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The effect of *Olea europea* L leaves extract and ZrO_2 nanoparticles on *Acinetobacter baumannii*

Prof. Dr. Rana Mujahid Abdullah*, Hiba Qasim Hameed*, Anaam Abdulqader Hasan*
** Department of biology, College of Education for pure science Ibn-Alhythm, University of Baghdad*

Abstract

The effect of *Olea europea* leaves extract on *Acinetobacter baumannii* was studied. The results of this study showed that there was an inhibitory effect of olive leaves extract against the three isolates of the studies bacteria (1, 2 and 3) with different rates of inhibitory zone. Moreover, the effect of ZrO_2 nanoparticles on *Acinetobacter baumannii* was determined, and the results observed that this compound had an inhibitory effect on the isolates (3) of bacteria but not with isolate (1, 2). When ZrO2 nanoparticles mixed with olive leaves extract, the results were higher in effect and the mixture showed a synergistic action against the three isolates of *Acinetobacter baumannii*.

The Fourier Transform Infere-Red Spectroscopy (FT-IR) test was performed for ZrO_2 solution and the analytic results indicated that this solution contained many active substances.

The X-Ray Diffraction Analysis (XRD) of ZrO_2 was performed also for ZrO_2 solution, and the results showed that the average size of each molecule of ZrO_2 nanoparticles was (29.8 nm).

Key words: - *Olea europea*, ZrO_2 nanoparticles, *Acinetobacter baumannii*.

INTRODUCTION

Thousands of years ago and nature is an important source of medical and therapeutic compounds, and today a large number of modern medicines and drugs have been produced from natural sources of different kinds of plants; these medicines have been produced due to the multiple uses of these plants this type of treatment called traditional medicine [1]. The high resistance of the bacteria for antibiotics and the widespread demand for using antimicrobial drugs that effect on immune system increased the incidence rate for bacterial infection, thus it is necessary to develop new antibiotics.[2], and the antibiotics should have a broad spectrum inhibition against microorganisms that given preference in the pharmaceutical industry in order to control the pathogenic microorganisms that have antibiotic resistance properties [3].

Among the plant sources that can be used as a treatment for infectious diseases is the olive plant (*Olea europea*), where throughout history and civilizations that olive plant is an important source of nutrition and medicine. The first official report about the uses of olives in the medical field was prepared in 1854 when it was found that the extract of olive leaves have been successful in the treatment of fever and malaria due to the presence of several highly effective compounds in this plant against bacteria, fungi and mycoplasma [4]. Recent studies have also focused on the medicinal uses of ZnO_2 nanoparticles. The fact that these nanoparticles have shown anti-microbial properties has contributed to the importance of these substances to be present in the pharmaceutical and medical industries, especially as we live the problem of the spread of infectious diseases and the difficulty of treating them due to the increase of bacterial strains resistant to antibiotics [5].

Nanotechnology has attracted remarkable attention in the past few years due to its unique physical, chemical and biological properties, as well as to its ratio of large surface area to small size, which make it interactive compounds with bacteria [8,7,6]. These studies that the compounds with low molecular weight such as nanoparticles generally have the potential to inhibit a wide range of bacteria [9] as well as their use in the medical field, and this may be due to surface area, low thermal conductivity, high rigidity, thermal insulation properties and flexibility, All these led to the entry of these materials as therapeutic especially in dentistry [10] while nanoparticles of ZrO_2 uses in the manufacture of dentures and cosmetic fillings [11]. Therefore, it is necessary to produce new antibiotics without side effects such as particles of ZrO_2 nanoparticles that can inhibit a wide range of microorganisms. This is indicated by many scientific studies that confirm the effective inhibitory action of ZrO_2 nanoparticles against gram-negative bacteria including *Escherichia coli*. In recent study found that ZrO_2 particles have the ability to inhibit gram-positive bacteria including *Bacillus* [13]. This study aimed to evaluate the inhibitory effect of *Olea europea* L. Leaves extract and ZrO_2 on *Acinetobacter baumannii*.

MATERIAL AND METHODS

1. Sources of isolates: *Acinetobacter baumannii* was obtained from the Microbiology and Molecular Biology Laboratory at the College of Education for Pure Sciences (Ibn Al-Haitham)/ Postgraduate Studies laboratory.
2. Collection of plant leaves: the leaves of olive plant were collected from olive trees, and then washed with water to remove the dust and impurities from them and dried in the open air. Then, leaves were grinded with an electric grinder and kept in the refrigerator in sterile sealed glass until the use.
3. Preparation of the hot alcoholic extract: the extract was prepared according to [14] procedure; 20 gm. of prepared powder of olive leaves was used and placed in the Thumb in the Soxhlet apparatus using 150 ml of ethanolic alcohol as solvent. And left for three hours, then the extract was dried at (50-60)° C and collect the dried extract placed in sterile tubes and kept until use.
4. Detection of active compounds: the following methods have been used to detect effective compounds: Alkaloids by method of [15], Flavonoids followed by [16], Tannins by method of [17] and glycosides and phenols by [16],

while Fatty acids was detected using the method [18], Terpenes and steroid following method of [19].

5. Preparation of Concentrations from the Extract of hot alcoholic olive Leaves: A stock solution was prepared from the hot alcoholic extract of the olive leaves as 1 g which was transferred to a new flask with distilled water and the final volume was up to 100 ml, then the mixture sterile using 0.22 µm filter papers [20].

6. Preparation of nanoparticles: Nanoparticles were suspended with distilled water using an ultrasound device for 15 minutes and attended with concentrations of 1g/100 ml [21].

7. Preparation of nanoparticles and olive leaves extract mixture: 1 ml of nanoparticles was added to 1ml of hot alcoholic olive leaves extract (1 g/ml) in ultrasound for 15 minutes [11].

8. Studying the inhibitory activity using wells Technique: the agar well diffusion methods was used to determine the activity of both nanoparticles and hot alcoholic olive leaves extract separately and in the mixture of both of them [22].

9. Fourier Transform Infra-Red Spectroscopy (FT-IR) analysis: FT-IR spectroscopy analysis was done for the studied compounds using (8300 FT-IR Shimadzu Spectrophotometer Perkin) device, where the wavelength of the spectrum was between 400 cm^{-1} to 4000 cm^{-1}.

10. X-ray diffraction (XRD) analysis: ZrO2 compound was used to determine X-ray diffraction (XRD) (Shimaduzu XRD-6000), and the analysis in the service laboratory of The Central Service Laboratory of college of Education for Pure Science (Ibn Al-Haitham)/University of Baghdad.

RESULTS AND DISCUSSION

The results found that ZrO_2 nanoparticles had a relatively low inhibitory effect against *Acinetobacter bumannii* for isolates (1,2,3) (Table 1), where isolates (1, 2) did not actually inhibited in the presence of ZrO_2 nanoparticles, while isolate 3 of the bacteria was affected by these nanoparticles with high efficiency. Many previous and recent studies have been indicated the ability of nanoparticles of ZrO_2 to inhibit certain species of bacteria. Both [23 and 24] studies found that the nanoparticles of ZrO_2 inhibited the growth of pathogenic *Escherichia coli* and *Staphylococcus aureus*, as well as their antifungal efficacy against fungi such as *Candida albicans* and *Aspergillus niger*, which were found to be better compared to conventional organic antibiotics; the inhibitory action of these compounds is may be due to its ability to break down the cell membrane by increasing of lactate dehydrogenase production according to the results 0f [25] which studied the effect of ZrO_2 on *E. coli*, *Klebsilla*, *S. aureus* and *Salmonella*. Moreover, the interaction between nanoparticles and cell components in bacteria may lead to synthetic changes and damage in the membranes and then cell death [26]. Recently, new observation proved that ZrO_2 nanoparticles are considered as excellent inhibitory agents against both positive and negative bacteria [27].

Interestingly, the importance of olive plant is well known in the medicinal fields and the results of this study indicated that the hot alcoholic leaves extract of *Oleo europea* was more effective in inhibiting the three isolates of *Acinetobacter bumannii*. In addition, [28] revealed that the extracts of the olive plant have a high inhibitory effect against some bacteria, and this effect is variant according to the type of the extract, where the methanolic extract was found to be more effective against Gram positive and negative studied bacteria, while the inhibition against the same bacteria were different after using the aqueous extract, chlorophorm and Ether Petroleum extract where the researcher found that methanol probably allows for the extraction of all phenolic compounds from the olive plant. In another study on olive leaves extracts, the results referred that the type of solvent used in the extraction was affected on the inhibitory efficiency of the extracts against *S. aureus*, *E. coli*, *S. entritidis*, *S. typhimurium* and some other bacteria [29]. The latest study observed the solvent type can effect on the distribution and concentration of phenolic compounds in the extracts as well as on the inhibitory action against the bacteria. Furthermore, [30] found that the aqueous extracts of the olive leaves have high inhibitory properties and inhibition zones with a diameter of 11.5 mm against *Salmonella typhimurium*.

The results also showed a synergistic action of ZrO_2 and olive leaves extract mixture against the studied bacteria and for the three isolates. Many studies pointed the high inhibitory activity of nanoparticles and plant extracts in the case of mixing them together in comparison to their effectiveness if they were used alone; this in agreement with results of [31] that revealed the inhibitory effect of *Eucalyptus tereticornis* leaves extracts was low against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (MRSA and MSSA), but when it mixed with silver nanoparticles it gives a significant inhibitory effect against all studies bacteria, and the inhibition zone was (9-12 mm) compared with (8-10) mm in case of use of the extract alone (Table 1).

Table (1): Diameter of inhibition zones of ZrO_2 and olive leaves extract alone and in combination together against *Acinetobacter bumannii*.

Isolates	Diameter of inhibition zones (mm)			
	Nanoparticle ZrO2	Olive leaves extract	*Combination	**Control
Acinetobacter baumannii 1	-	+	+	-
Acinetobacter baumannii 2	-	+	+	-
Acinetobacter baumannii 3	+	+	+	-

*** Combination: Mixture of nanoparticles with the olive extract ** Control: sterile distilled water**

The detection results of the active compounds revealed that the hot crude alcoholic extract of olive leaves contained alkaloids, flavons, tannins, clicosides, phenols, fatty acids, turbines and steroids (Table 2). This results are in agreement with [32] which identified seven phenolic compounds in the olive leaves extract and also [33] found that the olive leaves are rich with polyphenol compounds such as Oleuropin and Tyrosol and these compounds are responsible for antimicrobial properties [34, 35].

Table (2): Detection of the active compounds in hot alcoholic extract of *Olea europea*

Active compounds	hot alcoholic extract of *Olea europea*
Alkaloids	**+ve**
Flavonoides	**+ve**
Tannins	**+ve**
Glycosides	**-ve**
Poly Phenols	**+ve**
Fatty acid	**+ve**
Terpenst steroid	**+ve**

Fourier Transform Infra-Red Spectroscopy of the ZrO_2 solution was performed. The results showed that this solution contains chloride group at 592 cm^{-1}, Amide CN group at 1637 cm^{-1}, O_2 group at 2065 cm^{-1} and OH group at (3448 cm-1) as shown in (Fig. 1). These results are consistent with [36] study, which indicated that there was a high and broad absorption centered around (3413 cm^{-1}) and three clear absorption bands at (1044, 1352, 1630) cm^{-1} and also two weak absorption bands at (454, 580) cm^{-1} .

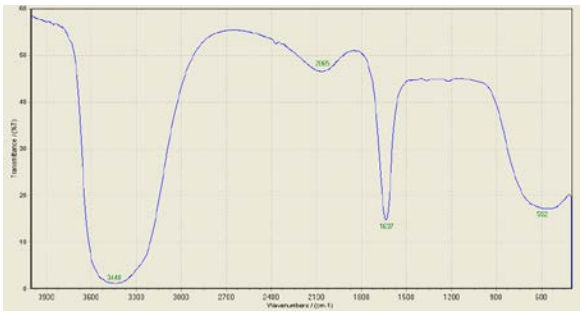

Figure (1): Fourier Transform Infra-Red Spectroscopy (FT-IR) analysis of ZrO_2 nanoparticles.

X-ray diffraction analysis was performed for ZrO_2 as shown in (Fig. 2) and the diffraction peaks were indicated to the small size of the crystals. Scherrer equation [37] was used to calculate the average size of the nanoparticles for ZrO_2 as well as the capacity of nanoparticles.

$D=K\lambda/\beta \cos\theta$

D= size of particles

K= Scherrer constant (1-0.9)

ʎ= the wave length of X-ray (A 1.5418)

β= width of peak

cosθ= Bragg angle

Based on the above equation, the results found that the average size of nanoparticles of ZrO_2 was 29.8 nanometers and this in agreement with [38] observation where found that the size of ZrO_2 nanoparticles was 18.1 nanometers according to Shearer equation. Moreover, [39] found that the size of nanoparticles was up to 20 nanometers using the Shearer equation.

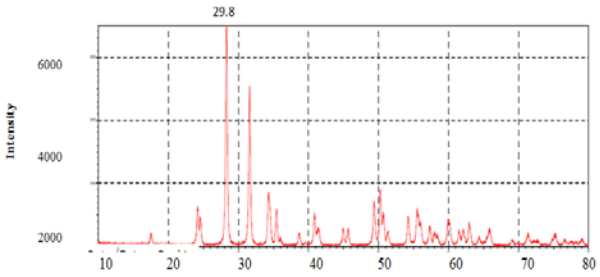

Figure (2): X-Ray Diffraction (XRD) of ZrO_2 nanoparticles and measurement of nanoparticles capacity.

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