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Research Article

Biosynthesis and Antibacterial Activity of ZnO Nanoparticles by Artemisia Aucheri Extract

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Background: Green approach to nanoparticles, including metal oxides Because of an inevitable disadvantage of physical or chemical synthesis routes is attractive nowadays. ZnO nanoparticles play a key role in the medicals and drugs area.
Objectives: In this study, biosynthesis of ZnO nanoparticles with new approach to enhanced the Antimicrobial properties against gram-negative and gram-positive was performed by use of a new type of plant extract, *Artemisia aucheri*, in an environmentally friendly, cost-effective, simple procedure way.
Materials and Methods: By adding $Zn(NO_3)_2$ to *A. aucheri* methanol extract followed by stirring The resulted solution and final heat treatment in 200 °C the ZnO nanoparticles were synthesized. Disc diffusion method was applied to evaluation the Antimicrobial properties of the extract and nanoparticles towards resistance into *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive).
Results: X-ray diffraction pattern (XRD) result showed all of the peaks proportion to ZnO and no other peaks were detected, also demonstrated nanostructure nature with crystallite size about 9 nm. In the Fourier transform infrared spectroscopy (FTIR), there is a band in the 550 cm^{-1} which is corresponded to ZnO. Also 76 nm average particle size obtained by DLS experiments. Energy-dispersive X-ray spectroscopy (EDS) analysis showed strong peaks for Zn and O, support supposition of ZnO nanoparticles. Field emission scanning electron microscopy (FESEM) images indicated spherical rounded particles with the size of average 30 nm. Antibacterial tests showed effective diameter about 11 and 10 mm for plant extract and also 7 and 5 mm for ZnO nanoparticles against *E. coli* (gram-negative) and *S. aureus* (gram-positive) in agar disc diffusion method, respectively.
Conclusions: Biosynthesized ZnO nanoparticles could be a good candidate for antibacterial activity, both against *E. coli* (gram-negative) and *S. aureus* (gram-positive) especially for versus *E. coli.*
Keywords: Antimicrobial Agents, *Artemisia*, Biosynthesis, Plant extracts, ZnO, Nanoparticles.

1. Background

Nanotechnology recently is in the focus of interest in the area of multifarious research such as medicine, engineering, environments and agriculture. Basically, this high degree of sublimity for nanomaterials could be attributed to remarkably their extraordinary surface area(1, 2). There are three main methods for producing nanoparticles which are including physical, chemical and biosynthesis routs to reach the favorite product such as oxide or metal nanoparticles. Due to the limitation like as require many equipment's or excess energy for physical routs or necessity and undesirable by-product in chemical procedures, lately, biosynthesis methods are developed (3, 4). Green chemistry is the one of subdivision in the topic of sustainable chemistry. The major purpose of this subject is approach to synthesis methods which are produce no or low amount of hazardous chemicals or contain dangerous processes, but the challenge is in the evaluation cost or performance is emerged by use of this new technique(5). Among many materials, ZnO is a very favorite candidate for catalyst and adsorption, magnetic properties, fabrication of sun-screens due to its UV filtering properties, ceramics, wastewater purification, electronic and biomedical systems such as drug delivery, anticancer, anti-diabetic, antibacterial and antifungal applications (4, 6, 7). There are many preferable methods for production of ZnO nanoparticles categorized in 4 main groups, contain plant or plant extract use, microorganism, biotechnology and biochemistry have been reported (7). By taking advantages of ease of the procedure, eco-friendliness, and mostly low cost and of the precursor, biological routs using plants or plant products has been

widely used for synthesis ZnO nanoparticles (1, 8). Many Plants or plant extracts were used to have synthesized ZnO, such as neem leaves (9-11), aloe vera extract (12, 13), red rubin basil (14), kapurli (15), waterhyacinth (16), dog rose (17), crown flower (18), rambutan (19), nochi (20, 21), coconut (22), gossypium (malvaceae) (23), and Mexican mint (24).

The genus *Artemisia* is one of the largest of a diverse genus of largly perennial herbs in the Asteraceae family. It contains more above than 800 species that are spread widely throughout the world. In Iran, thirty-four species of this genus have been reported (25, 26). *Artemisia aucheri* Boiss, is an aromatic plant, 20 to 25 cm in height and abundant in northern Iran and in the mountainous areas (27). This species is a native-growing plant which is widely used in Iranian traditional medicine (28), Indeed the methanolic or ethanolic extract of this plant showed higher antibacterial effects compared to aqueous extract (29-31).

Recently, many literatures focused on the impacts of ZnO nanoparticles in antibacterial activity. This is due to excellent inhibition growth properties against bacteria such as *E. coli*, *S. aureus* and fungal pathogens. The main mechanism supposed in the literature for this positive effects on inhibition growth by metal oxide nanoparticles is related to the generation of active oxygen species by these materials hereupon of this opportunity is limited by concentration, size (32), shape (33-35) and nature of extract used for synthesis these particles in other words, surface properties is critical issue in this application (36, 37). In this paper, authors investigated the effect of *A. aucheri* extract in the biosynthesis of ZnO nanoparticles and its ability to inhibition the microorganism growth also will be tested in order to develop the future application.

2. Objectives

In this study, biosynthesis of ZnO nanoparticles with new approach to enhanced the Antimicrobial properties against gram-negative and gram-positive was performed by use of a new type of plant extract, *Artemisia aucheri*, in an environmentally friendly, cost-effective, simple procedure way.

3. Material and Methods

3-1. Plant Material

The plant materials were collected during the flowering stage and in whole blooming time from Bardsir, the Bidkhan area, Kerman Province, Iran in June 2017. It was identified by Research Center of Agriculture and Natural Resources of Kerman, Iran.

3-2. Preparation of the Extract

The aerial parts of *A. aucheri* were thoroughly washed with double distilled water and shade dried in dust free condition for one week at room temperature before being grinded to a fine powder. Finely powdered plant material was extracted with methanol under shaking incubation (25 ˚C, 48 h). The extract was filtered and stored at 4 ˚C for further experiments.

3-3. Gas Chromatography (GC) and GC/MS analysis

The constituents of the extract were analyzed by GC and GC/MS. GC analysis of the components was carried out using a Hewlett-Packard 6890 device equipped with a flame ionization detector (FID). Compounds were separated on an HP-5 capillary column. The column temperature was kept at 60 ˚C for 3 min and programmed to 220 ˚C at a rate of 5 ˚C/min. Injector and detector temperatures were 270 ˚C, and the flow rate of the helium as carrier gas was 1 $mLmin^{-1}$. The amount of percentage composition of the individual components was calculated from the GC-FID peak areas without the use of correction factors. GC/MS analysis was performed using an Agilent 5975C mass spectrometer coupled to an Agilent 7890A gas chromatograph equipped with an HP-5MS capillary column. The carrier gas was helium, and the chromatographic conditions were as above (28).

3-4. Synthesis of ZnO Nanoparticles

The amount of 1 g of $Zn(NO_3)_2$ was added directly to 15 ml of *A. aucheri* methanol extract. The solution was stirred for 20 min. Then, the $Zn(OH)_2$ was precipitated from the solution and the residual precipitate was heated in a muffle furnace in 200 ˚C for 20 min. The overall efficiency of final sediment remains from the reaction occurred on the precursor plant is determined as 10%.

3-5. Characterization of ZnO Nanoparticles

The synthesized zinc oxide nanoparticles purity and crystallite size were identified by X'-Pert PRO (Philips, the Netherlands) X-ray diffractometer. The supplementary analysis of structural parameter from diffraction pattern was done by the High score plus v3.0 software. X-ray diffraction Cu Kα radiation (k = 0.15406 nm) in 2Θ range from 10 to 80. Fourier transform infrared spectroscopy was employed for assessment of functional groups on ZnO nanoparticles and was carried out by using a Bruker Tensor 27 FTIR & OPUS Data Collection Program (v1.1) FTIR spectrometer at a resolution of 4 cm^{-1}. FTIR spectra done under equivalent conditions in the limitation of 400–4000 cm^{-1} range. Field emission scanning electron

microscopy analysis and elemental analysis was carried out using fine powder of ZnO nanoparticles by use of Mira 3-XMU field emission scanning electron microscope equipped by the second generation of energy-dispersive X-ray spectroscopy (EDS) detector. Indeed particles size distribution and zeta potential analyziz was performed by Dynamic Light Scattering by use of Malvern zeta sizer device.

3-6. Antibacterial Activity

In order to measure the amount of antibacterial activity of the extract against *S. aureus* and Escherichia coli, the agar well diffusion method (38, 39) was used. The results were presented by measuring the diameter of inhibition zones.

The wells of 2.5 mm diameter were placed into the Mueller-Hinton agar (which have the concentration of test microorganism about 1.5×10^8 $CFUmL^{-1}$). Then 100 µL of extract at the concentration of 150 $mg.L^{-1}$ was poured into the well. Ampicillin and DMSO were used as a positive and negative control, respectively. The plates were incubated at 37 ˚C for 2 days. The concentration of the ZnO nanoparticles was 12 $mmol.mL^{-1}$, the specimen was prepared in sterile distilled water. A blanket disk was then placed in an ultrasonic bath for one hour. For Gram-negative bacterium, a standard plate and in the case of gram-positive one, the standard penicillin plate was used, each of these plates simultaneously compared by disk containing ZnO nanoparticles. Then these plates incubated 24 h at 37 ˚C.

4. Results

4-1. GC and GC/MS Analysis

The main constituents of the methanol extract of *A. aucheri* were monoterpenes, camphor (43.8%), 1, 8-cineole (35.4%) and camphene (8.6%).

4-2. X-ray Diffraction Pattern

From the structural information and the crystallinity of the XRD diffraction pattern of synthesized material which is depicted in **Figure 1** and comparing by (JCPDS CARD NO: 01-079-0205), it is concluded that there are good agreements between two patterns and by this method ZnO nanoparticles s were successfully synthesized. The broaden and wider almost all of the peaks detected, could represent very low crystallite size and nearly nanoscale, but it is notable that we could see seven different peaks corresponding to a diver's plane direction, that is good enough to confirm favorite crystallinity of formed structure. Beside the point, there are no any remarkable other peaks such as impurities or untreated precursor.

The Scherer formula was applied to evaluate the crystallite size the result shown in **Table 1**. The size of crystallite in all the peaks is very low and in the range of 5 to 9 nm indicating the nanostructure for ZnO material.

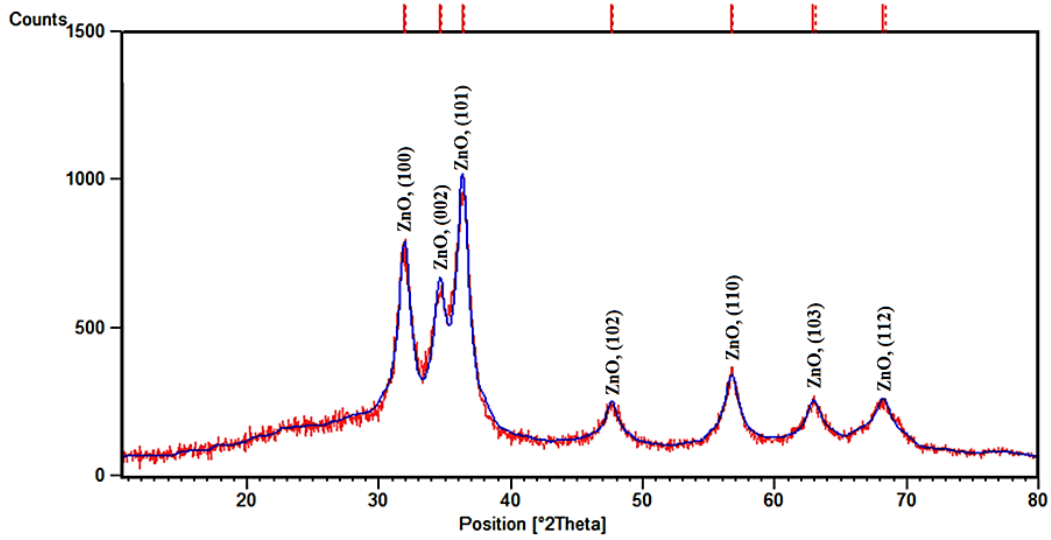

Figure 1. XRD patterns of ZnO nanoparticles synthesized by use of the extract.

Table 1. The crystallite size of different Peaks in the sample.

Peaks	(100)	(002)	(101)	(102)	(110)	(103)	(112)
Crystallite size (nm)	8	5	9	9	7	5	4

4-2. FTIR Analysis

The FTIR spectra analysis illustrated in **Figure 2**. Generally, the peaks in the range of 400 to 600 cm^{-1} bolster the probability of metal–oxygen bond. The band around the 550 cm^{-1}, which corresponds to ZnO forces could be seen in this spectrum. Also, band in the 1118.58 cm^{-1} (C-N stretch), 1373.93 cm^{-1} (N-O bond), 1571.40 cm^{-1} (N-H bond), 2205.9 cm^{-1} (Alkynyl C≡C Stretch), 2850-2950 cm^{-1} (C-H stretch) and finally broad peak in 33963.50 cm^{-1} could be attributed to the O-H groups(40, 41)

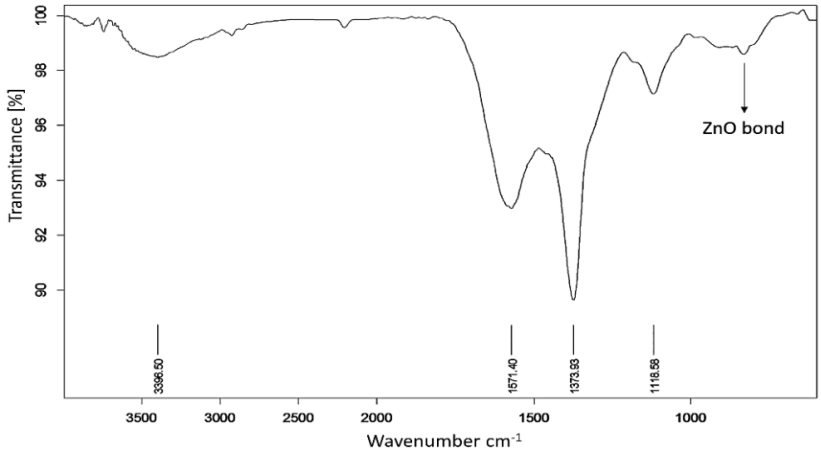

Figure 2. FTIR spectrum of ZnO nanoparticles synthesized by use of the extract.

4-3. FESEM and EDS Analysis

The FESEM images of ZnO nanoparticles in different magnification are shown in **Figure 3A, B**. The particles are in the seabed like spherical and granular shapes which are in the size of the range 15-40 nm. In addition, to somewhere partially agglomeration or clustering of particles could be seen. Also, elemental analysis of two different points (**Fig. 3C, D**) that shows the existence of elements such Zn and O, taking this besides X-ray diffraction would imply the formation of ZnO nanoparticles. In both EDS also peaks by low intensity of C and K was detected, these peaks could indicate the presence of alcohol or phenolic compound.

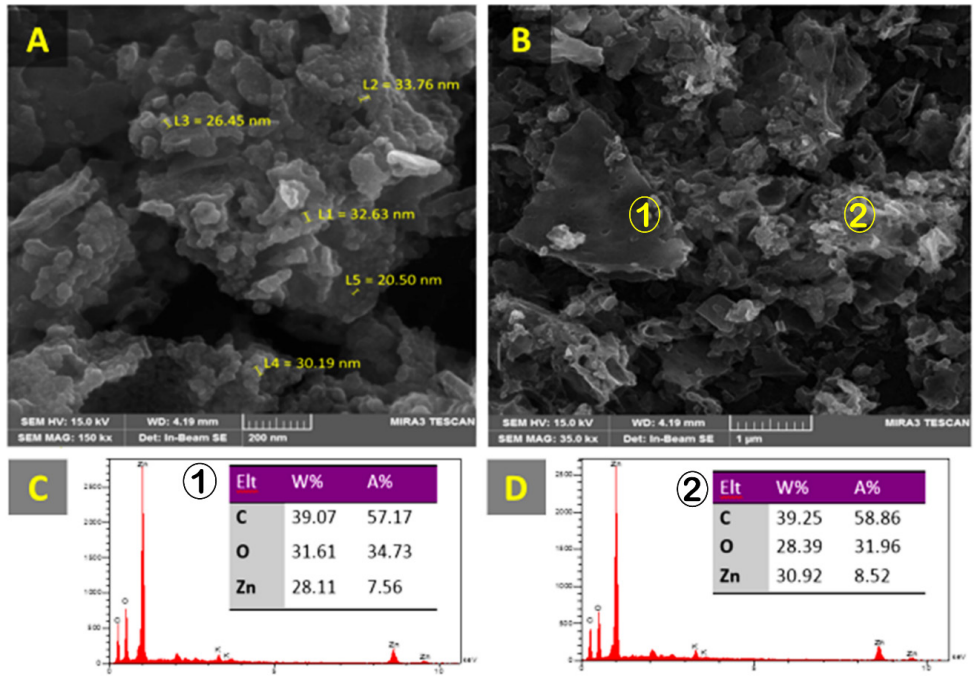

Figure 3. FESEM image in different magnification (A, B), EDS analysis of ZnO nanoparticles synthesized by the extract in two different selective point (C,D).

4-4. Zeta Potential and Particle Size Analysis

Dynamic Light Scattering analysis measurement was used to determine particles size in dilute suspension of ZnO nanoparticles. In **Figure 4 (A)** the negative zeta potential of ZnO nanoparticles is obtained as 38 mV which is resulted by the negative charges of the capping of polyphenols in the extracts. Also **Figure 4** (B) shows the ZnO nanoparticles size distribution. Based on this Figure the average of particles size is determined as 76 nm. Which this high and negative hydrodynamic amount of Zeta potential indicating that the nanoparticles of ZnO contain agglomerates and there is no significant of aggregates in aqueous condition(42, 43), by the way the very fine particles are fulfilling satisfactory result for such a method in order to produce these ZnO nanoparticles.

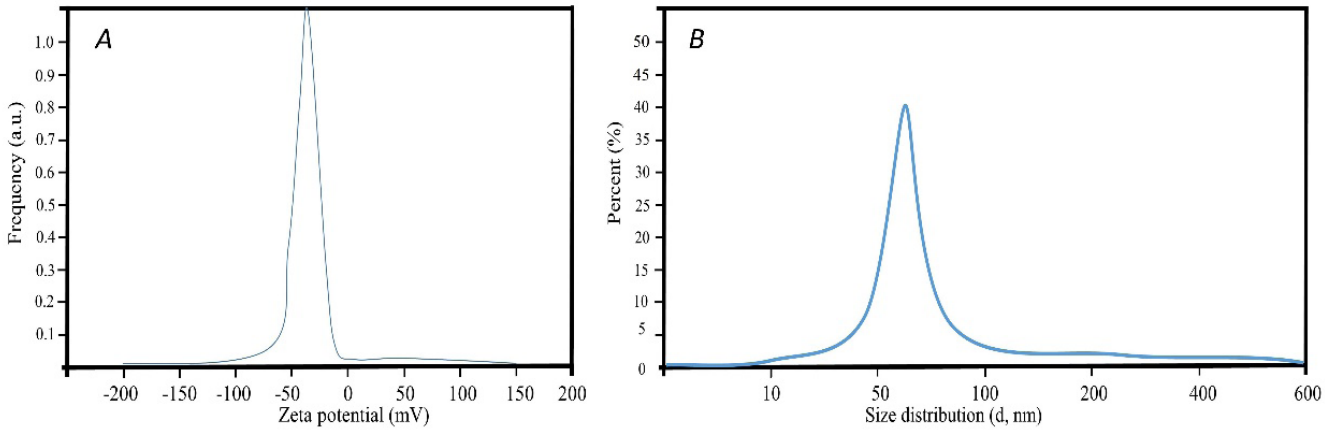

Figure 4. Zeta potential (A) and particle size analysis.

4-5. Antibacterial Activity

The antibacterial activity of *A. aucheri* extract was determined by measuring the diameter of affected areas for inhibition growth in agar plates, and the optical pictures and the result acquired is depicted in **Figure 5** and **Table 2**. The extract shows good inhibition zone growth for two bacteria, but it seems to better performance of this extract for S. aureus, which is 10 mm in diameter of affected zone compared to 10 mm for *E. coli* bacteria is recorded.

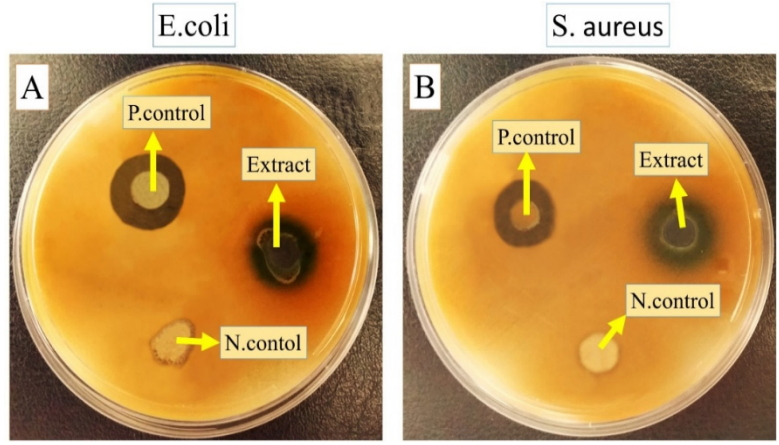

Figure 5. Antibacterial activity of the extract using agar diffusion methods against **(A)** E, coli and **(B)** S. aureus.

Table 2. Antibacterial activity of *Artemisia* aucheriextract.

Type of bacteria	Diameter of affected area around wells (mm)	
	Extract	Antibiotic
Escherichia coli	10	14
Staphylococcus aureus	11	12

The **Figure 6** and **Table 3** indicating the inhibition growth test of ZnO nanoparticles against two types of prevalent pathogenic bacteria with one gram-negative *E. coli*, and one gram-positive *S. aureus* by 7 mm and 5 mm in diameter of inhibition zone respectively. There are impressive antibacterial effects on both type of gram negative or positive.

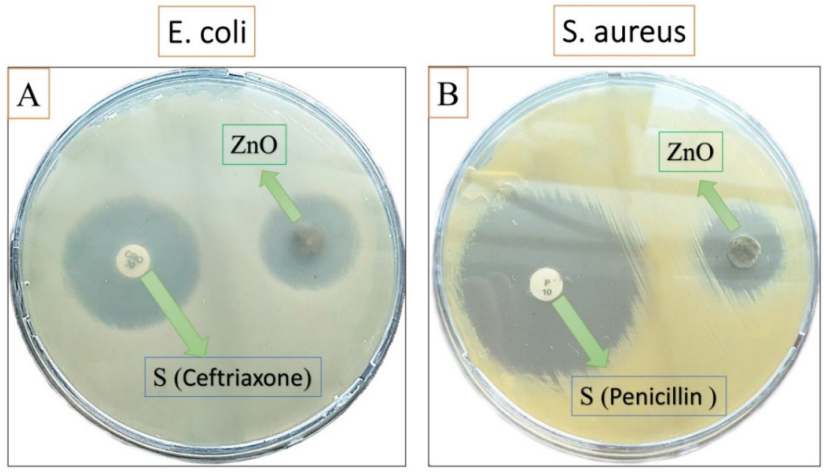

Figure 6. Antibacterial activity of ZnO nanoparticles using agar diffusion methods against **(A)** E, coli and **(B)** S. aureus.

Table 3. Antimicrobial activity of ZnO nanoparticles.

Type of bacteria	Diameter of inhibition zone (mm)		
	ZnO nanoparticles	Antibiotic	
		ceftriaxone	penicillin
Escherichia coli	7	10	-
Staphylococcus aureus	5	-	16

5. Discussion

This method successfully provided us pure ZnO in nanoscale size distribution also in nanostructure type. Since the main purpose of this article is how these nanoparticles /extract manners versus various bacteria agents, the result of antibacterial activity which was examined showed good inhibition zone growth for two types of common bacteria (gram-negative and gram-positive), but it seems to better performance of this extract for S. aureus is recorded.

In the case of the extract of *Artemisia*, this dominance in antibacterial behaviour is arising from the potential of composition of this matter which consists of important components of monoterpene, camphor cineole (44, 45) in the amount of 43.8%, 1.8-cineole in the amount of 35.4%, and the camphene in the amount of 8.3% as be determined by GC analysis (3-1) which is involved the preeminent portion of extract. The vital functions of cells, including energy production, food consumption, activity, coarse molecule synthesis is based on a healthy cell membrane (46). Camphor, which is a terpenoid group, and camphene, a monoterpene bicyclic compound, exhibit a variety of behaviours such as destruction and surface changes, holes on bacterial surfaces, or small and irreversible changes. When a cell membrane is exposed to an antimicrobial extract that contains the terpenoid compounds, cell permeability has increased and the degradation of a thick, transparent colloidal substance that creates the main building blocks of living cells and leads to loss of cytoplasmatic cell materials. Any change that damages the cell structure can have a negative effect on normal functioning and cellular activity, which ultimately leads to complete cell destruction (47, 48). The presence of these potential compounds in this extract is resistant to both gram-negative and gram-negative bacteria, and by comparison with the positive control sample **(Fig. 5)** and **Table 2**, which are in the equivalent range limit, indicating the potency of this substance for antimicrobial activity.

One drawback which is perceptible is the lower of antibacterial activity against *E. coli* into the other previous research about this area (49, 50).

The reason for this can be related to the type of structure

of this nature of bacteria. Basically, the difference in the cell wall structure of these two types of bacteria causes such behaviour. The gram-positive bacterial cell wall is naturally formed from peptidoglycan, which forms 80% of the cell wall. For gram-negative bacteria, these materials account for only 10% of the cell wall, but the outer membrane is equivalent to 50% of the lipopolysaccharides, 35% of the phospholipids and 15% of the lipoproteins. Thus, this superiority causes the destructive effect of such particles (ZnO) became more difficult compared to gram-positive type and causes more resistance to these drug agents (51, 52).

The bacterial cell walls consist of peptidoglycan porous layer which has the role of cell strengths and also the morphology of cell determined by this component. The thickness of this layer in gram-positive higher than the Gram-negative one. This superiority causes the destructive effect of such particles became more difficult compared to gram-negative type (49, 53-55).

Also, ZnO nanoparticles which synthesis by taking advantage of extract shows good resistance in opposition of E. coli and S. aureus. Many hypotheses involved for mechanisms in the antimicrobial activity of ZnO nanoparticles. The first mechanism is related to release of Zn^{2+} ions, because of the release of these ions could change the active transport inhibition and amino acid metabolism and have devastating effect on the enzyme system which is this property is a consequence of size and solubility of Zn^{2+} dependent used in the culture (54, 56), reactive oxygen species (ROS) generation which is tightly dependent in photocatalytic induction and under UV exposure the antibacterial activity of ZnO nanoparticles increased (57) and surface features (58, 59).

Table 4 indicating some prominent ZnO nanoparticles synthesized by different plant materials. Comparision this work by other works would be resulted in synthesis in a low temperature and time, very fine particles, especially the most lower crystallite size will be obtained, by the way for antimicrobial test, it provides significant antimicrobial properties against E.coli and S. aureus bacteria.

In the previous works, also has taking into account that one directly factor is concentration of ZnO nanoparticles which is affecting the amount of inhibitory activity (32, 37), in another sight, ZnO nanoparticles has good photocatalytic induction, which is the cause of increasing the generation of ROS in the surface of particles also oxygen from surface modification such as annealing in high temperature which that interferes the amount of oxygen in the surface (15, 60).

Table 4. ZnO nanoparticles synthesized by different plant materials.

ZnO nanoparticles synthesized by different plant materials									
Plant material	**Synthesis time (min)**	**Synthesis temperature**	**Annealing temperature/ time**	**Crystallite size (nm)**	**DLS (particle size, nm)**	**SEM (particle size, nm)**	**E. Coli Inhibition size (mm)**	**A. Areus Inhibition size (mm)**	**REF.**
Trifolium Pratense flower	240	90 ˚C	400 ˚C for 60 min	60-70	-	100-190	31(st:31)	31(st:31)	(37)
Catharanthus roseus	30, 60, 120 and 240	30, 60, and 90 ˚C	-	36.83	Op:50.73	62-94	11.10 (st:10.07)	11.74 (st:11.15)	(61)
P. caerulea L.	180	RT	80 ˚C for 120 min	37.67	-	70	13(st: 13)	-	(62)
Nyctanthes arbor-tristis	120	60, 70, 80 and 90 ˚C	60, 70, 80 and 90 ˚C for 24hr	16.58	74.36	12-32 (TEM)	-	-	(63)
Atalantia monophylla	120	60 ˚C	80 ˚C for 24 hr	33.01	-	30 (TEM)	11(st: 8)	10(st: 13)	(64)
Parthenium hysterophorus	24 hr	RT	-	-	-	16-45	20(st: 35)	11(st: 19)	(65)
Costus pictus D. Don	240	80 ˚C	40 °C for 8h followed by 450 ˚C	29.11	20-80	11–25	10(st: 36)	10(st: 32)	(66)
Artemisia Aucheri	20	RT	200 ˚C for 20 min	7	76	20-30	7(st:10)	5(st:16)	This work

St: standard, op: Optimum.

6. Conclusion

This study shows that by use of new class of plant extract, *A. aucheri*, synthesis of ZnO nanoparticles, successfully performed. This biosynthesis routs, is very low cost, fast and eco-friendly to environment. Complete synthesis of nanostructure of ZnO nanoparticles with crystallite size in the range of 4-9 nm is confirmed by XRD and FTIR spectroscopy. Besides FESEM images shows seabed like spherical particles in the range of 15-40 nm and EDS analysis indeed shows high intensity peaks of Zn or O elements, indicating presence of ZnO nanoparticles. In antibacterial test, satisfactory level of inhibition growth for two materials, extract or ZnO nanoparticles compared to control samples, for *E. coli* and *S. aureus* were acquired. Results show the remarkable potential of these nanoparticles especially against E. coli and It could be used as an effective alternative to commonly used chemical drugs and covering drug resistance issues resulted from persistent use of chemical drugs by applicants.

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