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GREEN SYNTHESIS OF SILVER NANOPARTICLES (AGNPS) BY *TRIGONELLA FOENUM-GRAECUM* (FENUGREEK) SEEDS EXTRACT

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Abstract

This study was designed to evaluate the green method synthesis of silver nanoparticles (AgNPs) as a method an easy, ecofriendly and convenient synthesis of (AgNPs) of an average diameter of 56.00 nm using the aqueous solution of *T. foenum-graecum* (fenugreek) seeds extract as a reducing and increases the stability of these nanoparticles. The reaction was carried out at 1mM concentration of silver nitrate, and the effect of temperature on the synthesis of AgNPs was investigated by stirring at 75^{o}C for 20 min. The AgNPs synthesis was confirmed by their change of colour of the solution from pale yellow to brown, which gives indication for the creation of AgNPs. For characterization and size measuring of silver nanoparticles (AgNPs), several measurements were used; UV-visible absorption Spectrophotometer, atomic force microscope (AFM), fourier transforms infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). UV- visible spectra gave a surface plasmon resonance (SPR) at 425 nm which silver ion (Ag+) reduction to silver (Ag^{o}) and silver nanoparticles (AgNP) formation.

***Keywords*:** Green synthesis, *T. foenum-graecum* (fenugreek) and AgNPs

Introduction

Fenugreek, *Trigonella foenum-graecum* L. (Fabaceae), a medicinal plant of Mediterranean origin, used by ancient Egyptians and grown globally. Its seeds are used as a flavoring agent in food preparations; it is presumed to have nutritional and restorative characteristics (Petit *et al*., 1993) and has been used in folks medication for hundreds of years for a wide range of diseases including diabetes (Renuka *et al*., 2009). Previous studies have revealed the hypoglycemic and hypocholesterolemic effects of *T. foenum graecum* aqueous seed extract of type 1 and type 2 diabetes mellitus patients and experimental diabetic mice (Laila and Murtaza, 2015). The synthesized nanoparticles event is a rapidly increasing sector with excellent potential and applications in several medical specialty areas, including nanomedicine. Nanomaterials have unique characteristics and applications that can be used sensibly in drug delivery and imaging to improve diagnosis and medical care for several human illnesses (Alkaladi *et al*., 2014). The use of metal nanoparticles is a fresh age in the management of polygenic disorder. Each gold and silver nanoparticles were assessed for their antidiabetic potential and showed promising outcomes (Alkaladi *et al*., 2014).

This study was aimed at: Synthesis of silver nanoparticles with a natural ingredient, aqueous Fenugreek, seeds extract.

Materials and Methods

Preparation of aqueous extract of *T. foenum graecum* seeds

Trigonella foenum-graecum (Fenugreek) seeds were bought from the local market, and washed. The seeds were recognized by the Biology Department at University of Baghdad, Baghdad.

Dried fenugreek seeds (4 g) were well cleaned and soaked overnight in (200) mL of boiled water. The sample was centrifuged at room temperature for (7) min at (8000) rpm and filtered again when used to prepare the nanoparticles (Promy, 2018).

Preparation of ($AgNO_3$) solution

To prepare 1mM of $AgNO_3$ solution, 0.017 g of ($AgNO_3$) it was dissolved in 100 ml of deionized distilled water.

Synthesis of green silver nanoparticles

Green silver nanoparticles synthesis: 50 ml of water was used to dissolve 0.001 M silver nitrate under vigorous stirring, resulting in a pale yellow solution. To this six ml *T. foenum graecum* aqueous seed extract was added, which altered the color of the solution to brown under vigorous stirring for 20 min at 75 Co, which confirmed the reduction of Ag^{+} ions and the formation of green (AgNPs) (Meena and Chouhan, 2015).

Characterization of green silver nanoparticles

UV-visible absorption Spectrophotometer of AgNPs : UV-VIS double beam spectrophotometers from SHIMADZU evaluated the absorbance spectra (SPE spectra) of AgNPs solution. All spectra were evaluated at room temperature in a quartz cell with an optical path of one cm. Deionized H_2O was used as a blank. The absorption was taken from (200-800nm). Some samples became too concentrated and that they were diluted 1: 10 in deionized water (Luis *et al*., 2014).

Atomic Force Microscope (AFM) Analysis : The surface morphology of green (AgNPs) was visualized in the Atomic Force Microscope Contact mode under normal atmospheric conditions. AFM analysis was done using scanning prop microscopy AA3000. A sample of AgNPs solution was sonicated with H_2O, a alittle drop of this sample was placed on glass slide (2x1cm) allowed to dry. Then the slide was placed on the AFM sample stage, analysis was applied in line with the quality procedure (Nishida *et al*., 2008).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis : The characterization of functional groups on the AgNP surface by plant extracts was explored by FTIR assessment (Shimadzu) and the spectra was also screened at a resolution of 4 cm^{-1} within the range of 4000–400 cm^{-1}. The samples were prepared by evenly dispersing the AgNPs in a dry Potassium bromide matrix, compressed to create an almost transparent disk. Potassium bromide was used as a standard for analyzing the samples. All the samples used in this analysis were in their indigenous liquid form throughout the analysis (Awwad *et al*., 2012).

Scanning Electron Microscopy (SEM) Analysis : A scanning electron microscope (SEM) was used to evaluate the morphology of the nanoparticles that were created. The morphological characterization of the samples was performed in science and technology ministry using Tescan Vega III for SEM analysis. The samples were distributed on a slide and then covered in an auto fine coater with platinum. After that, the material was analyzed (Prasad *et al*., 2011).

Results and Discussion

Synthesis and Characterization of silver nanoparticles (AgNPs)

Silver nanoparticles were prepared by the green method and characterised by using UV-VIS spectroscopy, Atomic Force Microscope (AFM), Fourier transform infrared spectroscopy (FTIR) and Scanning Electron Microscope (SEM).

T. foenum graecum aqueous seed extract functions as a reduction agent that reduces metallic silver to nanosilver and therefore the color modification was obtained, figure (1). It is well known that AgNPs exhibit brown color in aqueous solution this could be attributed to the excitation of surface plasmon vibrations in AgNPs. Silver nitrate ions Ag^{2+} are discovered to be reduced to Ag^{o} atoms (Thombre *et al*., 2013). Similarly, Krithiga *et al.* (2015) reported that the AgNPs exhibited striking colors, from colorless to yellowish brown.

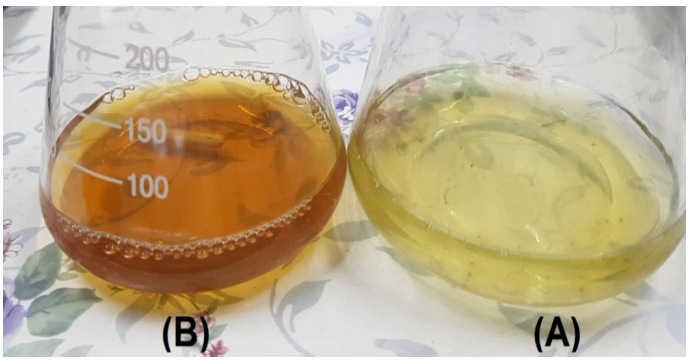

Fig. 1 : (A) *T. foenum graecum* Aqueous seed extract (B) green synthesis of AgNPs with *T.foenum graecum* aqueous seed extract after stirring for 20min at 75ºC

Formation and stability of prepared AgNPs in deionized water was approved by UV-Vis spectrophotometer during a range of 200-800 nm of wavelength. Figure (2) shows the UV-Vis spectrum collected from the reaction medium after one hour and results in an absorption band at 425 nm.

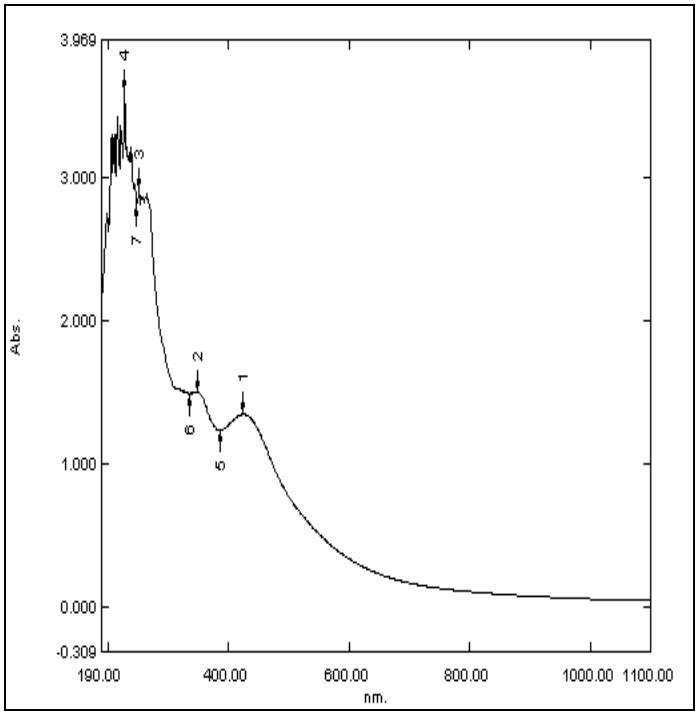

Fig. 2 : UV-Vis spectra showing absorption of AgNPs with *T. foenum graecum* aqueous seed extract after 1h in a range of 200-800 nm of wavelength

The sample spectrum was obtained for wavelength ranges from 400 nm to 580 nm. This is due to a phenomenon known as Surface Plasmon Resonance (SPR) displayed by AgNPs. When the silver nanoparticles are subjected to electromagnetic radiation and this oscillation provides a typical peak value (Smitha *et al*., 2008).

No modification in color was observed and also the silver nanoparticle (AgNPs) analyzed by UV-Vis spectra and it is stable even after 4 months, due to the binding force (ionic strength) between AgNPs and capping molecules in the reaction (Khalil *et al*., 2014).

Atomic Force Microscopy (AFM)

Atomic Force Microscopy was one amongst the first tools for measuring, imaging and manipulating matters at the nanoscale (Li *et al*., 2011). It was utilized to characterize the size and morphology of AgNPs. Figure (3) shows AFM images and the corresponding size distribution of the prepared AgNPs. It was found that the average diameter was about 56.00 nm.

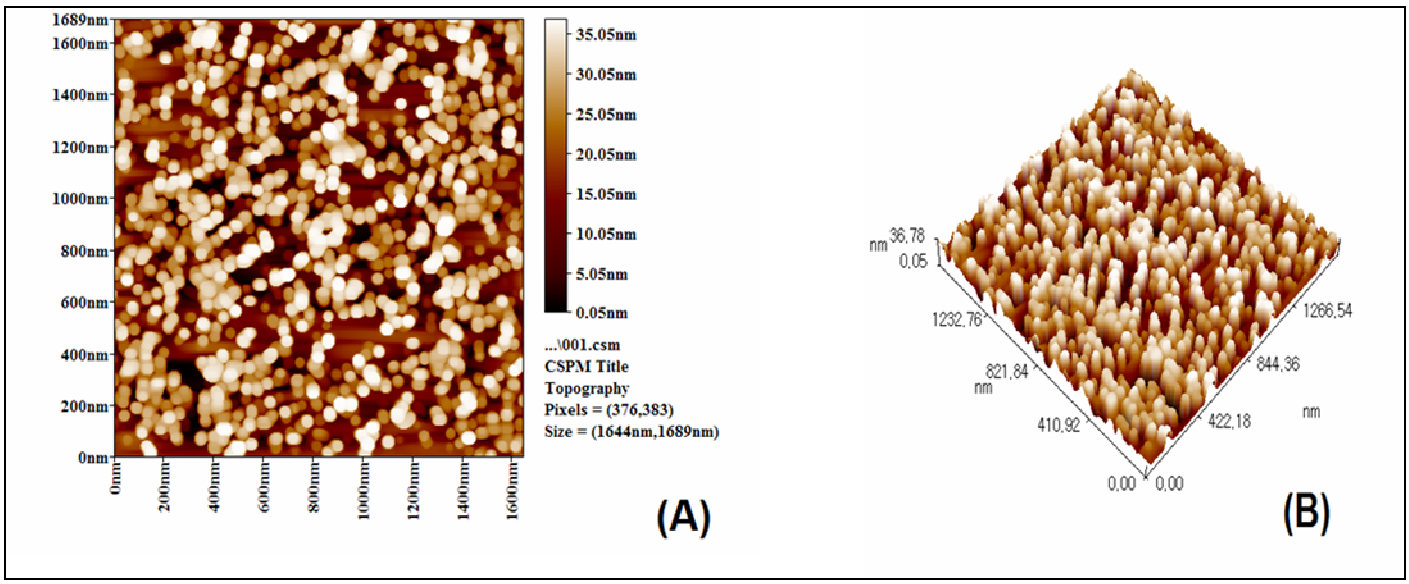

Fig. 3 : (A and B) atomic force microscopy image of AgNPs with *T. foenum graecum* aqueous seed extract

The AFM measurement results shown in Figure (4) refer to the granular distribution of AgNPs with *T. foenum graecum* extract, and it shows that 10% of the particles are 0 nm in size, 50% of the particles are 55 nm, 90% of the particles are 60 nm and the average particles size is about 56.12 nm.

Avg. Diameter:56.12 nm **<=10% Diameter:0 nm**

<=50% Diameter:55.00 nm **<=90% Diameter:60.00 nm**

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
50.00	16.20	16.20	60.00	25.70	73.74	70.00	9.50	100.00
55.00	31.84	48.04	65.00	16.76	90.50			

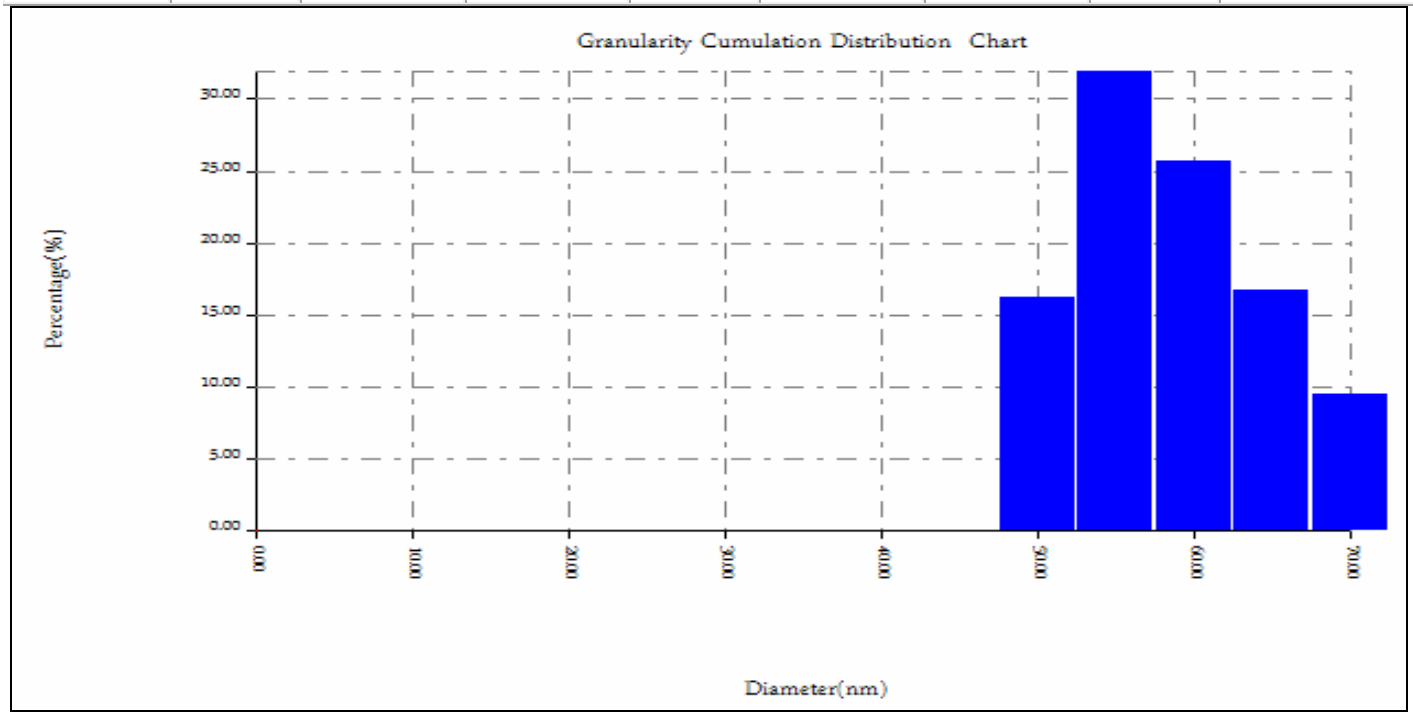

Fig. 4 : Distribution of AgNPs with *T. foenum graecum* aqueous seed extract in Atomic Force Microscopy

Fourier Transform Infrared Spectroscopy (FTIR) Spectrum for Biosynthesized Silver Nanoparticles

Analysis of Fourier transform infrared spectroscopy is used to substantiate the existence of visible plant peptides owing to bending generated by amide bonds. The biological synthesis of silver nanoparticles is an option to chemical synthesis and used the reduction characteristics of biological products for silver nitrate synthesis to AgNPs. Biological synthesis of nanoparticles was earlier recorded using seed extracts from Fenugreek. The phytochemical in the Fenugreek seed reduces silver nitrate and not only produces silver nanoparticles but also stabilizes it by capping the

nanoparticles with the plant peptides. The antimicrobial activity of nanoparticles is therefore improved owing to the presence of plant proteins and phytochemical (Meena and Chouhan 2015).

Fourier transform infrared spectroscopy measured from 400 cm^{-1} to 4000 cm^{-1} to determine the functional group of the nanoparticles. The synthesized AgNPs showed absorption bands at 3445 cm^{-1}, 1643 cm^{-1}, 2400 cm^{-1} and 459 cm^{-1}. The peak at 3445 cm^{-1} and 1643 cm^{-1} showed the presence of -OH or -COOH and amid group stretching in the silver nanoparticles Figure (5). These absorbance bands were also associated with an O-H stretch of phenol or alcohol group primary aminoalkane, N=O bend of nitro group, $-NO_2$ of aliphatic group [16]. The existence of the functional group in the AgNPs was similar to the report of Kumar *et al.* (2011) and Venkatachalam *et al*. (2013),Where the FTIR spectrum showed the functional group of hydroxyl, acid and amide in the synthesized AgNPs (Kumar *et al.,* 2011; Venkatachalam *et al.,* 2013).

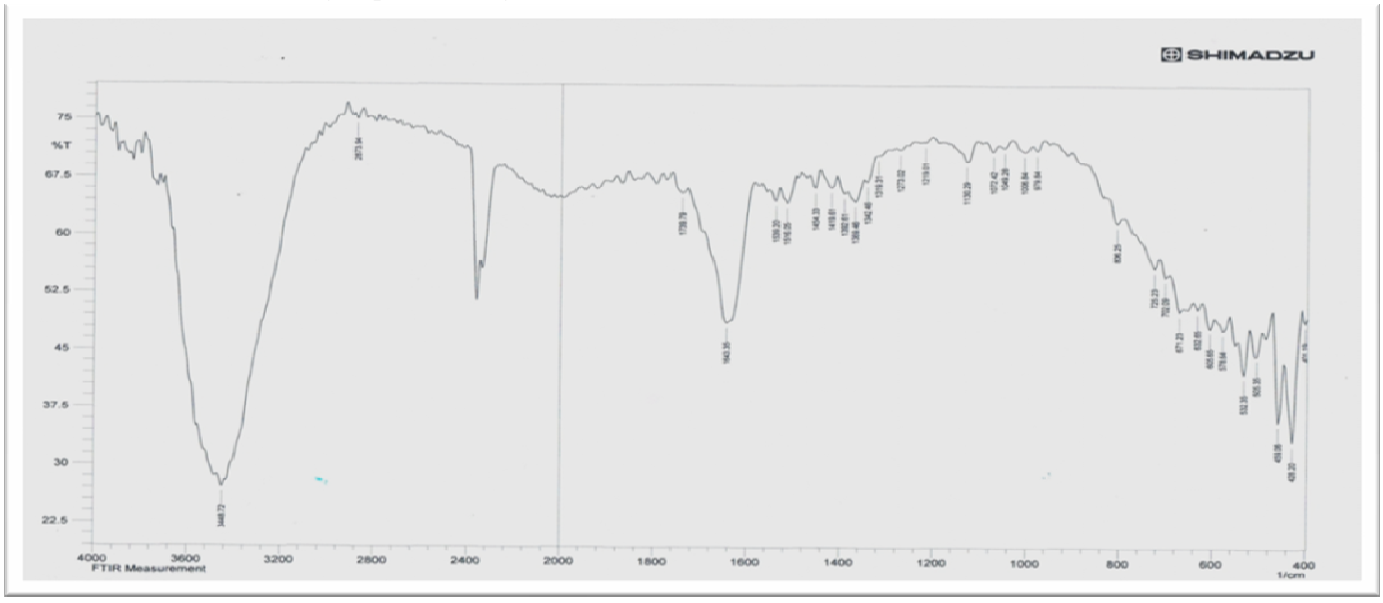

Fig. 5 : FTIR spectrums of AgNPs with *T. foenum graecum* aqueous seed extract

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is one amongst the wide used techniques for the characterization of the synthesized nanoparticles. And this microscopy also analyzed the form and morphology of the formed green nanoparticles. The observations ensure that the morphology of nanoparticles is very variable with a range of sizes and shapes. The surface deposited silver nanoparticles is clearly seen at (x50,000). The SEM image showing the high density silver nanoparticles synthesized by *T. foenum graecum* seed extract confirmed the event of silver nanostructure. Figure (6) shows the SEM analysis of the synthesized silver nanoparticles from *T. foenum graecum* seed extract, Average size of the particle synthesized was 48nm and this result agreed with Jithesh (2013).

And the form of nanoparticles was irregular and spherical and this result agreed with Promy (2018) mentioned that the shape being spherical and irregular, Suggested biomolecules like protein in *T. Foenum-graecum* extract was used as a capping agent to support the development of spherical nanoparticles and adhered to their surfaces. Davi *et al*. (2013) also mentioned that the AgNPs synthesized by *Sargassum longifolium* were cubical. It was well-known that the form of metal NPs considerably modification their optical and electronic properties (Xu and Kall, 2002). A similar phenomenon was reported by Chandran *et al.* (2006).

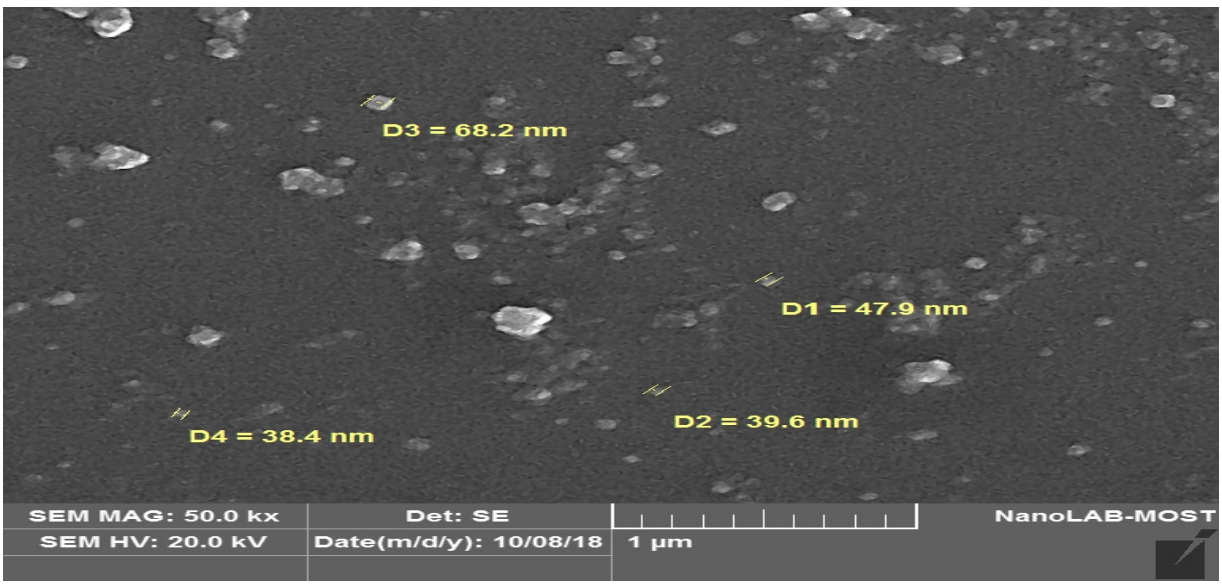

Fig. 6 : SEM image of AgNPs synthesized from *T. foenum graecum* aqueous seed extract at 50000x of magnification power.

Conclusion

Developing an easy, ecofriendly and convenient green synthesis of (AgNPs) of an average diameter of 56.00 nm using the aqueous solution of *T. foenum-graecum* (fenugreek) seeds extract after stirring for 20 min at 75C^{o} and it can be characterized by UV-visible absorption Spectrophotometer, Atomic Force Microscope (AFM), Fourier transforms infrared spectroscopy (FTIR), and Scanning Electron Microscopy (SEM).

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