Biosynthesis of Alumina Nanoparticles by Plant Extract for *Ocimum basilicum* Leaves and Evaluation of its Antibacterial Efficiency Against Some Bacteria Isolated from Skin Infections

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Abstract

This study includes the isolation and identification of bacteria that cause skin infections in addition to preparing alumina nanoparticles in the biosynthesis method and testing its efficiency as an anti-bacterial. compared inhibition zones between the prepared nanoparticles and commonly used antibiotics. The prepared nanoparticles were characterized by various techniques, including UV-visible spectrophotometry, X-ray diffraction (XRD), and field emission scanning electron microscopic (FE-SEM) technology was also used to verify the morphology of the crystal structure. UV- visible spectroscopy showed success in the synthesis of alumina nanoparticles by observing the absorption peak that was (248,250) nm, which belongs to alumina nanoparticles, and X-ray diffraction confirmed the presence of Al_2O_3NPs in the crystal structure of the prepared particles. FE-SEM showed the formation of alumina nanoparticles in the size range of (15-63) nm and in a spherical shape. When evaluating the efficiency of alumina nanoparticles as anti-bacterial, it was found that it has a high activity by measuring the diameter of inhibition, while the aqueous plant extract did not show any inhibition diameter. Alumina nanoparticles showed high efficacy against Acinetobacter baumannii which are resistant to all types of antibiotics used in the current study.

The antibiotics Imipenem (IPM), amikacin (AK), Ciprofloxacin (CIP), Gentamycin (GM) were used to test their effect on gram-negative bacteria while Imipenem (IPM), amikacin (AK), Vancomycin (VA), Amoxicillin/clavulanic acid (AMC) on gram-positive bacteria, where some bacterial isolates were resistant and others sensitive to these antibiotics.

***Keywords*:** Alumina nanoparticles, green synthesis, medical plants, *Ocimum basilicum,* skin

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Introduction

The skin is a complex barrier that serves as a protective interface between the interior part of the human body and the external environment. It is one of the largest and most varied organs in the body. Thus, it is exposed to a large number of various pathogens (Naveed & Abdullah, 2021). Despite numerous bacteria coming into touch with or adhering to the skin, people cannot be infected by them. (Egert et al., 2017). This barrier is an integral part of the immune system as it acts as the first line of defense against various bacterial infections. skin sites can be classified according to their physiological characteristics into oily, wet, or dry skin (Byrd et al., 2018). Bacterial skin infection occurs when bacteria penetrate the membranous system through hair follicles, cuts, scrapes, surgeries, and burns. Bacterial infection of the skin upon contact with other carriers of this infection (Barupal et al. 2019). Common skin diseases include acne, impetigo, folliculitis, necrotizing dermatitis, boils, skin abscesses, cellulitis, necrotizing fasciitis, and gangrene (Pizzuti et al., 2020).

Antibiotics are defined as compounds that can be produced by microorganisms, they have the ability to inhibit growth or kill bacteria. It also has the characteristic of selective toxicity towards the organism, that is, it is able to effectively affect the pathogen without harming the host tissue (Yujie et al., 2019). Antibiotic resistance occurs when a microorganism is able to grow or survive in the presence of a concentration of an antibiotic that is usually sufficient to inhibit or kill organisms of the same species (Stanford et al., 2020).

Since antibiotic-resistant bacterial strains have emerged as a result of the indiscriminate and inappropriate use of antibiotics to treat bacterial illnesses, these strains have become more difficult to manage, which has a detrimental effect on public health. (Zhou et al., 2020). Thus, the need to discover effective antimicrobial agents against which bacteria may not develop resistance has increased (Yahav et al., 2021, Mohamed et al., 2019). Therefore, nanomaterials have been used as antimicrobial agents due to their high surface area relative to volume and this ensures a wide range of interactions with the bacterial surface (Auda, 2020; MANYASREE et al., 2018). Microbes are unlikely to develop resistance to nanoparticles because they attack a wide range of targets that require the microorganism to undergo a series of mutations simultaneously in order to protect themselves (Singh et al., 2018).

Since medicinal plants are a significant source of medications, many biologically active medications derived from plants have been created to treat a wide range of diseases. (Obeidat., 2018). In recent years, the biosynthesis of nanoparticles using plant extracts has gained great interest, because the plant sources are toxic-free, low-cost, and environmentally friendly. It is also effective in treating infectious diseases while at the same time relieving many of the side effects often associated with synthetic antimicrobials (Ribeiro et al., 2020).

Nanoparticles ranging in diameter from 1-100 nm are of paramount importance in modern science because of their unique physical, chemical, and optical properties compared to larger particles (Song et al., 2021). Nanoparticles can be synthesized in several ways, namely physical, chemical, and biological (Grassian et al., 2020; Manikandana et al., 2018). Although physical and chemical procedures have a high production rate and allow for precise control over the size and form of the produced nanoparticles, they are not preferred because of their high cost and complicated mechanical requirements. It is also time-consuming, and uses toxic reagents, and generates hazardous waste. This has limited its use in clinical applications (Gour and Jain., 2019). However, the method of biosynthesis using plants is safe and non-toxic due to the use of secondary metabolites present within the plant components such as ketones, aldehydes, amides, and carboxylic acids as reducing agents (Fahimirad et al., 2019; Attia & Elsheery, 2020).

Procedure

Specimens Collection

Twenty samples were collected from patients who attended Al-Ramadi Teaching Hospital for both genders. The samples included bacterial isolates from different skin infections. Sterile cotton swabs containing sterile transport swabs were used to collect samples and transport them to the laboratory. The isolates were diagnosed based on cultivars, microscopy, and biochemical tests

Preparation of plant extracted

Fresh *Ocimum basilicum* leaves were collected from local markets and washed with plain water and then with distilled water several times to remove suspended impurities, then dried at room temperature, taking into account continuous stirring to prevent rotting. Then it was ground by the electric grinder to get a fine powder. 40 gm of powdered *Ocimum basilicum* leaves were dissolved in 400 ml of distilled water in a conical flask and placed on a magnetic stirrer at 45 ^{o}C for 2 hours. The solution was filtered in several layers of medical gauze and then by Whatman No. 1 filter paper. After that, the filtrate was collected in glass tubes and kept at refrigerator temperature until use.

Preparation of alumina nanoparticles by plant extract

Alumina nanoparticles were prepared by biosynthesis method using plant extract of *Ocimum basilicum* leaves, where 10 ml of plant extract was added to 90 ml of prepared aluminum nitrate solution at a concentration of

1mM, 2mM. Then they were mixed well using a magnetic stirrer at 45 °C for two hours. The gradual change in the color of the solution indicates the formation of alumina nanoparticles.

Antibacterial activity of nanoparticles test

The diffusion method on agar was followed to test the effectiveness of the prepared nanoparticles as antibacterial. 100 µl of the bacterial suspension after comparison with a McFarland tube (0.5) was spread on Muller-Hinton agar medium with a sterile cotton swab, then made holes with 6 mm diameter in each dish by cork borer and poured in each them 20 µl from a solution of alumina nanoparticles prepared in concentration (2mM). the plates were incubated at 37oC for 24 hours and the diameter of the inhibition zones around the hols was measured by a Vernier caliper with a millimeter scale (Kumar et al., 2019).

Antibiotic test

An antibiotic susceptibility test was performed by using the diffusion method on Agar Mueller-Hinton medium by Kirby-baucer. The bacterial suspension was spread on Muller-Hinton agar medium and incubated at 37°C for 24 hours. Then the diameters of the inhibition zone were measured to determine the sensitivity and resistance of bacteria to antibiotics and compared with (CLSI, 2020).

Results and discussion

Isolation and diagnosis

90% of positive growth was shown by the results of bacterial culture, while the remaining samples did not show any growth. This may be due to the presence of anaerobic bacteria that require special incubation conditions, the use of antibiotics, or the inappropriateness of the culture media used for culturing the sample. Various bacterial types of skin infections were isolated and the result of the isolation was (33.3%) Gram-positive bacterial strain and (66.6%) Gram-negative bacterial strain. Such results are consistent with those of previous studies (Atef et al., 2019). *Staphylococcus spp*. is among the various bacterial isolates which have the predominant percentage (27.7%), followed by *Escherichia coli* (22.2%) and *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (16.6%), and *Acinetobacter baumannii* (11.1%), and finally, it was *Kocuria rosa* (5.5%). This is consistent with the results of a study (Ghimire et al., 2020).

Diagnostics of nanoparticles

color change

Changing the solution color is a major indicator of nanoparticle formation due to surface plasmon excitation (Abod et al., 2017). The results of the current study showed that the plant extract of *Ocimum basilicum* has the ability to act as a reducing and stabilizing agent for the formation of alumina nanoparticles by observing the color productivity, which appeared in a yellowish-brown color as shown in Figure (1).

Figure 1: Color change of nanoparticles

UV-Vis Analysis of Al2O3 NPs Prepared by plant extract Method

UV-Vis Analysis is one of the important techniques for the detection of nanostructures. Ultraviolet-visible spectroscopy is used to determine the absorption of nanoparticles within the range (200-800) nm. Figure (2) shows the UV-visible spectrum of alumina nanoparticles biosynthesized by using *Ocimum basilicum* plant extract. The absorption peak was at the wavelength (250nm) for a concentration 2mM and (248nm) for a concentration of 1mM, which agrees with (Singh & Soni, 2014).

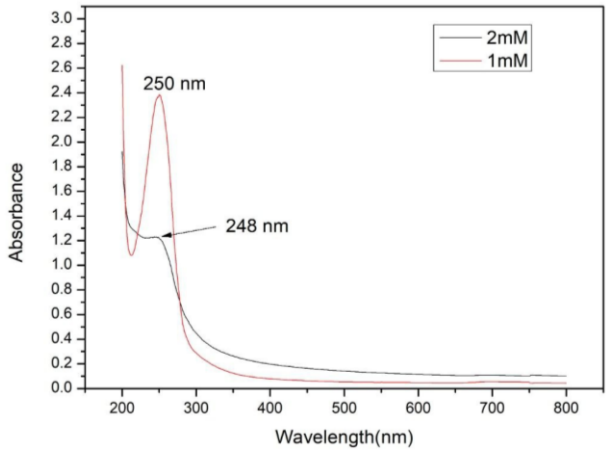

Figure (2): UV-visible absorption spectrum of the prepared alumina nanoparticles

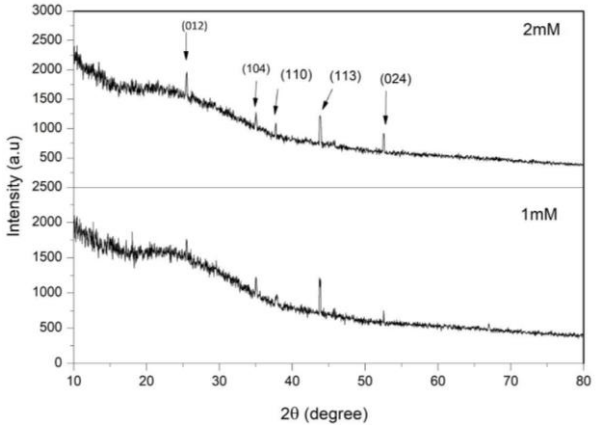

Figure 3: X-ray diffraction spectrum of alumina nanoparticles prepared using plant extract

X-ray diffraction analysis of the Synthesized Al_2O_3NPs using the plant extract method

The purity and elemental composition of the nanoparticles were determined by X-ray diffraction analysis, which is used for phase determination, crystal structure, and average grain size. The X-ray diffraction pattern of alumina nanoparticles prepared by *Ocimum basilicum* extract as a reducing agent was recorded at 2Theta values between 10^o to 80^o. Figure 3 shows the results of XRD patterns for alumina nanoparticles, from the results, it was observed that the crystal structures of the formed nanoparticles are polycrystalline and have a face-centered FCC cubic structure this agrees with (Alsumaidaie et al., 2017). The 2Theta values representing diffraction peaks are 26.14, 36.19, 36.95, 43.78, and 52.8 these peaks correspond to (012), (104), (110), (113), and (024) of the alumina patterns observed and compared with JCPDS cards for both concentrations. No additional peaks belonging to the other crystal facets were detected. This indicates the pure synthesis of the formed nanoparticles, which indicates that the prepared material is free of any impurities.

The average particle size of alumina nanoparticles was calculated by using the Scherrer equation and the average particle size was 43 nm at 1mM concentration and 32nm at 2mM concentration. We note that by increasing the molar concentration, the particle size of the prepared nanoparticles decreased. Therefore, 2mM concentration is used to perform the rest of the examinations and tests.

$$D= (K*\lambda)/(\beta*Cos\theta)$$

Granular size -D

Wavelength (nm) -λ

Θ- Prague corner

K- is a unitless constant equal to 0.9

β- Peak width at a mean height

Table 1: Granular size of alumina nanoparticles

التركيز	D (nm)	FWHM (β)	2 Theta
1 mM	43	0.20751	43.80
2 mM	32	0.27244	43.80

Field emission scanning electron microscopic analysis

FE-SEM analysis was used to characterize the shape and size of nanoparticles manufactured in the nanoscale. The microscopic image of the FE-

SEM analysis of alumina nanoparticles prepared by an environmentally friendly method shows that the obtained nanoparticles are spherical as Figure (4), and the particle size ranged between (15-63) nm.

The difference in the size of nanoparticles may be due to the presence of many secondary metabolites in the plant extract. The appearance of large nanoparticles may indicate the accumulation of small particles together, in addition to the presence of some agglomerations that may be due to the deposition method.

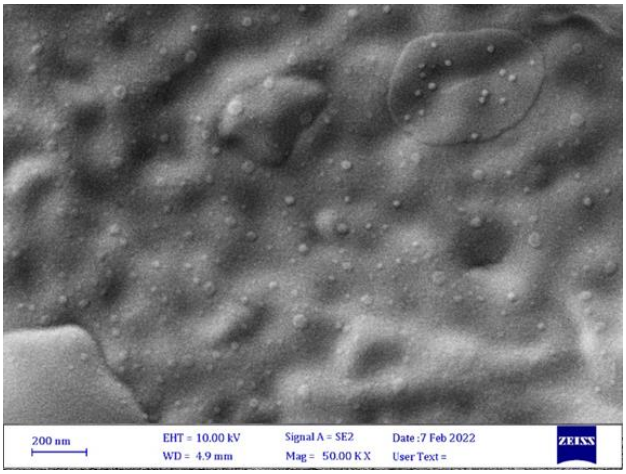

Figure 4: Field emission scanning electron microscope image of alumina nanoparticles prepared using plant extract.

Antibiotic sensitivity test of bacterial isolates

Some antibiotics were used to identify their effect on bacterial species isolated from various skin infections. It was found that there was a clear variance in the resistance where most of the isolates showed resistance to one or more of these antibiotics as shown in tables (2,3). The antibiotics Imipenem (IPM), amikacin (AK), Ciprofloxacin (CIP), and Gentamycin (GM) were used to test their effect on gram-negative bacteria while, Imipenem (IPM), amikacin (AK), Vancomycin (VA), Amoxicillin/clavulanic acid (AMC) were used on positive bacteria. The results of the current study showed that all isolates were resistant to the amoxicillin/clavulanic acid group by 100%. but all isolates were sensitive to Ciprofloxacin except *Acinetobacter baumannii*. *K. pneumoniae*, *E. coli*, and Staph. aureus, *Staph. lentus*, *Kocuria rosea* were sensitive to Imipenem, while others have shown resistance to it, such as *P. aeruginosa staph. epidermides*. Concerning Gentamycin antigen, *E. coli*, and *P. aeruginosa* isolates showed sensitivity to it, while *K. pneumonia* showed resistance to this antigen.

As for amikacin, some isolates showed resistance to it, such as *E. coli*, and *Staph. aureus*, while other isolates were sensitive to it. The cause of sensitive may be due to its lack of extensive use in clinical treatment due to its narrow therapeutic index, as low concentrations of it led to toxic effects. This reflected the group of cephalosporins, which are considered broad-spectrum antibiotics, as even in their high concentrations they do not lead to toxic effects (Roberts et al., 2012). On the other hand, all isolates were resistant to Vancomycin except *Staph. epidermides*. This indicates the development of bacterial resistance to this antibiotic as a result of excessive use. Long treatment regimens or inappropriate use of antibiotics can lead to bacteria gaining the ability to become drug-resistant. Vancomycin resistance occurs by altering the peptide ends of the peptidoglycan complex of the bacterial cell wall. This leads to a failure of Vancomycin binding and a lack of inhibition of the bacterial cell wall (Aldossary, 2021).

Acinetobacter baumannii was resistant to all types of antibiotics used in this study.

Table 2: Results of the susceptibility test for Gram-negative bacteria to antibiotics

Genus	IPM	AK	CIP	GM
K. pneumonia	S	I	S	R
E. coli	S	R	S	S
A. baumannii	R	R	R	R
P. aeruginosa	R	S	S	S

Table 3: Antibiotic susceptibility test results for Gram-positive bacteria

Genus	IPM	AMC	AK	VA
Staph. epidermidis	R	R	S	S
Staph. aureus	S	R	R	R
Staph. lentus	S	R	S	R
Kocuria rosea	S	R	S	R

Anti-bacterial activity of alumina nanoparticles

In this study, alumina nanoparticles were successfully synthesized by the biological method using *Ocimum basilicum* plant extract. Alumina nanoparticles showed antibacterial activity against microorganisms causing skin infections to various degrees as shown in Table 4. This is consistent with what was obtained (Sharma & Sharma, 2020; Manyasree et al., 2018; Ansari et al., 2015). The aqueous extract of the plant did not show any inhibitory activity against the microorganisms used in this study. The same is the case with aluminum nitrate solution. The antibacterial activity of alumina nanoparticles is attributed to their small size and large surface area. Whenever they are small, large numbers of them accumulate on the surface of cells, which leads to an increase in their toxicity against bacteria. Nanoparticles can alter the properties of the bacterial cell membrane, affect the permeability and respiration of bacterial cells, and they could damage DNA and release toxic ions leading to cell death (Auda et al., 2021; Yaqoob et al., 2020).

The mechanism by which alumina nanoparticles interact with bacterial cells is that the bacterial cell carries a negative charge while the nanoparticles carry a positive charge. This creates electromagnetic attraction between the bacteria and the surfaces of the nanoparticles. The nanoparticles release ions that interact with the thiol group (-SH) the proteins transporting nutrients that emerge from the bacterial cell membrane and reduce the membrane permeability leading to the death of the bacterial cell (Al-Janabi, 2020).

On the other hand, there are some factors that can influence the antimicrobial activity of Al_2O_3NPs including the type of phytochemical, size, shape, bacterial strain, conditions, and medium (Sharmin et al., 2021).

Table 4: Efficiency results of alumina nanoparticles prepared using plant extract (mm)

Bacterial Isolates	Plant Extract
K. pneumoniae	**25**
E. coli	**26**
A. baumannii	**22**
P. aeruginosa	**23**
Staph. epidermidis	**21**
Staph. lentus	**24**
Staph. aureas	**24**
Kocuria rosea	**22**

Figure (5): The inhibitory diameters of alumina nanoparticles

Conclusion

The nanoparticle biosynthesis method using plant extracts has confirmed its efficiency in the synthesis of alumina nanoparticles. The bio-prepared nano-alumina also showed anti-microbial effects against various types of bacteria. Thus, it can be used in the development of new antibiotics to treat various diseases. Alumina nanoparticles showed high efficacy against Acinetobacter baumannii which are resistant to all types of antibiotics used in the current study. It was observed that the antimicrobial activity of alumina nanoparticles prepared using *Ocimum basilicum* leaf extract was more effective than the corresponding antibiotics.

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