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Biosynthesis and Characterization of Silver Nanoparticles Using Olive Leaves Extract and Sorbitol

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Abstract: This study developed a rapid, ecofriendly and convenient green method for the synthesis of stable silver nanoparticles (AgNPs) with average diameter of 40 ± 5.0 nm and like spherical in shape, using the aqueous solution of Olive tree (*Olea europaea*) leaves extract as reducing and capping agent along with D-sorbitol. The reaction is carried out at 10^{-3}M of silver nitrate, and the effect of temperature on the synthesis of AgNPs was investigated by stirring at room temperature (25^{O}C) and at 60^{O}C. The AgNPs synthesized were confirmed by their change of color to (dark brown-grey). The characterization studied was done using UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Fourier Transmission Infrared Spectroscopy (FTIR) .

Key words: silver nanoparticles, olive leave extract, D-sorbitol.

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Introduction

"Silver nanoparticles (AgNPs) have received essential attention due to their chemical, attractive electronic and optical properties" (1). "They have been extensively used in many fields, including catalysis, optical sensing, and electronics" (2, 3). "As to antibacterial/ antifungal properties, AgNPs have been used in clothes, cosmetics, wound dressing, air- freshener sprays, water disinfectant, hygiene products and food containers, as a result increases the release of nanoparticles to environment which may cause exposure to human" (4). "Stability of AgNPs in the environment may be due to many factors including the type of capping agent (chemicals which used in the synthesis of NPs to prevent aggregation) that is used, and surrounding environmental conditions, like the ionic strength, nutrient levels, pH and the presence of binding agents" (5). Although physical and chemical methods are more popular in the synthesis of NPs, they have certain limitations such as increase cost of production, release of hazardous by-products, long time for synthesis and difficulty in purification (6). Therefore,

development of reliable, nontoxic and eco-friendly methods for synthesis of nanoparticles was required. The main mechanism considered for the synthesis of nanoparticles mediated by the plants is due to the presence of phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides (7). "A number of plants are being currently investigated for their role in the synthesis of nanoparticles" (8, 9,10). Olive tree (*Olea europaea* L.) is one of the most important fruit trees in Mediterranean countries. "This demonstrates the great economic and social importance of this crop and the possible benefits to be derived from utilization of any of its byproducts" (11). Olive leaves extract (OLE) are rich in biophenols (BPs), such as oleuropein (*Ole*) which is the major active components in leaves , and its derivatives, verbascoside, ligstroside, tyrosol or hydroxytyrosol , as well as cafeic acid , p- coumaric acid , vanillic acid, vanillin, leteolin, diosmetin, rutin , luteolin-7- glucoside , apigenin-7-glucoside and diosmetin-7-glucoside" (12). The green synthesis of silver nanoparticle includes mainly three steps; selection of solvent medium, selection of biological source related reducing agent, selection of nontoxic stabilizing agents .

Materials and Methods

Preparation of Olive Leaves Extract (OLE)

Olive leaves were collected from olive trees in Baghdad University, Baghdad, Iraq. The leaves were washed for several times with distilled water to remove the particles of dust, then dried to remove the residual moisture and cut it into small pieces .An amount of 100g from the small pieces of olive leaves were placed into the flask with 500ml of sterile distilled water, and heated in the water bath at 60^{o}C for 2hr . Then the extract was cooled to room temperature and filtered for several times with Whatman no.1 filter paper by Buchner funnel . The aqueous solution of the extract was concentrated by using rotary evaporator to remove the largest possible amount of water, and then put it in Petri dish at room temperature to dry. The stock solution of extract was prepared with a concentration of 1 and1.5 mg/ml.

Preparation of $AgNO_3$ Solution

To prepare 10^{-3}M of $AgNO_3$ solution, 0.0169 g of AgNO3 from BDH was dissolved in 100 ml of deionized water.

Preparation of D-Sorbitol Solution

To prepare 10^{-2} M of sorbitol solution, 0.1821 g of sorbitol from BDH was dissolved in 100 ml of deionized water.

Synthesis of Silver Nanoparticles

For synthesis of AgNPs, 40 ml of $AgNO_3$ (10^{-3} M) were mixed with 5ml of olive leaves extract (1 and 1.5mg/ml) as reducing agent and 2ml of sorbitol (10^{-2} M) as a capping agent (the color of the mixture was colorless at the beginning)(13) .The mixture was heated in a water bath at 60^{o}C till the color of the mixture solution changes to deep brown-grey with the time ,then the solution was centrifuged for 10 minutes at 15000 rpm(14) . The supernatant was

thrown out to get rid of any uncoordinated materials. The nanoparticles were washed with sterile deionized to remove any residue particles that were not the capping agents, after that the washed supernatant was thrown out after centrifuged for 10min. at 15000 rpm , dispersed the precipitated particles in 5ml of sterile deionized water and put it in a Petri dish to dry at 40^{o}C and then collected the particles (15).

Characterization of Silver Nanoparticles UV-Vis Spectroscopy

The silver nanoparticles were confirmed with UV-VIS Spectrophotometer 1800 Shimadzu from 200 to 800 nm.

SEM Analysis

The morphological characterization of the samples was done using JEOL Jsm-6480 LV for SEM analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater, after that the material was subjected to analysis (16).

AFM analysis

The surface morphology of the nanoparticles was visualized by Atomic force microscope (Veeco) under normal atmospheric conditions. The examined samples were dispersed on small slide and explored on contact mode of the instrument (17).

FTIR Analysis

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR analysis using IR Prestige-21, Shimadzu. The spectra were scanned in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. The samples were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc . KBr was used as a standard analyze the samples (18).

Results and Discussion

Synthesis and Characterization of Silver Nanoparticles

In this study, the prepared silver nanoparticles was characterized by using UV-VIS spectroscopy , Atomic Force Microscope (AFM), Fourier transform infrared spectroscopy (FTIR), and Scanning Electron Microscope (SEM). AgNPs were synthesized using green method, which olive leaves extract (OLE) was used as a reducing and stabilizer agent to reduce silver ion Ag^{+} in $AgNO_3$ to silver nanoparticles (Ag^{0}), and sorbitol as capping and stabilizer agent. After the addition of OLE and sorbitol to $AgNO_3$ and heated to 60^{o}C. The mixture color was changed from colorless to pale yellow, which indicated initial reduction, then to brownish yellow to light brown to deep brown - grey- with the time at 60^{O}C and 2 hr., which due to excitation of surface Plasmon resonance (19) .The reduction rate increased with increases of temperature (20).

Visual Observation and UV-Vis Spectral Study

UV-Visible spectroscopy was used to examine size and shape of nanoparticles in aqueous suspensions (21). Formation and stability of prepared AgNPs in sterile distilled water was approved by

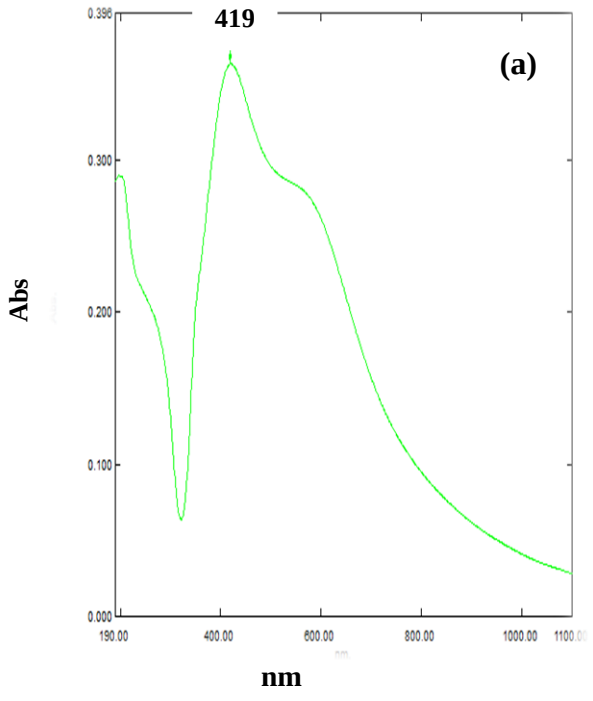
UV-Vis spectrophotometer in a range of 200-800 nm of wavelength . Once olive leaves extract was mixed with $AgNO_3$ and sorbitol, the reduction reaction of Ag^+ ions to Ag^o was observed by measuring UV-Vis spectrum for the reaction media. (Figure 1) showed the recorded UV-Vis spectra after the completion of reaction.

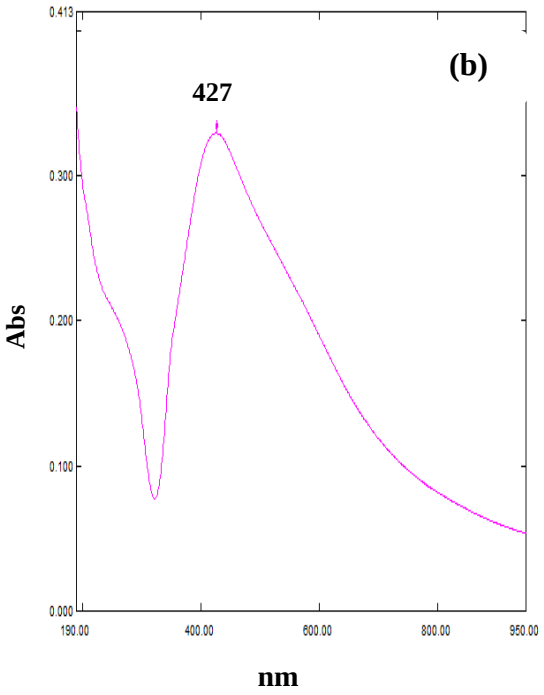
The surface Plasmon resonance (SPR) of AgNPs create a peak near 419 nm (fig.1.a) when the concentration of olive leaves extract was 1mg/ml clearly indicating the formation of spherical AgNPs in this plants extract. The occurrence of which occurs due to the excitation of the surface Plasmon present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field (13), while a peak near 427 nm was observed when the concentration of olive leaves extract was 1.5mg/ml (fig.1.b). As a shown in (Fig.1.c), there was a peak at 459 nm after 1 week at room temperature with 1.5mg/ml, while two peaks at 417 and 579 nm, was noticed after 14 months with 1.5mg/ml as showed in (fig.1.d). All these peaks indicate to biosynthesis of AgNPs (14).The size of AgNPs synthesized at 60^o C was smaller in comparison with those at room temperature (15).The presence of these peaks after this period of time indicated to existence and stability of AgNPs. The peak near 417 nm revealed to the blue shift which was mean a decrease in the size of AgNPs, which demonstrating the ability of capping and stabilizer molecule to prevent NPs to aggregate together, as the peak at 579 nm indicated to arise additional new particles with larger size, which is might be due to aggregations of the other smaller one. Shifts in Plasmon peaks over time indicate the growth of nanoparticles (22). "The frequency and width of surface Plasmon absorption depend on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium" (23). After 14 months, no change in color was observed, and the uv-vis spectra band of AgNPs was stable which is may due to binding force between AgNPs and capping molecules in the reaction. At higher extract concentrations the biomolecules act as reducing agent and capped the nanoparticles surfaces protecting them from aggregation (24). The spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of layer covering AgNPs and acting as a capping agent to prevent agglomeration and provide stability to the medium (14). Gole *et al*., (2001) reported that "proteins present in the extract can bind to silver nanoparticles through either free amino or carboxyl groups in the proteins" (15). As well as, Prasad *et al*., (2011) reported that "the carboxyl (–C=O), hydroxyl (–OH), and amine (–NH) groups of leaf extracts were mainly involved in fabrication of silver nanoparticles" (16). The function of the protective agent was to protect NPs from agglomeration (17). Previous studies have shown that the spherical AgNPs contribute to the absorption bands at around 400 nm in the UV-visible spectra (18).

Characterization by FTIR Spectrum

FTIR study was carried out to investigate the functional groups of olive leaves extract and identify the possible biomolecules which responsible for reducing of the Ag^+ ions

and capping of the bio reduced silver nanoparticles synthesized by OLE with sorbitol. The spectra are shown (in figure 2). OLE displays a number of absorption peaks, reflecting its complex nature. A peak at 3383cm^{-1} observed due to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl (-OH) group. The strong absorption peak at 2935 cm^{-1} could be assigned to –CH stretching vibrations of $–CH_3$ and $–CH_2$ functional groups. The shoulder peak at 1705cm^{-1} assigned for C=O group of carboxylic acids . "The peak IR bands observed at 3383 and 1705 cm^{-1} in OLE were characteristic of the O‑H and C=O stretching modes for the OH and C=O groups possibly of oleuropein, apigenin-7-glucoside and/or luteolin-7-glucoside present in the olive leaf " (19,24). The peak at 1604 cm^{-1} indicated the fingerprint region of CO, C–O and O–H groups, which exists as functional groups of olive leaves extract. The absorption peaks at 1604 cm^{-1} could be attributed to the presence of C–O stretching in carboxyl coupled to the amide linkage in amide I. The band at 1527 cm^{-1} was characteristic of amide II arises as a result of the N-H stretching modes of vibration in the amide linkage. The band at 1396 cm^{-1} assigned to the methylene scissoring vibrations from the proteins". "The intense band at 1076 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amines (19) or C-OH vibrations of the protein in the olive leaf " (24). FTIR study of the *Olea europaea* leaves extract indicated that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of Olea uropea leaves extract were mainly involved in reduction of Ag^+ to Ag^o nanoparticles. Proteins present in the extract can bind to $AgNO_3$ through either free amino or carboxyl groups in the proteins (15).





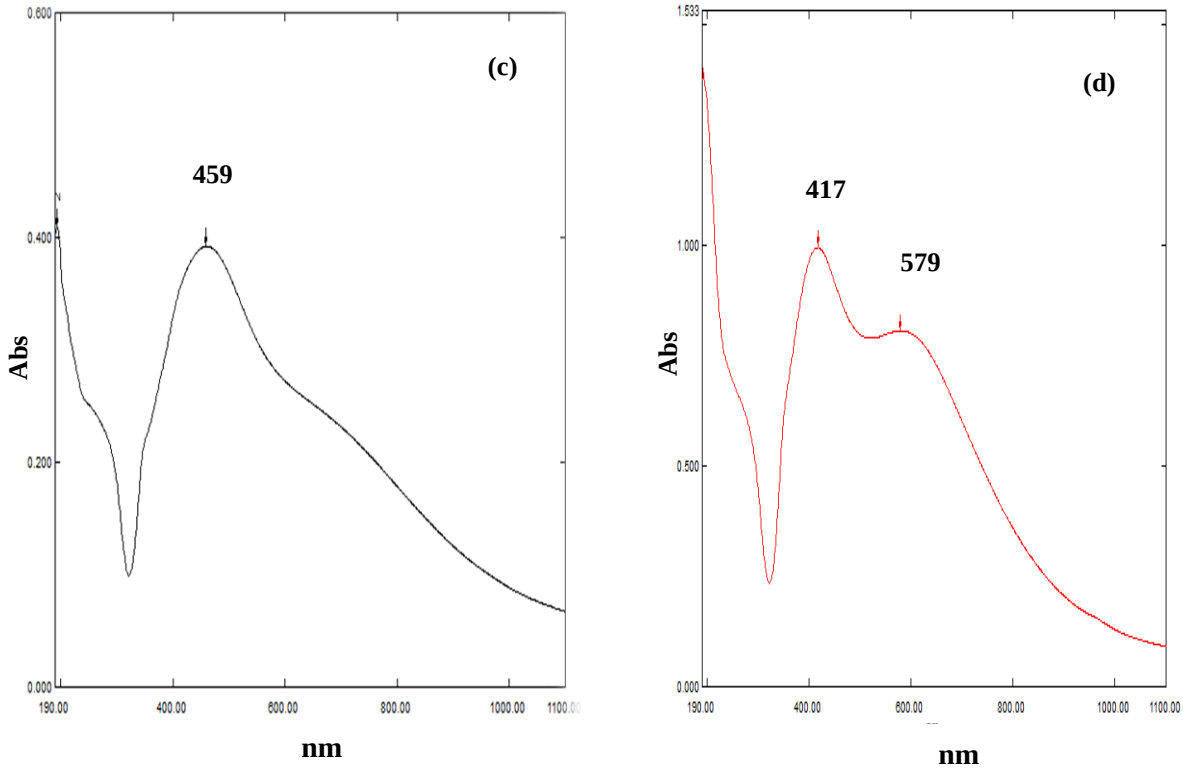


Figure 1: UV-vis spectra showing absorption of AgNPs (a) at 1mg/ml conc. of extract at 60 °C (b) at 1.5 mg/ml conc. of extract at 60°C (c) after 2 weeks at room temp with 1.5mg/ml (d) the storage after 14 month at 1.5 mg/ml of extract.

FTIR spectrum of D-sorbitol showed in (Figure 3). Many bands which at 3398 cm^{-1} is due to bounded hydroxyl, 2970 cm^{-1} (C–H stretching in alkanes), 1280 cm^{-1}due to C-O stretch and 1423 cm^{-1} due to C-H bending vibrations (24). Experimentally, D-sorbitol did not have the potential to reduce the silver ions in the solution, but it may cap the formed silver nanoparticles through electrostatic attraction or bind to the protein groups in the extract via hydrogen bond and increase the stability of the silver nanoparticles. It was indicated that the functional groups in biomolecules were mainly responsible for the reduction of silver ions (25).

In the case of silver nanoparticles, (figure 4) showed a strong band at 3414 cm^{-1}which is due to bounded hydroxyl (-OH) or amine (- NH) groups of olive leaves extract . The band at 2916 observed due to –CH stretching vibrations. A shoulder peak in 1674 cm^{-1} implying the binding of silver ions with C=O groups of the extract (19, 23). The band at 1597 cm^{-1} formed due to C=C bond at aromatic compound. The spectra also illustrate a marginal peak at 1546cm^{-1} and 1531cm^{-1}indicated the amide I and amide II arise due to carbonyl stretch and N-H stretch vibrations in the amide linkages of the protein.

Validates that free amino ($–NH_2$) or carboxylate ($–COO^{-2}$) groups in compounds of the olive leaf extract have interacted with AgNPs surface making AgNPs highly stable (24). FTIR bands of silver nanoparticles, confirm the presence of protein in the silver nanoparticles biosynthesized in this method, which coat the silver nanoparticles known as capping proteins (Figure 4**)**. Capping protein stabilizes AgNPs and prevents agglomeration in the medium. FTIR spectroscopy study confirmed that the *Olea europeae* leaves extract has the ability to perform dual functions of reduction of (Ag^{+}) to (Ag^{o}) and stabilization of silver nanoparticles (19). FTIR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver , suggesting the formation of layer covering AgNPs and acting as a capping agent to prevent agglomeration and provide stability to the medium (14).

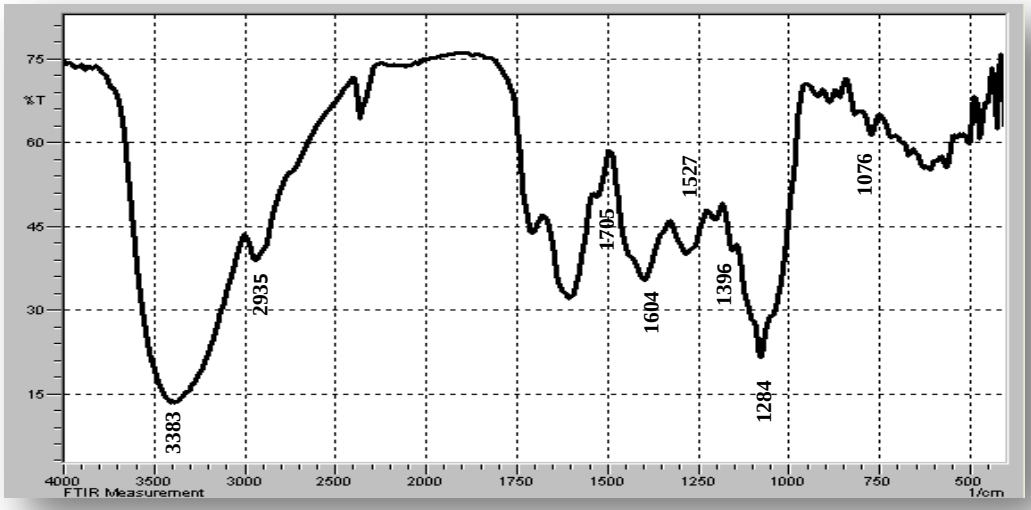


Figure 2: FTIR spectrum of olive leaves extract

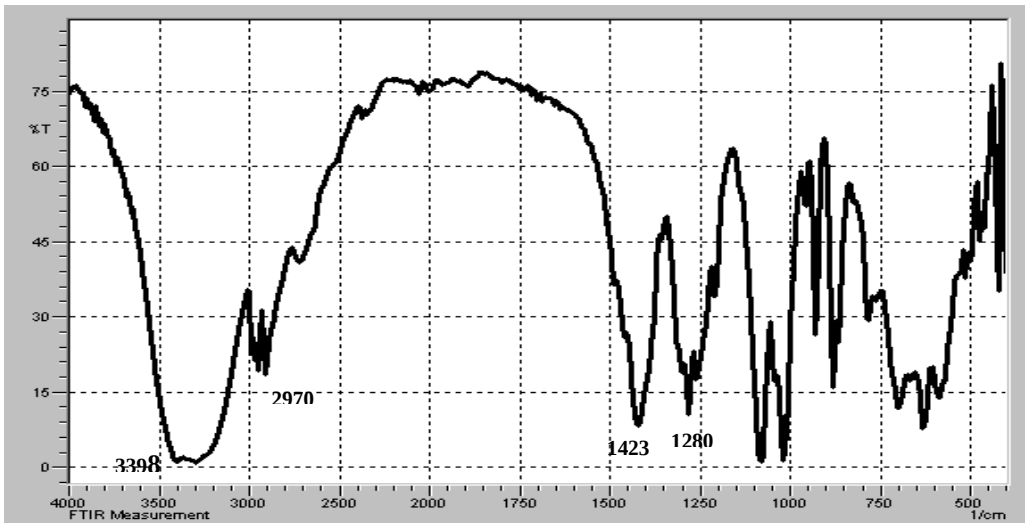


Figure 3: FTIR spectrum of D- sorbitol

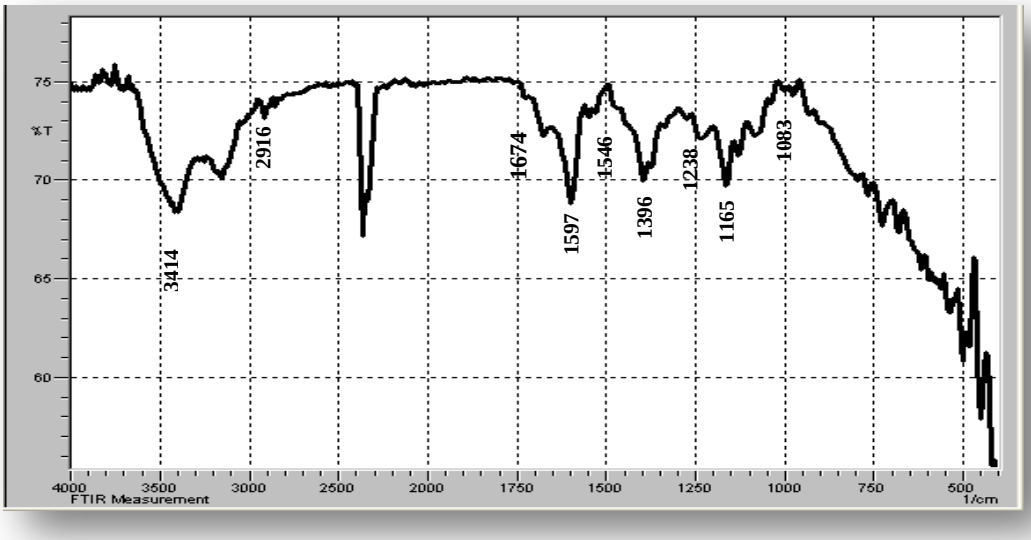


Figure 4 : FTIR spectrum of Silver nanoparticles

Characterization by Scanning Electron Microscopy (SEM)

Scanning electron microscope was employed to analyze the shape of the silver nanoparticles that were synthesized by green method. The surface deposited silver nanoparticles are clearly seen at high magnification (x100,000) in the micrograph. SEM analysis shows that the olive leaf plant have tremendous capability to synthesize silver nanoparticles which were predominantly spherical in shape and were uniformly distributed with an average size little less than 50 nm (Figure-5).

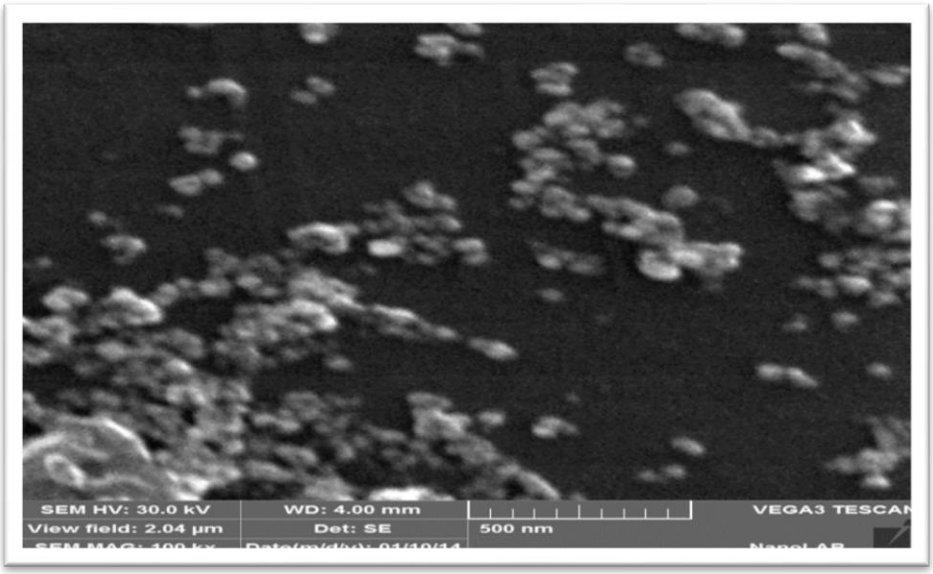


Figure 5: SEM image of AgNPs synthesized from olive leaves extract at 100kx of magnification power

Characterization by Atomic Force Microscopy (AFM)

AFM one was one of the primarily tools for measuring, imaging and manipulating matter at the nanoscale (10); it was employed to characterize the size and morphology of AgNPs. (Figure 6a.6b) showed AFM images and corresponding size distribution of prepared AgNPs, and it was found that the average diameter about 40.45 nm.

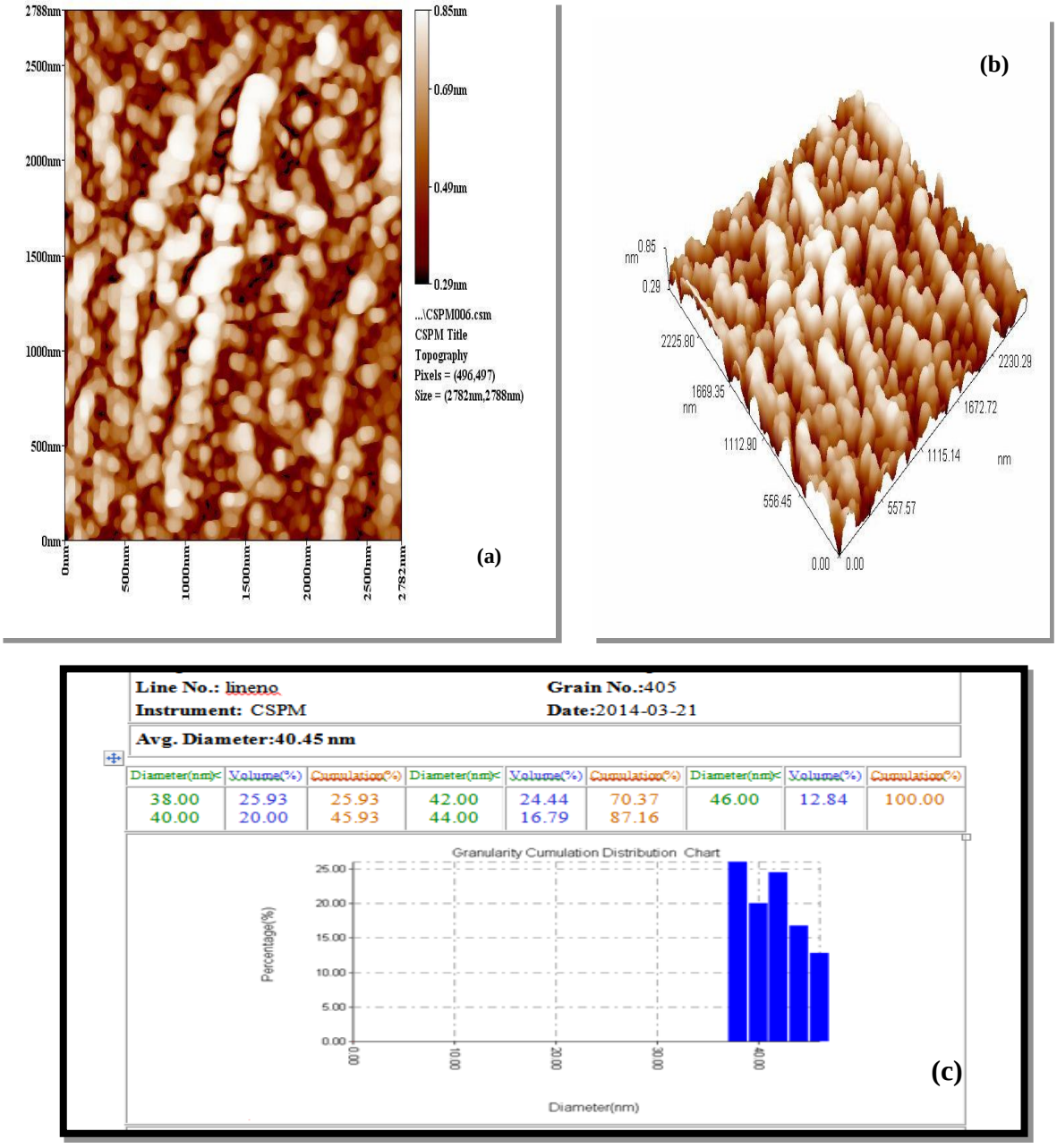


Figure 6: (a, b, c) AFM image of silver nanoparticles

Conclusion

Silver nanoparticles can be prepared in a relatively simple, ecofriendly way using the aqueous solution of Olive leaves extract and D-sorbitol as reducing and capping agent to reduce Ag^{+} to Ag^{o}. Size and shape of the particles were characterized by UV-VIS spectra , SEM, FTIR and AFM , with average diameter of 40 ± 5.0 nm and like spherical in shape .UV-VIS spectra gave surface plasmon resonance (SPR) at 419, in which indicate the best way to synthesize silver nanoparticles.

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