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Curcumin extract nanoparticles: preparation, characterization and antimicrobial effect

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Key words: Zinc oxide nanoparticles, Nano characterization, Toxic effect, Bacterial test, Curcumin extract

Abstract

In recent years, synthesized zinc oxide nanoparticles have been increasingly investigated for different medicinal uses. In the present study, we aimed at the biosynthesis of zinc oxide using a curcumin extract. Although, toxic effects of curcumin derivative and zinc oxide nanoparticles in different concentration have been studied specifically on animal models besides the antibacterial activity of synthesized curcumin extract and zinc oxide nanoparticles. The aim of the study was to synthesize extract combined zinc oxide nanoparticles. Methods: The synthesized nanoparticles and extract were characterized for the particle size distribution, morphology, optical properties and surface charge by using UVvisible spectroscopy, dynamic light scattering (DLS), (TEM) and (SEM). Elemental composition and structural properties were studied by energy dispersive X-ray spectroscopy (EDX) and X-ray diffraction spectroscopy (XRD). Results: The synthesized nanoparticles and curcumin were irregular shape and had a size distribution in the range of 50–100 nm. The in vitro toxicity effects of zinc oxide and extract showed no toxic effect with different concentration with antibacterial effect.

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Introduction

Nanoparticles now a day can be synthesized by physical, chemical and biological methods with desired specifications such as size and shape (Jayarambabu and Kumari, 2015). The chemical methods are not much explored due to expensive and toxic chemicals used for synthesis so the new study revealed to used metallic nanoparticles like silver, gold, titanium dioxide, copper oxide, iron oxide, aluminium oxide, zinc oxide (Senthilkumar and Sivakumar, 2014). Among these, zinc oxide nanoparticles (ZnONPs) have attracted attention of scientists worldwide due to its medicinal values when it combined with different herbal extract. Curcumin: 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5 -dione, is a lipophilic molecule that rapidly permeates cell membrane (Jaruga *et al.*, 1998). Curcumin has a long history of administration in China, India and Iran for the treatment of many diseases such as diabetes, liver diseases, rheumatoid diseases, atherosclerosis, infectious diseases and digestive disorders (Ammon and Wahl, 1991; Pandeya, 2005). Curcumin received high attention due to antioxidant, anti-inflammatory, antitumoral and antiangiogenesis effects. It does not appear to be toxic to animals (Shankar, 1980) or humans (Soni and Kuttan, 1992) even at high doses. Recent studies have discussed poor bioavailability of curcumin because of poor absorption, rapid metabolism, and rapid systemic elimination (Anand, 2007; Sharma, 2007). According to (Yang *et al.,* 2012) reported 1% bioavailability for 1g/kg of oral administration of curcumin in rats; the elimination more than 75% excreted in feces and negligible amount was detected in urine (Wahlstrom and Blennow, 1978). So according to FDA has declared curcumin as “generally safe.” Nanotechnology is considered as technology of the future and the use of nanoparticles combined with extract for enhancing the bioavailability and the solubility of lipophilic compounds such as curcumin when used as folk medicine or combined with chemical, improve the therapeutic effects through protecting from degradation and prolonged blood circulation, decreasing their toxicity (Freitas, 2005). Over a period of time, numerous emphases have been given to develop the biodistribution of natural curcumin, but it is only just recently that the application of the field of nanotechnology has considerably enhanced its therapeutic effects. Nanoparticles when combined with herbal extracts are emerging as one of the useful alternatives that enhance therapeutic effect of different concentrations of curcumin extract that are used in future and improved major problems as solubility or stability and increase rate of metabolism in wound healing and others. in present study was aimed to synthesize, characterize and check antimicrobial activity and toxicity of zinc oxide nanoparticles combined to curcumin extract for improved major those problems.

Material and method

The leaves of the plant were subjected to surface sterilization using 30% alcohol, and then dried in shade. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. The powdered sample (50 g) was boiled in hot water for 30 min after which it was filtered using a filter paper no.1. The filtrate was evaporated to dry at 40°C producing yellow color solid residue (yield: 5% w/w). The residue was weighed and stored in air and water proof containers, kept in refrigerator at 4°C. From this stock, fresh preparation was made whenever required (Yasmeen and Prabhu, 2012). The curcuma extract used at concentration 5% equals to 50000 ppm calculated by the equation: C(%)=C(ppm) /10000; 5% = C(ppm)/ 10000= 50000 ppm (Sun *et al.*, 2010).

Preparation of Zinc oxide nanoparticles

1.5g of ZnO was dissolved in 80ml of Sterile water then put the mix in Ultrasonic cleaner for 10 minutes then add 100μl of Hydrofluoric acid (H.F) + Ethanol 1: 1 ratio by Micropipette then put the mix in Ultrasonic cleaner for 30 minutes than add 0.05g of PVC then put the mix in ultrasonic cleaner again for 45 minutes then examined to make sure it became nanoparticles (Sangeethaa, 2011).

X-Ray Diffraction Analysis (XRD)

To determined structure and crystalinity of synthesized ZnO as nanoparticles by using X- ray diffractometer (XRD-6000, Shimadzu).

The source of X-ray radiation had been Cu-Kα radiation with 0.15406nm wavelength and device had been operated at 40KV and 30mA emission current which scanned the sample between (20 - 60 degree) analyzed in Ibn-Al-Haitham College.

Scanning Electron microscopy (SEM)(TEM)

The sample scanned by Scanning electron microscopy (SEM) is basically a type of electron microscope with (EDAX), the magnification power 250000 AIS2300C (Oxford instruments).

Atomic force microscopy (AFM)

To observe the surface roughness and topography of deposit thin films, checked by using Atomic Absorption Spectroscopy (AA3000, Angostrom Advanced Inc. USA AFM contact mode) in science College of Baghdad University (Kumar and Chen, 2008).

Preparation the mix of herbal extract with nanoparticle Zinc oxide

One concentrations of curcuma extract (5) ppm was mixed with Zno nanoparticle (21000 ppm), the concentration of ZnO nanoparticle was mixed with each concentration of extract in ratio 1:1.

Result and discussion

x-ray diffraction analysis of synthesized ZnO thin film

XRD diffraction patterns of synthesized ZnO nanostructure thin films prepared by simple chemical methods and deposited on glass are shown in Fig. 1. ZnO contain seven main peaks at diffraction angles (31.7°, 34.36°, 36.2°, 47.2°, 56.54°, 62.78° and 67.88°) corresponding to (100, 002, 101, 102, 110, 103, 112) planes. This result agree well with that presented in references. All the diffraction peaks are indexed to hexagonal structure and there is no trace of cubic face which were match well the standard peaks (JCPDS NO.00-005-0664). Crystallite size (D) in nm for a knowing X-ray wavelength at a diffraction angle of ZnO nanostructure is calculated by using scherrer formula (Sathyavathi *et al.*, 2010).

Scanning electron microscope (SEM)

Fig. (2) and (3) shows SEM images of ZnO nanoparticles and ZnO combined with extract prepared with simple chemical method.

The images confirm different morphological size of NPS. The morphology of ZnONPs not uniform with average size (35-64)nm; while the NPS combined with extract the average size ranges between (48-84)nm this result agree with (Agarwal *et al.*, 2017).

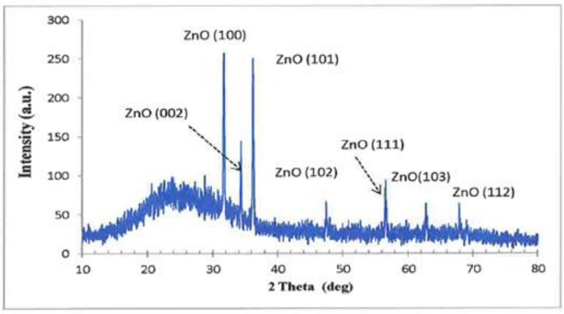

Fig. 1. XRD pattern of ZnO nanoparticles, which obtained by the chemical method is precipitated by drop casting method on a glass.

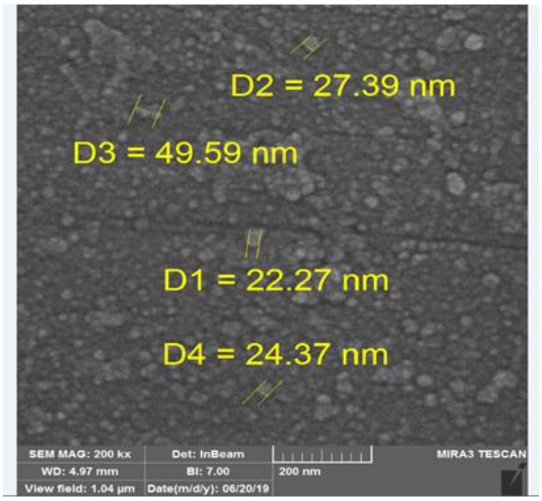

Fig. 2. SEM pattern of ZnO Nanoparticles and curcuma extract.

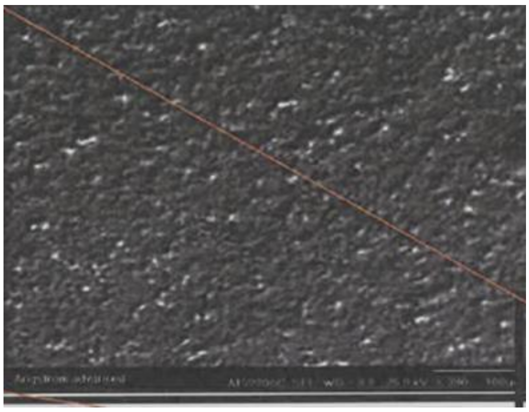

Fig. 3. SEM images of ZnO Nanoparticles thin films deposited on glass by drop casting method.

EDS pattern of zinc oxide nanoparticles was shown in Fig. 4 and percentage analysis were showed in Tables 1. The fig. and table, were observe the peaks of carbone and aluminum along with some other constituents as Sulphur, potassium, silicon and calcium which acted as a capping agent to nanoparticles.

Table 1. EDS analysis of ZnO Nanoparticles and curcuma extract synthesized.

Element	Line	Int	Error	K	W%	A%
C	Ka	1287.7	170.7383	0.2640	47.31	62.05
O	Ka	540.0	170.7383	0.1126	21.59	21.26
Na	Ka	97.9	125.0808	0.0211	1.46	1.00
Mg	Ka	111.2	125.0808	0.0243	1.34	0.87
Al	Ka	1318.0	125.0808	0.2917	14.76	8.62
Si	Ka	202.4	125.0808	0.0455	2.36	1.32
P	Ka	129.7	125.0808	0.0296	1.48	0.75
S	Ka	83.7	125.0808	0.0195	0.90	0.44
Cl	Ka	326.6	125.0808	0.0773	3.61	1.61
K	Ka	390.8	39.8506	0.0962	4.36	1.76
Ca	Ka	72.9	39.8506	0.0183	0.81	0.32

Atomic force microscope AFM

The 3D AFM images and granularity accumulation distribution chart of ZnO NPs are around 49nm and ZnO NPs herbal extract synthesized around 56nm and particles had one or more dimention. The ZnO NPs extract has irregular ball shape with good dispensability distributed within the scanning area (500x500)nm with individual columnar grains extending upwards and the average grain size is 80.48nm and the average roughness with root mean square about (11.7, 14) nm respectively as in table (2) by using special software (Imager size 2212.88nm) (Massimiliano, 2004).

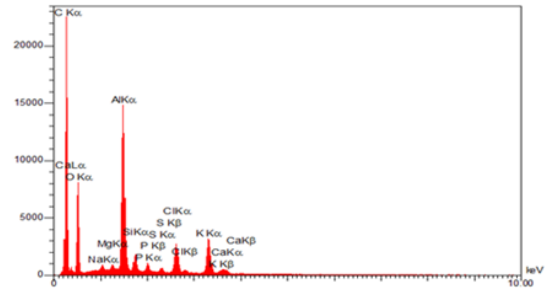

Fig. 4. EDX pattern of ZnO Nanoparticles and curcuma extract.

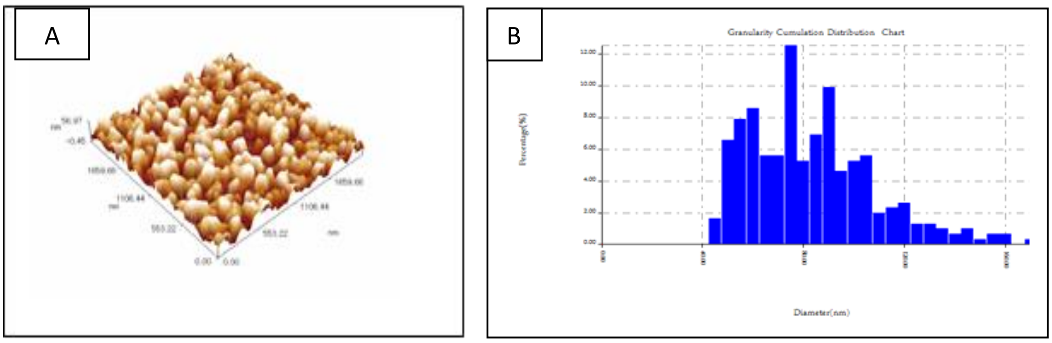

Fig. 5. A 3D AFM image B granularity accumulation distribution chart of ZnO Nanoparticles and curcuma extract.

Table 2. the grain size roughness average and root mean square ZnO Nanoparticles and curcuma extract.

Roughness average	11.7nm
Root mean square	14nm
Average diameter	80.48nm

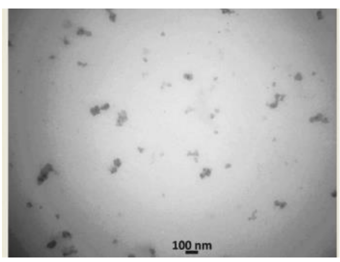

Fig. 6. TEM pattern of ZnO Nanoparticle and curcuma extract.

Transmission electron microscopy (TEM)

TEM analysis can be used to understand the characteristics and size of the synthesized NPs. The analysis was carried out using a Tecnai G2 20 with an accelerating voltage of 200 kV and the images with different magnifications are shown in Fig. 6. The fig. observed that the average particle size is found to be 50–200nm and confirmed that the particles are almost irregular crystal a slight variation in thickness, which supports the SEM results. The particle size determined from TEM analysis is almost close to the size obtained from XRD and SEM analysis.

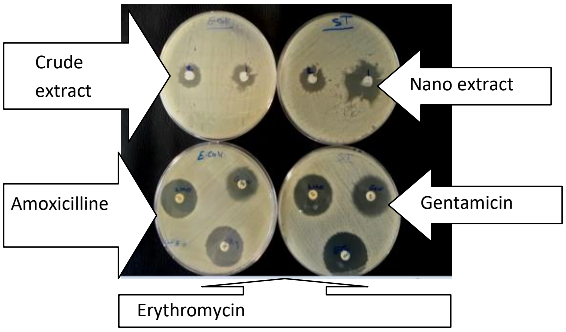

Fig. 7. Antibacterial activity of ZnO Nanoparticle and curcuma extract (1) compared to crude extract (2) by using *E. coli and ST. aurius*.

Acute Oral Toxicity of ZnO NPs and curcuma extract

In the study toxic effect of ZnO NPs and curcuma extract we used the limit range to evaluate the acute toxicity in mice. The concentration of NPS extract 5% was considered as stock solution were take 1ml and dissolved in 9ml of saline and administrated via gavage to different concentration (0.05, 0.1, 0.01, 0.001, 0.0001g/ml) after 24hr. and During 14 days of administration orally, no clinical signs of toxicity or mortality were observed. The median lethal dose (LD50) < 0.0001g/ml/mice in the present study which suggesting its safety (Song *et al.*, 2018)

Antibacterial effect

From the studies, it was observed that the NPS curcuma extract was found to have good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* [19] according to crude extract, amoxicillin, gentamicin and erythromycin (Droepenu *et al.,* 2019).

Conclusions

It is known that the green synthesis of ZnO NPs curcuma extract is much safer and environmentally friendly as compared to chemical synthesis. In response to this assumption, this study successfully used synthesis ZnO NPs combined with curcuma aqueous extract. FTIR and AAS confirmed the presence of ZnO and organic constituents in the fabricated samples. SEM and TEM reported the shape and size of the samples as ranging between 50-100nm, having agglomerated petal-like morphologies with organic materials from the plant extract surrounding the particles and serving as capping agents and this size synthesized is suitable for applications in the biomedical field beside the synthesized ZnO NPs and extract exhibited antibacterial activity against *Staphylococcus aureus* (Gram +ve) and *Escherichia coli* (Gram –ve). in other way; the nano extract didn't appear any toxic effect when given to albino mice in different doses. This shows that the synthesized ZnO NPs and extract can be used as medical alternative in future.

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