals (Raveendran *et al.*, 2003). All components of the plant like leaf, stem, flower, seed and skin of the fruits were used earlier for the synthesis of silver nanoparticles (AgNPs). Plants are used for the synthesis of nanoparticles were coated by the plant extract that has medical edges and may be used as drug and cosmetic applications (Mallikarjunaa *et al.*, 2011).

Fenugreek was reported to possess gastro protecting result, antimicrobial activities, anticancer effect, employed in treatment of arthritis, reducing weight, increasing milk production and may regulate gland disease. (Jasim, 2014). Fenugreek is a self-pollinating annual leguminous bean which belongs to Fabaceae family (Balch, 2003). Fenugreek seeds are the most vital and helpful part of fenugreek plant. The fenugreek, plant mainly shows the presence of saponin and alkaloids are anti-nutritional factors (Jani *et al.*, 2009). The rationale for choosing plant biosynthesis is to avoid problems of using dangerous substances and toxic reducing agents. To avoid the problem of agglomeration of Ag NPs in solution, it had been suspended in high salt concentration for clinical uses such as drug delivery.

The current investigation focuses on the aqueous seeds extract of Fenugreek used to synthesize AgNPs and evaluated for its antimicrobial activities by confirming the mechanism.

MATERIALS AND METHODS

Preparation of Dried Biomass

The seeds of Fenugreek local name (Helba) were collected from Omdurman market, Khartoum, Sudan. The seeds were thoroughly washed with deionized distilled water and crushed. The powder was further used for preparation of 10 g/L aqueous seeds extract. This extract was filtered with filter paper and stored at 4ºC until further use for present investigation.

Chemicals

Silver nitrate (AgNO3) was purchased from Lab Course Trading Enterprises, Khartoum Sudan and used without further purification. Deionized distilled water was used throughout the experiment. All other chemicals were of analytical grade.

Synthesis of nanoparticles

For biosynthesis of nanoparticles, 2.0 ml plant seeds extract were mixed with 25 ml of freshly prepared silver nitrate 10-3 M AgNO3 solution that was prepared in 250 mL of deionized water in a sterile conical flask and kept in dark place at room temperature. The reaction mixture was incubated for 30 min or until colour change to dark pink observed. The nanoparticles then synthesized by drying at 90oC.

Characterization of nanoparticles

The synthesized AgNPs were characterized by Scanning Electron Microscopy (SEM) and X- ray diffraction analysis

(XRD), by following the standard method (Nethradevi *et al*., 2012).

Test Organisms

The test organisms used for antimicrobial analysis of AgNPs extracts were four different species of bacteria. Pure isolates of these organisms were obtained from the Department of Microbiology, University of Bahri, Sudan. The bacteria include: *Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis* and *Escherichia coli.*

Antibacterial activity

The agar diffusion method was used for the antimicrobial activity study. Four types of agars were used in this experiment, sterile nutrient agar, Mac Conky, Eosin methylene blue (EMB) and mannitol salt agar. Six millimeters (mm) diameter wells were bored into the agar with sterile cork borer and filled with 0.4 ml. of various dilutions of the extracts: 40%, 60%, 80% and 100% in distilled water. The petri dishes were incubated at 37ºC for 24hrs. At the end of incubation, the zones of inhibition that developed were measured in (mm) with the help of a transparent ruler. Distilled water was used as negative control, and Ciprofloxacin was used as a positive one. Each sample repeated three times (A, B, and C), Diameter of zones inhibition ≥10 mm exhibited by plant extract was considered active (Alshafei *et al*., 2016).

Statistical analysis

Tests were performed in triplicate, and the results are expressed as means ± the standard errors of the means.

RESULTS

The current research work, biosynthesis of AgNPs was carried out using the aqueous extract of fenugreek seeds *(Trigonella foenum -graecum*) as reducing and capping agent. The principle is based on the reduction of $AgNO_3$ by the extract of fenugreek seeds.

Microscopic techniques

The scanning electron microscopy (SEM) and X- ray diffraction analysis (XRD) image of the nanoparticles presented the topography of the particle was showed in figure (1). SEM photograph of silver nanoparticles clearly indicates that synthesized silver nanoparticles have average size less than 100 nm, with different shape.

Table 1: Antimicrobial pattern of AgNPs on *Klebsiella pneumonia*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean ±SE
1	100%	36	17	18	16	17 ±0.6
2	80%	36	15	15	15	15 ± 0.0
3	60%	36	15	15	12	14 ± 1.7
4	40%	36	13	12	17	14 ± 1.7

Conc. = Concentration, (%) = Percentage, (mm) = millimeter zone of inhibition, (SE) = Standard Error

Table 2: Antimicrobial pattern of AgNPs on *Escherichia coli*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean ± SE
1	100%	25	17	16	15	16 ±0.6
2	80%	25	15	14	15	14.7 ±0.3
3	60%	25	16	17	15	16 ±0.6
4	40%	25	20	15	16	17 ±1.5

Conc. = Concentration, (%) = Percentage, (mm) = millimeter zone of inhibition, (SE) = Standard Error

Results of antimicrobial examination of the different concentration of the extract on the microbial isolates are shown in tables 1, 2 and 3 and figure 2. The antimicrobial activity of the AgNPs were evaluated by agar diffusion method and the primary screening revealed that the AgNPs suppress the growth of Gram positive and Gram negative bacteria at different concentrations (40, 60, 80, and 100) mg/mL; among the bacteria, *Staphylococcus aureus, Klebsiella pneumonia*, *Bacillus subtilis* and *Escherichia coli.* The results revealed that the growth of bacteria suppressed by the AgNPs and comparatively revealed better activity at concentration 100%.

This experiment showed the antibacterial effect of silver nanoparticles against some microbes. Silver nanoparticles have an antibacterial effect and the zone of inhibition was observed in figure (2). The following tables and figure show the size of zone of inhibition for the different concentration used.

Table 3: Antimicrobial pattern of AgNPs on *Staphylococcus spp.*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean± SE
1	100%	25	19	22	21	20.7 ± 0.9
2	80%	25	18	20	19	19 ±0.6
3	60%	25	17	18	16	17 ±0.6
4	40%	25	16	14	14	14.7 ±0.7

Conc. = Concentration, (%) = Percentage, (mm) = millimeter zone of inhibition, (SE) = Standard Error

Table 4: Antimicrobial pattern of AgNPs on *Bacillus subtilis*

No	AgNPs conc.	Control (mm)	A (mm)	B (mm)	C (mm)	Mean ± SE
1	100%	40	40	41	37	39 ± 1.2
2	80%	40	39	37	38	38 ± 0.6
3	60%	40	36	34	35	35 ± 0.6
4	40%	40	34	32	33	33 ± 0.6

Conc.= Concentration, (%) = Percentage, mm= millimeter zone of inhibition, SE = Standard error

DISCUSSION

Green synthesis of nanoparticles is advanced than alternative strategies because of its simple, comparatively consistent, and cost-efficient and sometimes leads to additional stable materials (Kalaiarasi *et al*., 2010). Plants have been used for the synthesis of nanoparticles were coated by the plant extract that has medical advantages and may be used as drug and cosmetic applications (Mallikarjunaa *et al*., 2011).

Aqueous seed extract of Fenugreek acts as a reducing agent (Thombre *et al*., 2013) that reduces metallic silver to Nano silver and hence the color modification was obtained. It's standard that silver nanoparticles exhibit red pink colourize solution due to excitation of surface Plasmon vibrations in silver nanoparticles. Ag^{+2} ions of silver nitrate are found to be reduced to Ag atoms. The synthesized nanoparticles were characterized using SEM and XRD spectroscopy analysis.

The spectrum of the sample was obtained for wavelength range in-between 1nm- 100nm. The λ max of the nanoparticles was observed at 75 nm. This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by silver nanoparticles. The silver nanoparticles oscillate when exposed to electromagnetic radiation and this oscillation gives a typical peak value (Smitha *et al*., 2008). The SEM images of the nanoparticles represents the topography of the particles is shown in images figure (1). The results obtained in this study is near to that found by (Jing *et al*., 2009) who mentioned that, the size of silver nanoparticles synthesized by green synthesis was estimated to be around 20- 50 nm.

The results obtained during this study and conferred in tables 1, 2, 3, 4 and figure 2 demonstrate that AgNPs at completely different concentrations were quite effective against test organisms. This means that the extract of seeds with silver nitrate contains substances, which will inhibit the expansion of some microorganisms. This analysis conducted on gram positive and gram-negative bacterium and also the results seem that enormous zone of inhibition and these finding more accept as true by alternative analysis groups, (Kong and Jang 2008) wherever it had been verified that silver nanoparticles exert identical result on Gram positive and Gram-negative strains.

Our result at identical line with that found by (Sung and lee, 2010) who obtained that, phytochemical constituents of the medicinal plants, principally concerned within the alteration of the gram positive and gram-negative microorganism cell walls by neutering the membrane. The phytochemical within the seeds reduce the silver salts and not solely manufacture silver nanoparticles however additionally stabilize it by capping the nanoparticles with the plant peptides. The antimicrobial activity of the nanoparticles is high increased because of the presence of plant proteins and phytochemical. (Rajesh and Neelu, 2015). The mode of action of silver nanoparticles is analogous to it of silver ions, which complex with electron donor groups containing sulfur, oxygen or nitrogen atoms that are normally present as thiols or phosphates on amino acids and nucleic acids (McDonnell 2007).

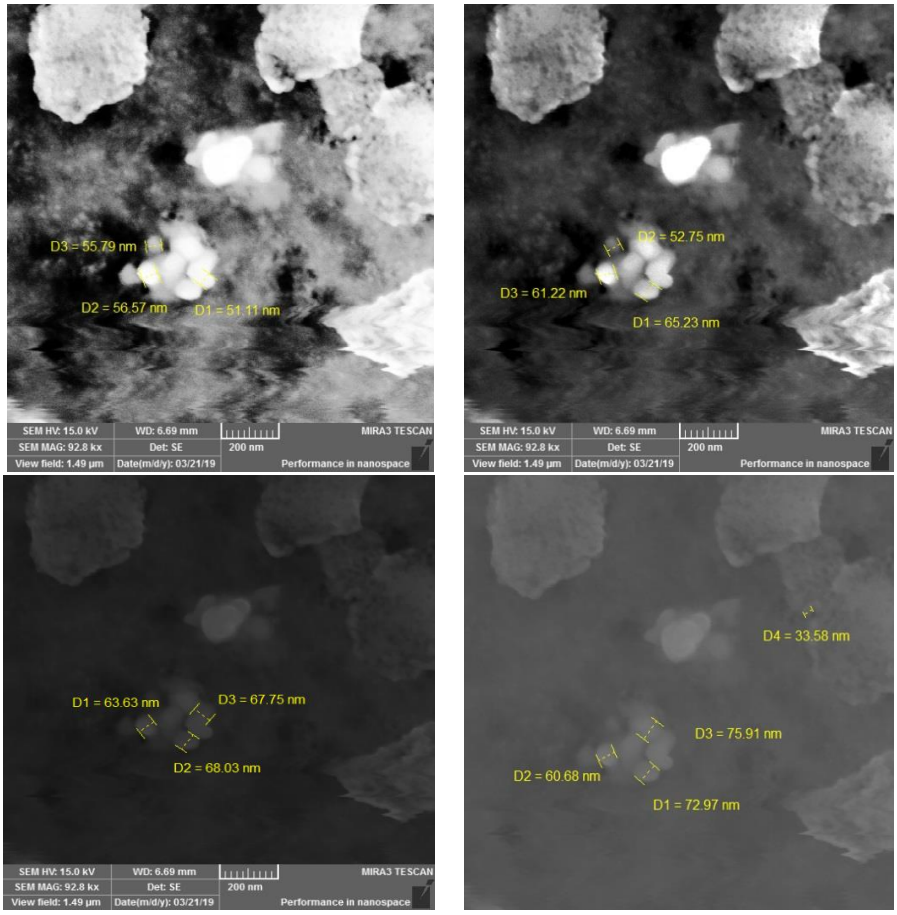

Figure 1: SEM images of silver nanoparticles produced by Fenugreek seeds extract

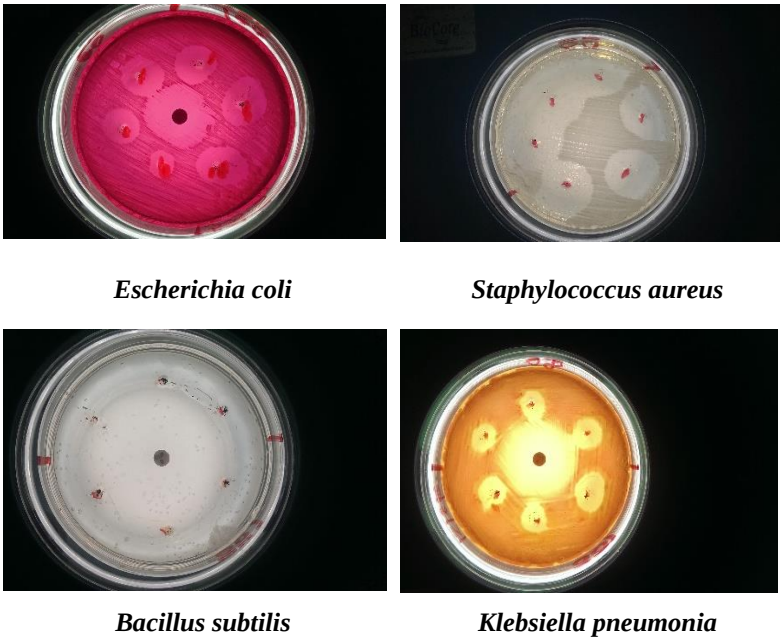

Figure 2: Zone of growth inhibition of the AgNPs against tested bacteria species

Like silver nanoparticles, silver ions additionally exert their activity through a broad vary of mechanisms, together with denaturing the 30s ribosome subunit, suppressing the expression of enzymes and proteins essential to adenosine triphosphate production. Inhibiting metabolic process enzymes thereby causing the assembly of reactive oxygen species (Yamanaka *et al*., 2005), binding and dimerizing ribonucleic acid RNA and deoxyribonucleic acid (DNA) (Rai *et al*. 2009). Consequently, silver nanoparticles got to reach the cytomembrane to realize an antibacterial drug result. Indeed, silver nanoparticles attach to the surface of the cell membrane and disturb it perform, penetrate bacterium, and unharness silver destabilizing and disrupting the outer cell membrane (Lok *et al*., 2006).

CONCLUSION

The present work reassessed that, green synthetic method was a low-cost approach and capable of synthesizing silver nanoparticles at room temperature. The size and structure of obtained silver nanoparticles were characterized by SEM and XRD. The stability and biocompatibility of the silver nanoparticles synthesized using biological protocols was found to be extremely high than the chemically synthesized silver nanoparticles. In addition, silver nanoparticles are broad-spectrum antibacterial agents. Since plants are widely distributed, readily available and at the same time safe to handle, there will be safe to use keeping food from animal origin, a lot to do to develop this methodology of synthesis inspired by several conventional ideas

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