



GREEN BIOSYNTHESIS, IDENTIFICATION AND CHARACTERIZATION OF AG AND ZN NANOPARTICLES USING IVY (*EPIPREMNUM AUREUM*) PLANT EXTRACT.

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Abstract

The present study aimed to biosynthesize silver (Ag) and zinc (Zn) nanoparticles (NPs) from the Ivy plant water extract and the diagnosis of the properties of optical and structural NPs using the UV-Vis spectrum, FTIR, SEM, and AFM. The results of the use of UV-Vis showed higher peaks of absorption of NPs of Ivy at wave length 365 nm for Ag NPs and at 427 nm for the Zn NPs. The results of FTIR spectroscopy revealed a change in the peak absorption of OH groups associated with phenol compounds and monomeric carbohydrates from 3317.98 cm⁻¹ of Ivy pure extract to 3268.32 cm⁻¹ when the extract used to synthesize Zn NPs, and to 3291.17 cm⁻¹ when it used to synthesize Ag NPs. While SEM results showed the presence of a NPs in the pure Ivy extract, and found that the shape of the Zn NPs of Ivy of regular/irregular rectangle and the Ag NPs of Ivy irregular, whereas the NPs of pure Ivy extract regular/irregular spherical. AFM data showed that Zn NPs, Ag NPs, and NPs of pure ivy respectively were 41.70nm,90.07nm, and 21.00nm size, 27.5, 45.00 nm and 12.5n size range, 21.23%, 6.88% nm, and 38.38% size ratio, 1.61nm, 15.9nm, and 6.14nm surface roughness, 85 nm, 60 nm, and 50.00 nm diameter range, and the distribution ratio were 8.04%, 9.69 % and 10.75% respectively.

Key words: Ivy plant, Silver nanoparticle, zinc nanoparticle.

Introduction

In the mid of the 20th century, the concepts of nanotechnology emerged from the principles of physics that saw the possibility of manipulating parts of atom with that of other, creating the belief that we could create anything that we could accurately define. Today, nanotechnology is one of the most important fields of investment among the modern science, for the rapid development of its concepts, the comprehensiveness and breadth of its applications covering all aspects of human life. This has created a sense of excitement and competition among countries, research institutions and researchers to invest effort and money, in further research on nanotechnology development (Singh *et al.*, 2010). Nanotechnology field therefore, give potential to several exploits by the medical and industrial sector, especially in the field of health, equipment and therapeutics (Prabhu *et al.*, 2010).

At the level of nanoparticles (NPs), the materials undergoes a radical change in its properties and in its multi-use applications. Synthesis of NPs of metals such as gold, platinum, silver (Ag), or zinc (Zn), and their oxides, has been investigated using different physical, chemical, and biological approaches (Khan *et al.*, 2016). The latest one is the best in the synthesizing of NPs using environmentally friendly alternatives such as microorganisms, bacteria and fungi (Verma *et al.*, 2010), enzymes (Willner *et al.*, 2007) and plants or their extracts (green processing) (Shankar *et al.*, 2004; Vigneshwaranm *et al.*, 2007 and Jiang *et al.*, 2009), which shows rapidity, safety, stability, and economic advantages, and utilizing especially the case with the plant extracts, its constituents of biomolecules of amino acids, proteins, enzymes, alkaloids, vitamins, phenolic, terpinoids, tannins, and saponins, in reduction and stabilization of metal ions (Jha *et al.*, 2009). Recently, the field of research on NPs with antibacterial effect (anti-BE) has witnessed a boom in the interest of pharmaceutical institutions and researchers in the evaluation of NPs synthesized from plant source that

have a wide control ability on the growth and reproduction, as well as on the pathogenicity both of gram-negative (G-) and gram-positive (G⁺) bacteria, without inducing their resistance through a genetic modification or mutation, in order to enable them avoiding the mechanism of the effects of these antibiotics. The phenomenon of bacterial resistance to traditional antibiotics is a global health problem that began with its use for the first time in the 1940s (Sepehri *et al.*, 2010). Pharmaceutical applications of NPs as antioxidants, antibacterial or inhibiting agents targeting the virulence mechanism of the pathogens without interfering with its resistance, growth or even the bacterial quorum sensing (QS) and biofilm formation, as well as Nano sensors for monitoring the quality and stability of food packing, and food preserving, are gaining attention as a novel next-generation antipathogenic and food preservatives class of agents (Hentzer and Givskov, 2003).

This has inspired us to select *Epipremnum aureum* (Ivy) plant extract for green synthesize AgNPs and Zn NPs and identified their characteristics by using the scanning electron microscope (SEM), Fourier Transmission infrared spectrometer (FTIR), Ultra Violet Visible Xray spectrophotometer (UV-VI) and Atomic Force Microscope (AFM).

Materials and Methods

Preparation of Ivy water extract

Ivy leaf water extract was prepared by collecting of a fresh leaf cleaned by thoroughly washing in distilled water and dried for chopping and grinding in a grinder. 10g of the homogenized extract was added to 100 ml double distilled water (deionized) and incubated with constant stirring (100 rpm) at 45 °C for 30 min. The resultant mixture then filtered using Whitman filter papers No. 1 to remove debris. The extract was stored at 4 °C for future uses. This extract was used either for synthesizing of Zn or Ag NPs (Manokari *et al.*, 2016).

Synthesis of ZnNPs of Ivy:

Zn NPs of Ivy leaf water extract was prepared by adding of 5g (0.05 M aqueous solution) of zinc nitrate (99.999%; Sigma Aldrich) to 50ml Ivy water extract heated on hot plate up to 40-45°C. Heating of the mixture was continued until it changed to a deep yellow paste. The paste then dried in a serving dish by heating in domestic oven at 300°C for 2 hours. The dried paste then grinded by a mortar to achieve of light yellow powder, which then used for characterization purposes (Vidya *et al.*, 2013).

Synthesis of Ag NPs of Ivy:

Ag NPs of Ivy leaf water extract was prepared by mixing of 10ml Ivy leaf water extract with 100ml (1mM aqueous solution) of silver nitrate (Sigma Aldrich), heating of the mixture on hot plate of 40-45°C for 15-20 minutes, and observing the change in the colure of the mixture as a primary sign of building of Ag NPs. 100ml of the silver nitrate solution was kept to use as a control latter (AL-Othman Monira *et al.*, 2017). All of the reagents involved in the experiments were of analytical grade purity and utilized as received without further purification.

Characterization of Ag and Zn NPs of Ivy:

Samples of synthesized Zn and Ag NPs of Ivy were prepared for the detection and characterization by adding 100 ml deionized water to 1g both of each of the sample, then filtered using the Millipore filter measuring 0.22 μm , and 1 ml of the supernatant of prepared solutions after diluted 1:3 by deionized water and centrifuged at 222 rpm for 5 minutes were used to record the room temperature optical adsorption spectrum in the range of 200–1100nm using UV-Vis spectrophotometer (APEI PD303 Japan) (Bharathidasan and

Panneerselvam, 2012), and to record the Fourier transmission infrared (FTIR) spectra of the solutions using a FTIR spectrometer (SHIMA DZU, Japan) in the range of 350-500 cm^{-1} at room temperature (Doaa, 2015). Moreover, detection of the particle size and surface morphology of the synthesized NPs was achieved by the scanning electron microscope (SEM; Meiji Japan) operated at different magnifying forces (Vanmathi and Sivakumar, 2012), whereas further morphology detection of surface, diameter, and size of the NPs was achieved by using the Atomic force microscope supported by advanced angstrom (AA2000, USA) scan (Doaa, 2015).

Results

Characterization of Ag- and Zn-NPs by UV-Vis:

The results of the detection of NPs in Ivy extract were shown by analyzing the visible-UV spectroscopy at wavelengths of 200-1100 nm. The results (Fig. 1) showed the emergence of spectral absorption peaks for both Ag and Zn NPs of Ivy. In Figure 1A, the Zn NPs of the Ivy extract show the highest absorption peak at 365 nm and confirms the presence of Zn NPs in the solution, as the absorption peaks that appear at wavelengths ranging from 300-380 nm are characteristic of the diagnostic properties of Zn NPs due to Surface Plasmon Resonance (SPR). In Figure 1b, the Ag NPs of Ivy shows the highest absorption peak at 419 nm wavelength, and confirms the presence of Ag NPs in the solution, as the absorption peaks at wavelengths ranging from 400-450 nm are characteristic of the diagnostic properties of Ag NPs due to SPR.

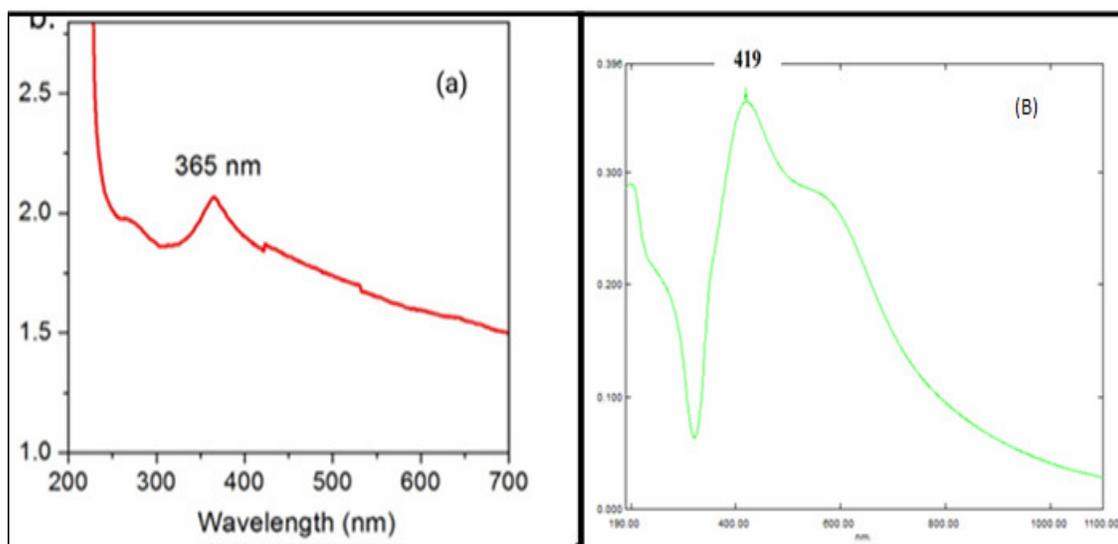


Fig. 1 : Shows the UV-Vis spectrum of nanoparticles of the Ivy extract (A) zinc (B) silver.

UV-Vis is one of the most important techniques used in the diagnosis of NPs. UV spectroscopy was used to examine the size of NPs in the water extract of different plant species (Muthukrishnan *et al.*, 2015 and Logaranjan *et al.*, 2016). In the light of the accumulation of knowledge of the wavelength both of Ag and Zn NPs prepared by UV-Vis spectra at a range of 200-700 nm, the results of this study for UV wavelength spectroscopy of Zn NPs (365nm; figure 1a), and of Ag NPs (419 nm; figure 1B) are differ slightly.

The accuracy of the diagnosis of NPs of the two minerals confirms the results of a large number of similar research (Muthukrishnan *et al.*, 2015; Logaranjan *et al.*, 2016 and Vijay Kumar *et al.*, 2014). However, the appearance of these values slightly different for Zn or Ag NPs of Ivy, is familiar if these sources agree on the extent of the values and not on their compatibility. The wavelength of any Nano scale medium depends on the SPR, which in turn depends on the size, shape and mass of the NPs reflected from ultraviolet

rays in spectra. The difference in the values of the UV spectral analysis of the absorbance peak of NPs may also be partially dependent on different amounts of reducing agents present in the solution, as is the case with 486nm when analyzing the lingonberry and 520 nm when cranberries juice are analyzed, although they contain Ag NPs. It is known that the amount of phenols, anthocyanin, and benzoic acid have been identified in berry juice and were responsible for the transition from Ag⁺ to NPs (Puisoa *et al.*, 2014).

In this study, the formation and stability of both Ag and Zn NPs of Ivy were verified by UV-Vis spectra at a range of 200-700 nm of wavelength. Once the extracts were mixed with AgNO₃, the agonizing reaction of Ag⁺ to Ag was measured by measuring the UV-Vis spectra of the reaction media. The formation of Zn NPs was also verified by UV-Vis spectroscopy results. The results of this study were consistent with the results of Manokari *et al.* (2016) in the preparation of Zn NPs from the extract of the *Micrococca mercurialis*, and obtaining a peak absorption at wavelength 311 nm and 350 nm (Henglein, 1993). The UV-Vis spectroscopy is an important analytical tool in the detection of nanoparticles in solution as a result of the optical properties of nanoparticles resulting from Surface Plasmon Polarization Resonance (SPPR) and that the absorption at specific wavelengths is strongly related to size and dimensions relative to NPs (Mohammed *et al.*, 2009). The UV-Vis spectrum is highly absorbent for the bulk of SPPR, thus determining the bio-reduction process of Ag⁺, as well as determining the shape and size of NPs by determining absorptive locations at wavelengths, that increase the size of NPs leading the SPPR peak to a longer wavelength (Prathna *et al.*, 2011).

Characterization of Ag- and Zn-NPs by FTIR:

Analysis of the FTIR spectroscopy results which used to determine the effective functional groups of Nano molecules of the pure Ivy extract and the stability of Ag and Zn NPs of Ivy, showed the existence of bundles of peaks expressing the existence of a number of these groups. Fig. 2 showed the presence of active functional groups in the pure Ivy leaves extract.

In figure 2A, the spectral analysis of the pure Ivy extract revealed presence of large peaks at (3317.98cm⁻¹) of vibrational bundles of OH groups associated with phenol compounds and monomeric carbohydrates, at (2107.75cm⁻¹) of -C≡C- groups associated with alkane compounds, at (1636.40cm⁻¹) of the carbonyl-C = O group in the amides, at (1344.19 cm⁻¹) of the nitro-N = O groups, and the beam of the convex vibrations at (576.02cm⁻¹) of the C≡C- associated with alkenes.

In Fig. 2, the spectral analysis of the NPs of Ivy showed a change in the absorption packs of C=O- groups of carbonyl and amine compounds from 1636.40 cm⁻¹ in pure Ivy extract to 1632.35 cm⁻¹ in Zn NPs of Ivy (Fig. 2B), and to 1634.46 cm⁻¹ in Ag NPs of Ivy (Fig. 2C). The results of the FTIR spectroscopy also showed the appearance of the spectrum of -C≡C- groups associated with Zn NPs of Ivy in beam 515.34

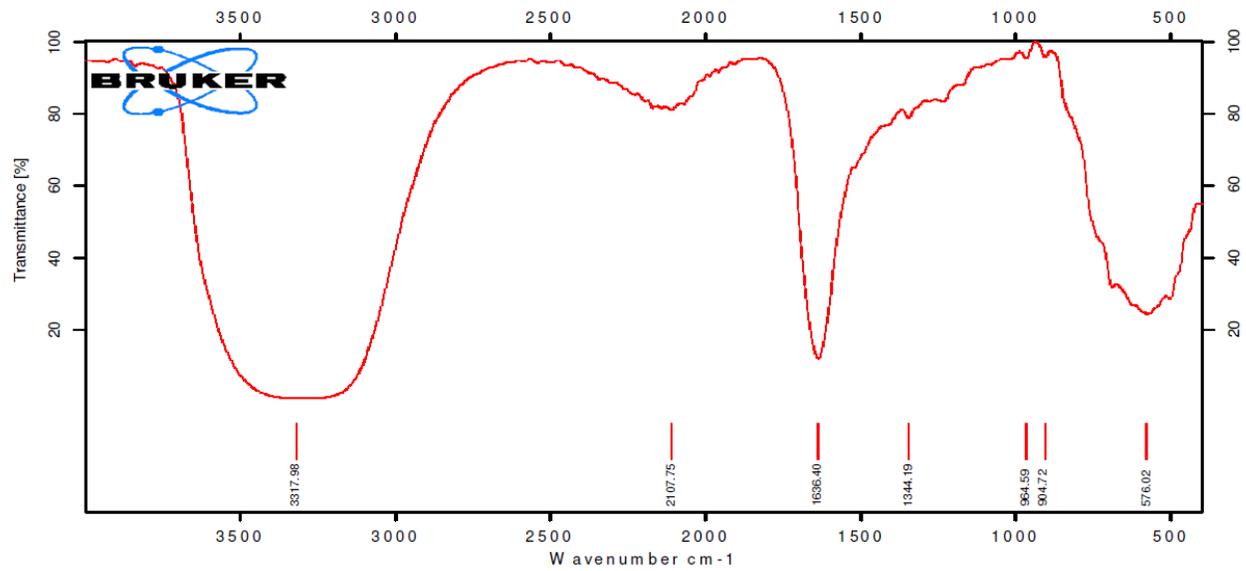
cm⁻¹ (Fig. 2B). Further changes in the spectrum values illustrated elsewhere (Hammodi *et al.*, 2019).

Many researchers have recently used plant or herb extracts such as neem (Zhang *et al.*, 2011), *Aloe vera* (Roldan *et al.*, 2013), *Phyllanthus* (Sotiriou and Pratsinis, 2010), turmeric (Sotiriou *et al.*, 2011 and Tien *et al.*, 2008) as reducing agents for the synthesis of AgNPs, since these sources not only act as reducing agents in the processes but also as stabilizers. The results of our study suggest that the reduction factors in the Ivy extract have a similar stabilizing effect, in terms of the narrow limits of changes in the values of the peaks of the active functional groups of Ivy sample after the synthesis of the nano-zinc and silver- particles. Also, changes may indicate that the extension of the carbonyl that has been assigned is associated with amine compounds of the proteins that are strongly absorbed by NPs (zinc or silver) and form a layer with organisms that ensure interaction with NPs (Gole *et al.*, 2001). A similar study suggested that the infrared spectra of Ag NPs contained a suspension at 1644 cm⁻¹ and 1533 cm⁻¹ together to determine the range of amide I and amide II of the protein corresponding to the -C= O- and NH (Karatoprak *et al.*, 2017). The reading of the results of the current study of FTIR spectroscopy can be assessed by the nano-zinc particles of the Ivy extract in light of the results of the FTIR spectroscopy of the Zn NPs synthesized in sol-gel method, and appeared in the 400-4000 cm⁻¹, and the beam was in range 450-500 cm⁻¹ correlated with ZnO, and the increase in the temperature of the processes was sharpened from the characteristic peaks of the metal oxide, suggesting that the crystalline nature of ZnO increased by increasing the temperature of calcification. Note that the peaks in the 1400-1500 cm⁻¹ range correspond to the C=O. bonds and that the adsorbed range at 1626 cm⁻¹ is set to the O-H bending oscillations. The peak is 1319 cm⁻¹ and 1530 cm⁻¹ with the bending vibrations C=O and O-H, respectively, which gradually decreases to the plasticized sample at a higher temperature.

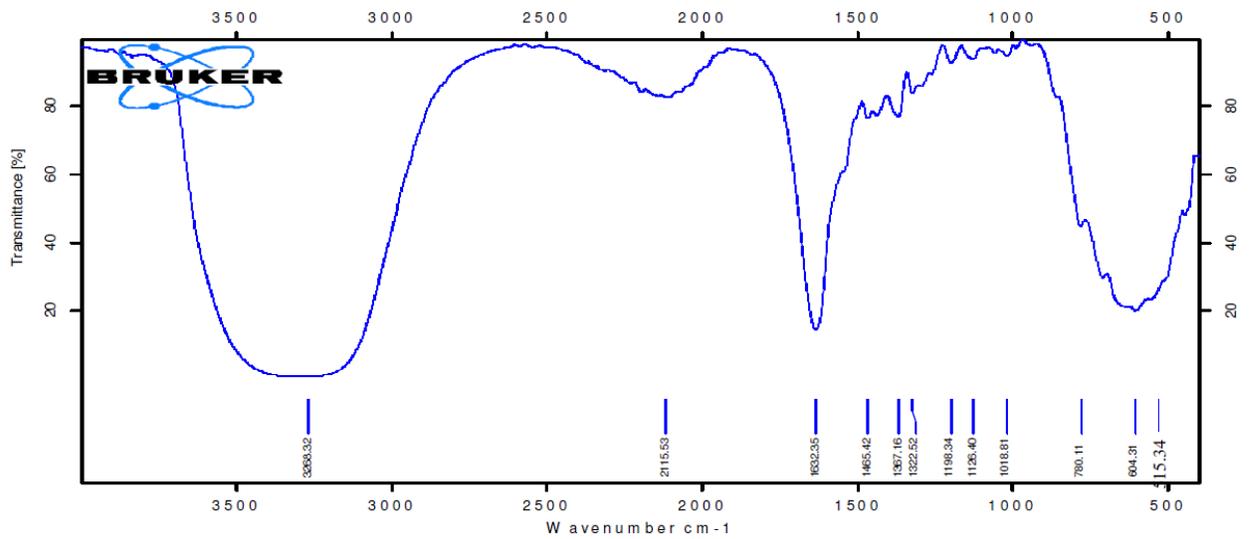
The FTIR spectroscopy of Zn NPs of Ivy shows that the beams 515.34 cm⁻¹, may be related to the vibrational groups associated with the Zn NPs. Carbonyl groups which are associated with amino acids and peptides, have a strong bonding ability to silver, and the proteins located above the surface of NPs can act as stabilizers (Balaji *et al.*, 2009). However, there is no study on the diagnosis of NPs of Ivy extract can be guided in the discussion of the results of the diagnosis of the particles of this plant, and the results of this study remain a pioneer for future studies on this plant.

Characterization of Ag- and Zn-NPs by SEM:

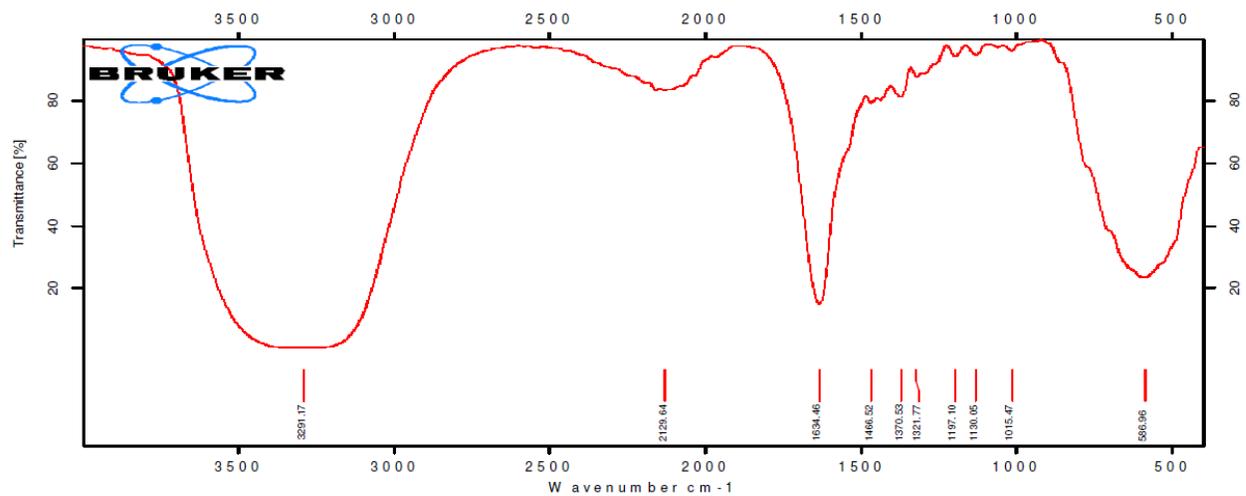
The image of the SEM shown in figure (3) determined the structural form, surface features and composition of both the Ivy pure extract and the Zn and AgNPs of Ivy. It illustrates presence of NPs in the Ivy pure extract of a regular\irregular spiracles shape (Fig.1A), Zn NPs of Ivy of regular\irregular rectangle shape (Fig. 1B) and Ag NPs of Ivy of irregular shape (fig.1C). Further data concerning the nanoparticles size, size range, size ratio, and nanoparticles roughness available in (Hammodi *et al.*, 2019)



Sample Name: Sample description



Sample Name: Sample description



Sample Name: Sample description

Fig. 2 : Shows the FTIR spectroscopy for (A) Pure Ivy extract (B) zinc nanoparticles and (C) silver nanoparticles of Ivy.

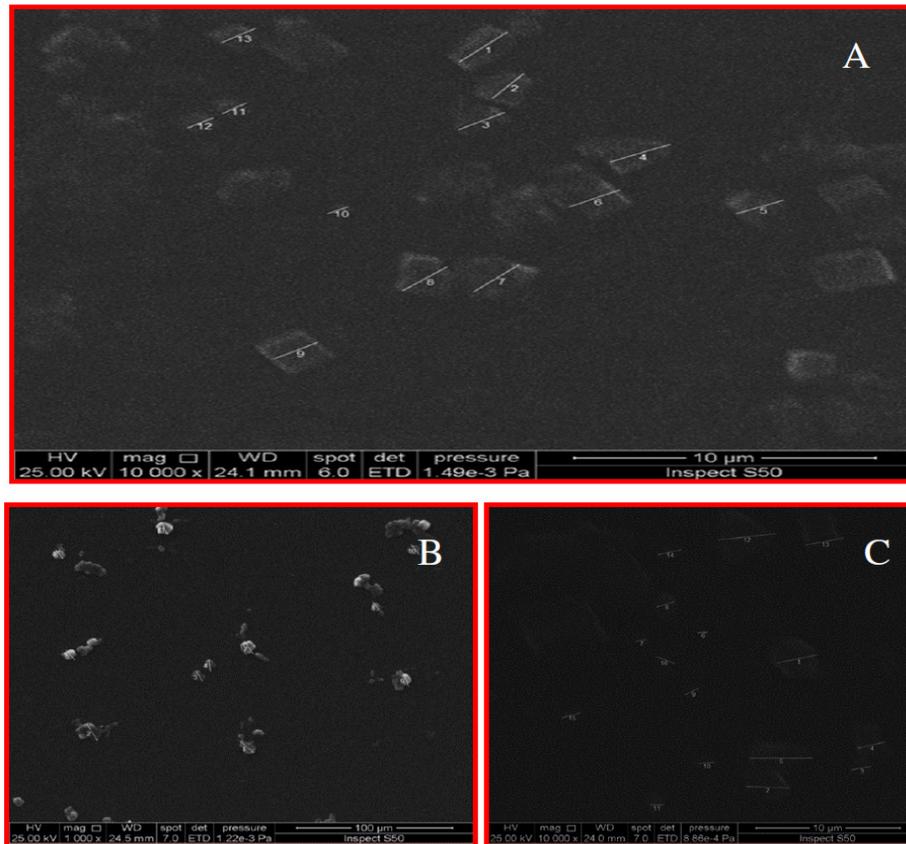


Fig. 3 : Shows the SEM image of the (A) pure Ivy extract (B) biosynthesised Zinc-nanoparticles (C) biosynthesised Silver nanoparticles of the Ivy plant extract.

Characterization of Zn- and AgNPs of Ivy extracts by AFM:

The data shown in figure (4) of the AFM, shows three-dimensional images of different measurements of the surface, diameter, and the size of the NPs. The AFM data showed the presence of a NPs in the pure Ivy extract and the size of these particles were determined at 21.00nm, whereas that of the Zn NPs and Ag of the ivy plant were 41.70nm and 90.07 nm respectively (figure 4 A, B & C).

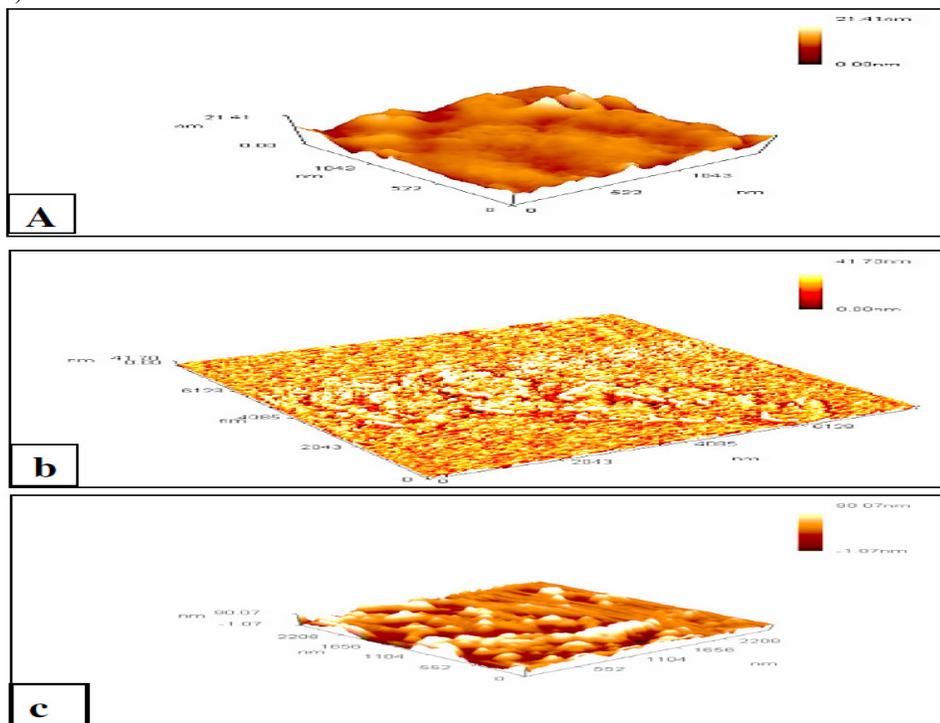


Fig. 4 : Shows three-dimensional images of the surface of (A) nanoparticles of Ivy pure extract, (B) biosynthesised Zinc and (C) silver nanoparticles of the Ivy plant extract.

The data of the AFM shown in Fig. (5), shows surface roughness (RMS) of the particles of Ivy pure extract, and Zn and Ag NPs of the Ivy, respectively were nm 1.61, 6.14 nm, and 15.9 nm.

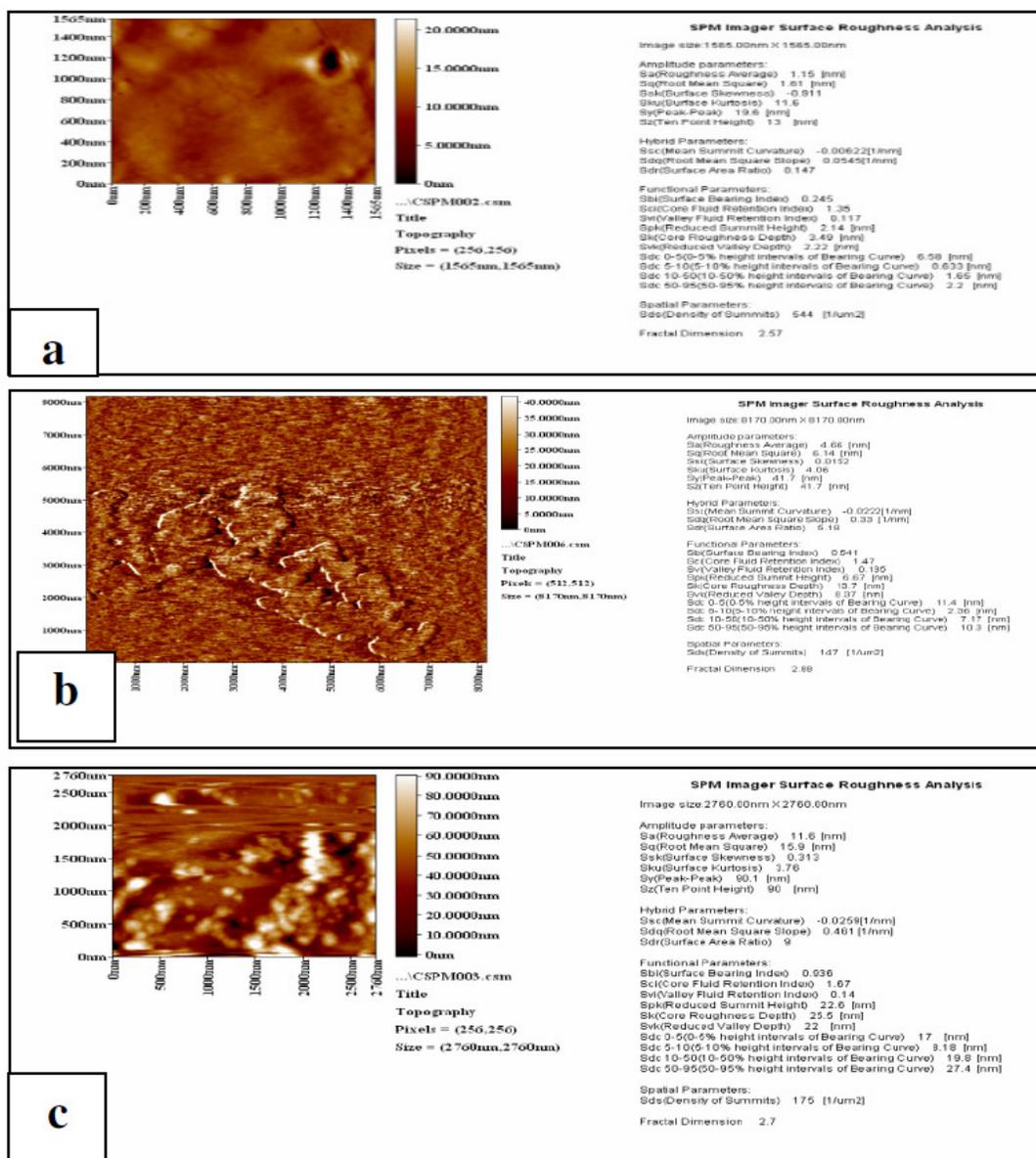


Fig. 5 : Shows surface roughness (RMS) (A) pure Ivy extract particles (B) zinc nanoparticles and (C) Ag NPs of Ivy extract

AFM data showed that Zn NPs, Ag NPs, and NPs of pure ivy respectively, of size 41.70nm, 90.07nm, and 21.00nm, size range 27.5, 45.00nm, and 12.5nm, size ratio 21.23%, 6.88%, and 38.38%, surface roughness 1.61nm, 15.9nm, and 6.14nm, diameter range 85nm, 60nm, and 50.00nm, and distribution ratio 8.04%, 9.69%, and 10.75%

The data accompanying the photogrammetry of AFM were clear and can be inferred from their compatibility with the Zn and Ag NPs of Ivy survey data of using the SEM scanner, and other data of UV-vis spectroscopy and FTIR Spectroscopy in describing the formal properties of the synthesized Zn and Ag NPs. This is evidenced by the success of the process of synthesizing Ag and Zn NPs from the water extract of Ivy plant, and that the results of the present study are consistent with the results of a number of previous studies (Manokari *et al.*, 2016; Vidya *et al.*, 2013 and AL-Othman Monira *et al.*, 2017) who synthesized NPs from different aquatic extracts.

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