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SYNTHESIS OF ZnO NANOPARTICLES FROM OLIVE PLANT EXTRACT

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Abstract

The zinc oxide nanoparticles (ZnO NPs) green synthesis were prepared by using *Olea europaea* leaves extract as bio-reductant agent. The ZnO NPs were detected visually by changing in color from yellow to brown color. Biological and chemical ZnO NPs were characterized by several techniques. The UV-visible spectrophotometric showed absorbance peak at 300nm, Fourier Transformer Infrared analysis (FTIR) showed the present of active groups as carboxylic groups, polyphenolic groups and others are coated on the surface zinc oxide nanoparticles producing stabilized of ZnO nanoparticles, and the mean size of ZnO nanoparticle synthesized by olive leaf extract was approximately 20-50 nm with spherical shaped by using X-Ray Diffraction XRD and Field Emission Scanning Electron Microscope (FESEM), and Energy Dispersive X-Ray Crystallography (EDAX) shows the quantitative and qualitative analysis of elements that formation of zinc oxide nanoparticles which identified as zinc (70.47%), oxygen (20.07%), and carbon (9.46%).

***Key words* :** ZnO nanoparticles, olive leaves extract, characterization of ZnO NPs.

Introduction

Nanoparticles is a small particles that have range of size 1-100 nm (nm=10^{-9} m) (Laurent *et al*., 2008). Because of their distinctive characteristics like optical, catalytic action, electrical and magnetic properties, the nanoparticles of metal oxide and metal characteristics were have a great interested (Singhal *et al.,* 2010). The establishment of nanoparticles/ biological series is resulting from the interaction between nanoparticles and biological materials; and the interfaces of the interaction is dependent on the forces of colloidal and interactions of dynamic bio-physicochemical. These interactions lead to the development of new nanomaterials with control size shape, coatings' surface, hardness and surface chemistry (Nel *et al*., 2009). As a result, the production of nanomaterial and application from (1-100) nanometers (nm) is an emerging research field (Dahl *et al*., 2007; Hutchison, 2008). One of the prodigious material from the technology side is Zinc oxide (ZnO) which is used in a wide applications range like magnetic material, semi-conductors with (Eg = 3.37 eV), material of electroluminescent, gas sensor, sensor and actuator of piezoelectric, varistor nanostructure, material display field, material thermoelectric, products of cosmetics (Prasad, *et al*., 2009). The use of plants to synthesize nanoparticles is novel and offers an alternative to chemical and physical synthesis that is costly-effective and ecofriendly for the environment. Additionally, plant use for synthesis with large-scale can be easily scaled up without using of toxic chemicals or the needing for energy, temperatures and high pressures (Bhainsa *et al*., 2006). In this research, the *Olea europaea* leaves extract utilized as a capping agent for the ZnO nanoparticles synthesis. Olive tree (*Olea europaea* L.) is one of the Mediterranean's most important fruit trees, illustrating the great economic and social importance of this crop and the potential benefits of using any of its by-products (Guinda, 2006).

Materials and Methods

Plant Collection and Identification

Olive fresh leaves from the house gardens in Hilla City, Babylon, Iraq were collected in July 2019. The plant specimen was identified in Plant Herbarium / Biology Department / College of Science / Babylon University. *Olea europaea* L. leaves. Where dried air and then ground into a fine powder with the helping of an appropriate grinder.

Preparing of aqueous leaves extract of olive

The extract of Olive Leaves was produced by adding 0.01 kg of olive leaves powder in 0.1 l sterile purified water and heated for 30 minutes using a magnetic stirrer at 60 °C and 700 rpm, then filtered overnight utilize Whitman filter paper No.1 and stored and collected at 4 °C for additional use (Vaishnav *et al*., 2017).

The nanoparticles of Zinc oxide Synthesis

Prepared 100 ml of 100 mM zinc sulphate heptahydrate solution ($ZnSO_4.7H_2O$) and stored on a magnetic stirrer at around 60 °C and 700 rpm. Dropwise 25 ml of leaves extract has been added and color changes are observed. By adding 1 M NaOH solution, the pH is checked and adjusted to 12. White cloudy appearance observation marked the formation of ZnO nanoparticles. The solution is left for two hours in same condition. Then incubated Overnight at room temperature. Dispersing in sterile purified water and 700rpm centrifugation for 30 minutes three times purified the suspended particles. The particles with white color were subsequently wash by ethanol for removing the impurities from the final products. After drying for six hour in the vacuum oven at 60 °C, in order to obtain a white powder (Vaishnav *et al.,* 2017).

Results and Discussion

Preparation of ZnO nanoparticles

The reaction between olive extract and salt of zinc sulfate dihydrate solution salt has been reduced from Zn (II to 0) and the size maintenance of the olive leaves extract utilized as a stabilizer and reducer agent to synthesize ZnO nanoparticles from nano-formed precursor particles by placing them together to make each one in contact with other.

The formation of a solution of cloudy has been referred for occurring reaction decrement. Where the adding of Sodium hydroxide (NaOH) as an accelerator to increase the decrement rate and the process of nucleation by the directly Zn^{2+} deposition to be $Zn(OH)_2$ when placed in high (pH=12) alkaline environment which is following by water loss to produce ZnONPs (Nishimura *et al*., 2011; Balavandy *et al*., 2015). The producing of the ZnO with nano size particles from olive leaves extract was initially observed by the changing in color, which occurred during the reaction period from yellow-brown after completing the reaction mixing, where no change in color was noted in the olive leaves extract without $ZnSO_4.7H_2O$ solution.

ZnO Nanoparticles were synthesis after 4 hours under heating at 60°C and pH 12, the nano particles were precipitate in white colour figure (1). The color change is caused by the excitement of the metal nanoparticles ' plasmon's surface resonance, indicated the formation of nanoparticles of ZnO (Sutradhar and Saha, 2015). The reaction mechanism demonstrates that ZnO nanoparticles can be received by reducing Zn^{+2} using olive leaf rich in biophenols, such as oleuropein (*Ole)*, which is the main active ingredient that acts as both the reduction and the capping agent. This also provided strong evidence to support the involvement of olive leaf polyphenol content in rapid biosynthesis and the metal nanoparticles stability in the aqueous media (Chandran *et al*., 2006).

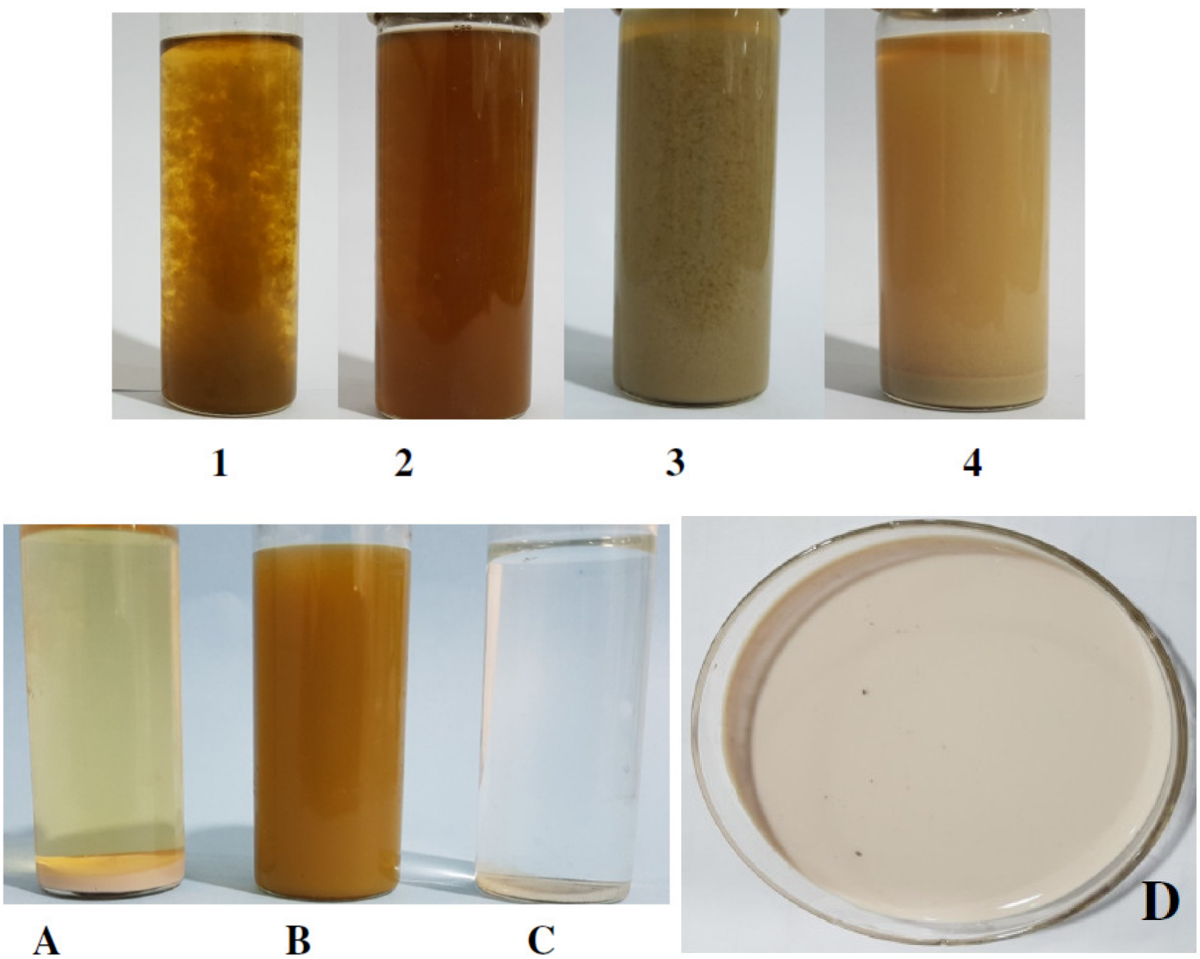


Fig. 1 : ZnONPs synthesis stages (1,2,3,4) mixed with olive leaves extract. (A) ZnONPs. (B) olive extract. (C) $ZnSO_4.7H_2O$ solution. (D) ZnONPs powder.

UV-visible Spectrum

ZnO NPs synthesis characterized by spectroscopy UV visible. Using double distilled water as blank, the maximum synthesized ZnO NPs absorption was noticed at 300 nm in UV spectrum zone. ZnO's UV-visible spectrum demonstrates the absorption peak at 300 nm figure (2) that corresponded to the probable ZnO nanoparticles value (Susan *et al*., 2013). Reduction of zinc sulfate to oxide of zinc is observed by spectrum of UV-vis, the band was detected about (300–450) nm that was recognized as a " plasmon's surface resonance band" and this band is attributed to excitement of ZnO arrangement of the valence electrons in nanoparticles (nanocrystal /nanosphere) (Sutradhar and Saha, 2015). The result also correlates with the already reported results (Jayarambabu *et al*,. 2014).

Similarly the λmax of ZnO NPs in a spectrum of UV-Vis have been assessed at 283 nm utilize double purified water as blank. (Dobrucka and Dugaszewska, 2016)

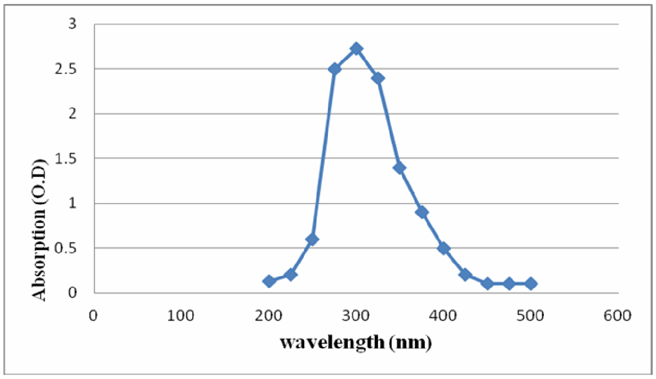


Fig. 2 : Absorption spectrum of synthesized ZnO NPs by olive leaf extract

Fourier Transform Infrared (FT-IR)

Figures (3-A,B) show the functional groups in olive leaf extract and identify their role in zinc oxide nanoparticles synthesis, *Olea European* leaves extract FT-IR spectrum and synthesized ZnONPs. The FTIR analysis results indicates to existence of bio-components on the nanoparticles' surface, mainly alkaloids, tannins, glycosides, ursolic acid flavonoids and others. FTIR measurements were utilized to determine the interaction between zinc salts and protein molecules capable of reducing zinc ions and stabilizing ZnONPs zinc oxide nanoparticles. Analysis of FTIR revealing some significant bands of vibration like hydroxyl group, C-O, Zn-O and others. This corresponds to the ZnONPs and bio-compounds on the surface (Aqil, *et al.*, 2006; Joshi, *et al.*, 2010; Devendran, *et al.*, 2011) .

Figure (3) demonstrates the FTIR spectrum of zinc nanoparticles with absorption peaks situated between 4000 cm^{-1} and 400 cm^{-1}. Bands detected at 3423,65 cm^{-1} assigned to the stretching of O-H phenol group vibrations. Bands detected at 2926,01 cm^{-1} could be assigned to the stretching C-H alkaline vibrations. The bands detected at 2357.01 cm^{-1} could be allocated to-C≡C- stretching. The bands identified at 1597.06 cm^{-1} could be allocated to C=C /amine – NH stretching. The bands (1492.90, 1408.04) cm^{-1} can be allocating to C–C stretching of aromatic ring. The band 1265.30 cm^{-1} can be allocated to C–O stretching vibrations of guaiacyl ring. The bands discovered at 1083.99 cm^{-1} could be allocating to stretching amine. This result rivals the already reported results of ZnO nanoparticles biosynthesis using leaves extract from *Acalypha indica* (Gnanasangeetha and Thambwani, 2013).

Si–o–si stretching protein vibrations could be allocated to the bands identified at 1016.49 cm^{-1}. The bands can be allocating to the secondary amine waging at 867.97 cm^{-1}. C–N stretching amine could be allocated to the bands at (759.95, 684.73, 609.51, 493.78, 464.84) cm^{-1}. The shift of bands to significantly lesser frequency give indications for the depositions of these compounds in ZnO NPs synthesis. for instance 1614cm^{-1} reduced to 1597 cm^{-1}, 1539 cm^{-1} to 1492 cm-1^{-1}, 1442 cm^{-1} to 1408 cm^{-1} and 781 cm^{-1} to 867 cm^{-1}. In addition, a substantial band acted at 536.21 cm^{-1}, owned by ZnO vibrational stretching, which more detection on ZnONPs formation. This finding associates with the existing reported results of ZnO nanoparticles biosynthesis using Olive leaves extract (Awwad *et al.*, 2014).

The existence of phenolic compounds and proteins was assured by the functional vibrations bands group as demonstrated in the FTIR spectrum. (Macromolecules) that surrounding the nanoparticles with the distinctive Zn-O band (Liang, 2016).

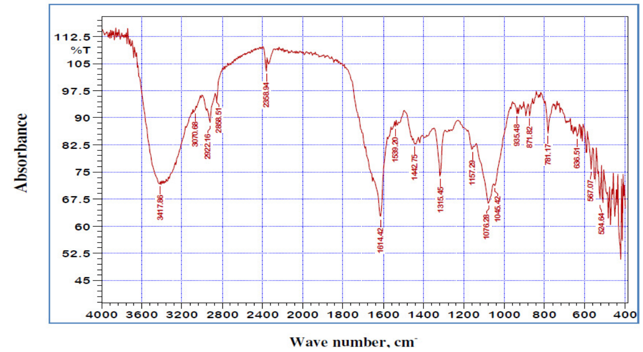


Fig. (3-A) : FT-IR spectrum of the olive leaves extract

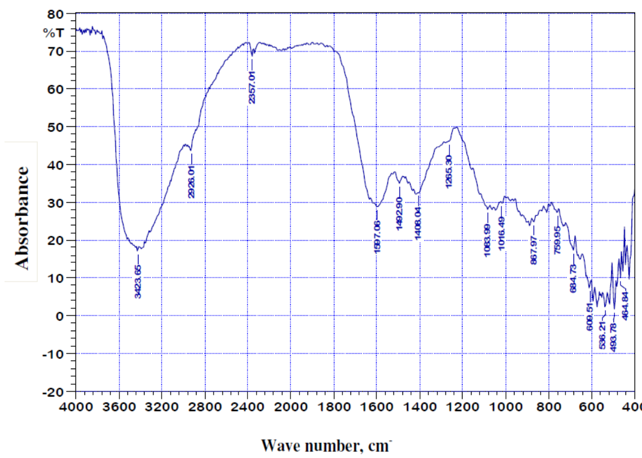


Fig. 3-B : FT-IR spectrum of the biosynthesized anoparticles of ZnO by olive leaves extract.

X-ray Diffraction

The crystallography of ZnO NPs examined by X-ray diffraction, X-RD Spectra offers an insight into the crystallinity of nanoparticles figure (4) representing XRD spectra of ZnONPs synthesized with extract of olive leaves. Appearing at around 2θ of the sharp and narrow diffraction peaks are at 31.8 °, 34.44°, 36.29°, 47.57° and 56.61°. Similar findings have also been reported (Talam *et al*., 2012). These diffraction peaks have also been detected to expand attributable to small particle sizes in nano-scale with a moderate crystallinity of 36.29 percent. The dry powders of the zinc Nanoparticles were used for X-RD analysis. The diffracted intensity was documented at 2theta angles from 20° to 80°. Size of nanoparticle was calculated using Debye–Scherrer equation that suggesting the spherical in shape crystal structure of the nanoparticle.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

D- particle size in nm, K is the constant is Sherrer (0.9), λ- X-ray wavelength, β- FWHM, θ- Reflective angle of Bragg.

The mean crystalline size of the synthesized ZnO NPs is 19.22 nm based on the most intense diffraction peak FWHM at 36.28°. Earlier studies also acquired similar X-ray diffraction patterns for green synthesis of zinc oxide nanoparticles (Vaishnav *et al*., 2017).

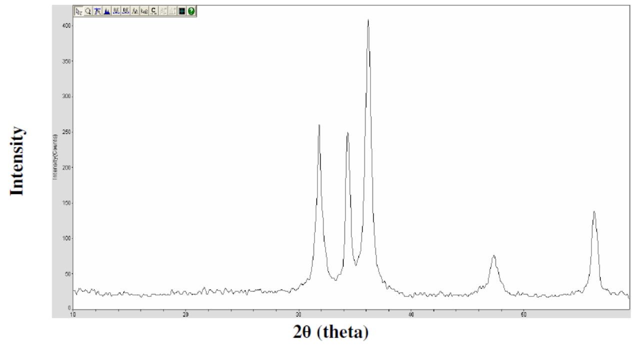
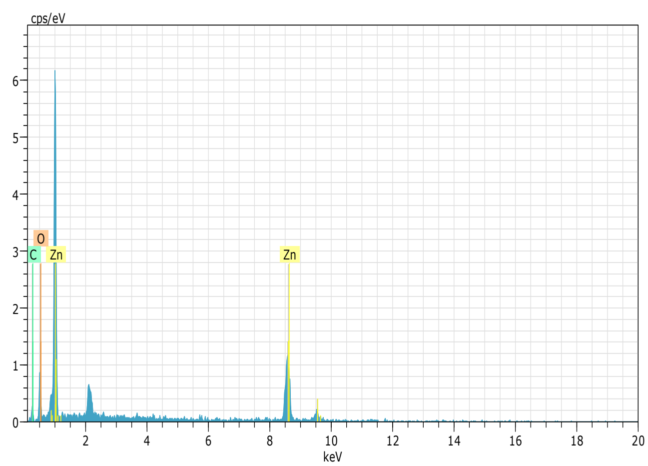


Fig. 4 : X-ray diffraction (XRD) results for zinc oxide nanoparticle extracted by olive leaves extract

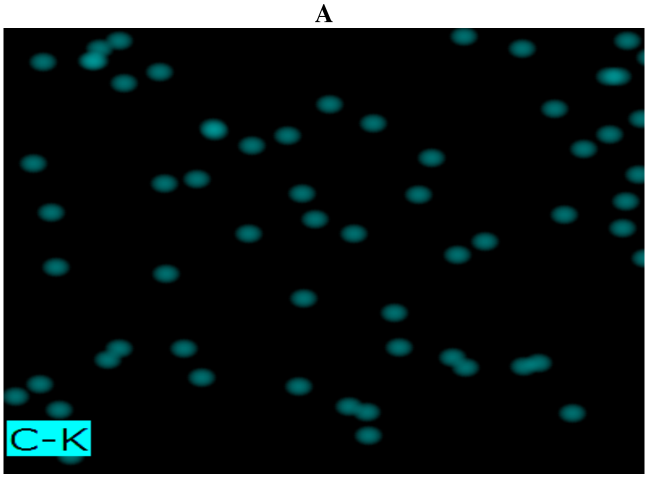
Energy Dispersive X-ray Crystallography

The structural characterization of ZnONPs was implemented utilizing an analysis of dispersive energy X-ray crystallography (EDAX). Figur (5) shows the element quantitative and qualitative analysis may involve the formation of ZnONPs. Metallic zinc oxide is confirmed in biosynthesized ZnONPs. The obtainable composition from the analysis of EDAX were three peaks which identified as Zinc (70.47%), Oxygen (20.07%), and Carbon (9.46%). The Zn shows the absorption peaks of higher counts. The trace amounts existence of carbon demonstrates that plant phytochemical groups are involved in reducing and capping of synthesized ZnO NPs. (Bala *et al.,*2015).

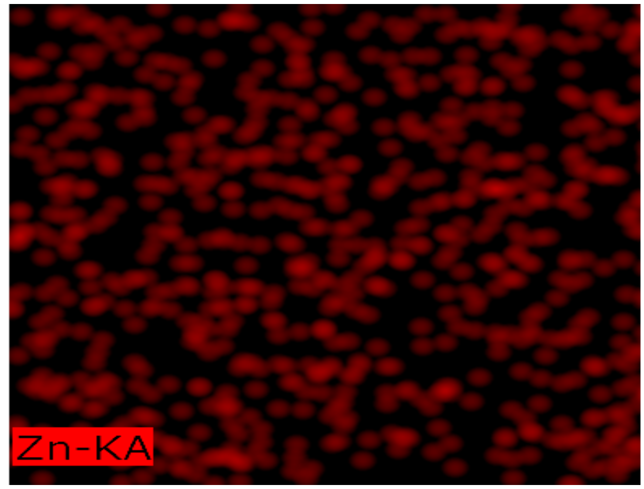
These results correspond with the results that reported in which the same peaks were demonstrated with *Acalypha indica* leaves extract in ZnO NP synthesis (Gnanasangeetha and Thambwani, 2013).



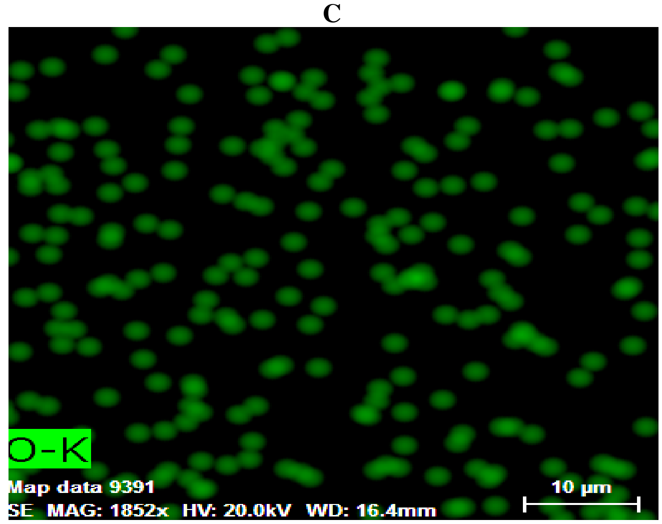
A



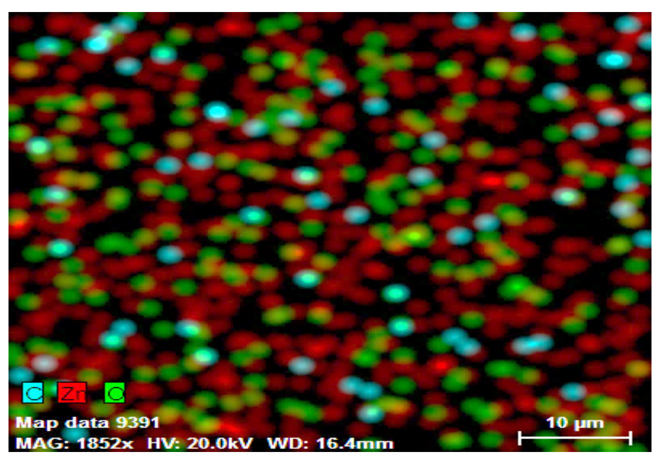
B



C



D



E

Fig. 5 : EDAX Spectrum showed the purity of ZnONPs. (A)Curve of EDAX. (B)Carbon. (C)Zinc. (D)Oxygen. (E) Distribution of C, Zn, O in ZnONPs sample.

Field Emission Scanning Electron Microscope

The second method used to identify the shape, size and distribution of the synthesized nanoparticles of zinc oxide was the field emission scanning electron microscope (FESEM). Figure (6) display individual ZnO nanoparticles and a number of aggregates, the particle shape was spherical.

In the present study, the diameter of the ZnONPs in the solution was found to be within 20-50 nm which is revealed by FESEM. This result is constant with previously reported results showing the formation of spherically shaped aggregated molecules and nanoparticles in the leaves extract of *Calotropis* (Vidya *et al*., 2013). The analysis of the FESEM was utilized to assess the structure of the products reaction that produced. Because of aggregation, large nanoparticles were observed. This aggregation occurs on the nanoparticles' surface by the presence of a cell component and represents as a capping agent. (Helen and Rani, 2015). FESEM has been used to reveal the shape of olive leaves extract capped ZnONPs Figure (6). The images of FESEM were seen in varying magnification ranges such as 2 μm–200 nm that illustrated the existence of nanoparticles spherical shaped with a diameter average (Raut *et al*., 2013).

The nanoparticles have not been directly in contact even with the aggregates, indicating that the nanoparticles were stabilized by a caping agent (Priya *et al*., 2011) Analysis of X-ray diffraction providing a perfect induction of the composition of high-quality crystalline ZnONPs utilizing olive leaves extract mediated biosynthesis procedure which was also the simplest method and ZnONPs seem within a few hours. The results presented in this study from UV-vis., XRD, FTIR, EDAX and FESEM clearly determine the formation of ZnONPs.

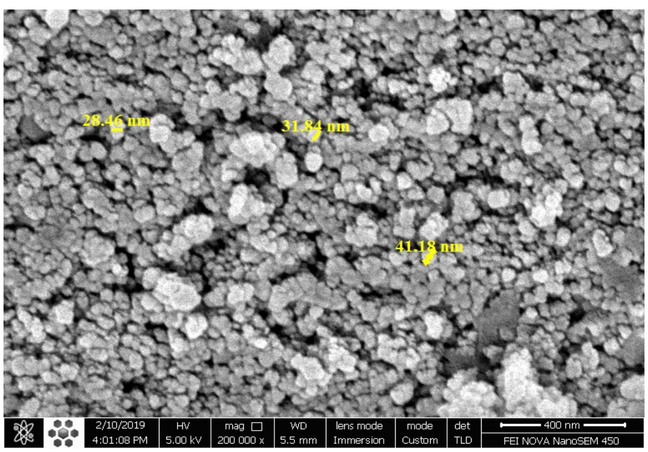


Fig. 6 : FESEM of ZnO NPs in different magnification ranges 400 nm shows size and shape of ZnONPs.

Conclusion

Biological method using Olive plant leaves extract showed ability to synthesis ZnO nanoparticles and acts as a reducing agent to reduce to nanosize particles. The synthesized ZnONPs were subjected to analysis such as FESEM, UV-Vis. Spectroscopy, XRD, and FTIR in order to characterize them. This opens a way to understand the synthesis mechanism of ZnO nanoparticles formed from other plant seeds extracts.

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