

# Green Synthesis, Characterization, and Applications of Aluminum Oxide Nanoparticles Using Aqueous Extract of Clove

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## Abstract

In this work,  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs were successfully biosynthesized, mediated aluminum nitrate nona hydrate Al(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O, sodium hydroxide, and aqueous clove extract in alkali media. The  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs were characterized by different techniques like Fourier transform infrared spectroscopy (FT-IR), UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy, transmission electron microscope (TEM), Energy-dispersive X-ray spectroscopy (EDX), and atomic force microscopy (AFM). The final results indicated the  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs nanoparticle size, bonds nature, element phase, crystallinity, morphology, surface image, particle analysis – threshold detection, and the topography parameter. The identified of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> bands were detected by the FT-IR spectroscopy. The UV-visible spectrum of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs exhibited an absorption band and (energy gap, Eg = 4.91 eV). It was found that the size range of nanoparticles was (28-37) nm and cubic with agglomerated particles. Antimicrobial activity study showed an excellent inhibition activity of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs against *Escherichia coli* negative (G-), *staphylococcus aureus*, positive (G+), and *Candida albicans* fungal. The effectiveness of the adsorption experiment was proven on binary metal ions, such as cobalt, nickel, and copper, by removing them from water using a nanostructured active surface of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs. The efficiencies removal of Co<sup>+2</sup>, Ni<sup>+2</sup>, and Cu<sup>+2</sup> ions were 93.22%, 87.49%, and 93.17% respectively.

**Keywords:** Adsorption, antimicrobial, clove,  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> NPs, green synthesis

## 1. INTRODUCTION

In recent years there are several articles have documented the use of the plant extract for the green synthesis of Al<sub>2</sub>O<sub>3</sub>NPs for its wide variety of applications, for example, in the field of ceramics, batteries, capacitors, chemical, biological, and defense sensors, as well as in the field of optoelectronics, in addition, to be involved in the manufacture of foodstuffs and textile industries<sup>1</sup>. Green synthesis has attracted significant attention in the research field, leading to the reduction of derivatives, pollution, prevention, and minimization of waste. Green synthesis, which utilizes plant extract to synthesize NPs metal oxide, is non-toxic, environmentally beneficial, and less expensive. It can be employed as a reducing agent during synthesis and has uses in industry, agriculture, medicine, and the environment<sup>2</sup>. In this paper, we used clove, which is a fragrant, dense

spice that is an aromatic flower bud of the tree also known as *Syzygium Aromaticum* and is a member of the *Myrtaceae* family order *Myrtales*, genus, Kingdom *Plantae*<sup>3</sup>.

This plant is full of antioxidants, including eugenol (phenolic components). The main chemical components of clove oil are phenolic hydroxyl, double bond (C=C), carboxylic acid, and the benzene ring. Also, clove leaves are components of non-phenolic compounds<sup>4</sup>. It is an anti-inflammatory, helping the body fight free radicals that damage cells. It also helps reduce the severity of various diseases such as (diabetes, heart, ulcers, and some types of cancer). In addition, it protects the liver and strengthens its functions. It also protects the stomach from developing ulcers. Scientific research has also proven that eugenol in cloves reduces liver fat or cirrhosis<sup>5</sup>.

Alumina  $\text{Al}_2\text{O}_3$  occurs in two forms: transition or metastable phases Alumina. The metastable phases of  $\text{Al}_2\text{O}_3$  were eta ( $\eta$ ), chi ( $\chi$ ), theta ( $\theta$ ), kappa ( $\kappa$ ), gamma ( $\gamma$ ), and delta ( $\delta$ ); these phases were related to the biosynthesis methods conditions, temperature, pH, weight et al.,  $\alpha$ - $\text{Al}_2\text{O}_3$  is alumina stable phase <sup>6</sup>. prepared  $\gamma$ - $\text{Al}_2\text{O}_3$  from ammonium alum by a recrystallization method <sup>7</sup>, used a novel eco-friendly method to obtain  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs which have high purity from waste aluminum., synthesized  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs by laser ablation of the aluminum target in ethanol with size ranging from 50 to 90 nm <sup>8</sup>. Synthesized  $[\text{NiO}]_{0.5} [\gamma\text{-Al}_2\text{O}_3]_{0.5}$  using the heat treating the spinel NPs at 800 °C to create spinel nanoparticles using liquid-feed flame spray pyrolysis, with an average particle size of 40–60 nm and adorned with Ni metal particles (~ 10 nm in diameter) scattered on the surface, with flowing 5/95  $\text{H}_2:\text{N}_2$  100ml /min in a fluidized bed reactor. The supposed activity of the substrates is activated by setting the tone of the developing stimuli <sup>9</sup>. Synthesized  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs by using the (sol-gel) technique, and mean diameter = (60 nm) with employing aluminum nitrate nonahydrate, triethanolamine (TEA,  $\text{NC}_2\text{H}_5\text{O}$ ), and citric acid <sup>10</sup>.

$\gamma$ - $\text{Al}_2\text{O}_3$  NPs have been synthesized by electrochemical using a rectangular aluminum plate as the anode and an aluminum plate as the cathode <sup>11</sup>, A practical and environmentally friendly method for preparing alumina nanoparticles was inserted using derivative leaves of *Calligonum comosum* L. The main goal of their study was to treat organic pollutants by evaluating their effectiveness through photocatalysis. The surface morphology analysis also showed that  $\text{Al}_2\text{O}_3$  NPs are crystalline and have crystalline arrangements. Crystalline form. The XRD study showed an average crystal size of 25.1nm, while the band gap value for  $\text{Al}_2\text{O}_3$  NPs was 2.86 eV. These particles have also been shown to be applicable in improving the mechanical performance of vehicles by enhancing the properties of compression and mechanical aerodynamics.

It is important to note that the biosynthesis of nanoparticles using plant extracts instead of conventional chemical methods leads to the formation of highly homogeneous and stable particles in size and shape <sup>12</sup>. The possibility of treating water pollution was highlighted by the research team of Ali and others, who conducted new studies and research linking the relationship between the absorption of cationic dyes and the effect of  $\text{Al}_2\text{O}_3$  NPs on them <sup>13</sup>.

Tween-80 and Formamide were used to synthesize gamma-alumina particles, which proved effective in increasing the absorption of methylene blue dye with an increase in the initial concentration from 50 to 400 mg/L and an increase in capacity from 490 to 2210 mg/L at specific conditions (time 10

minutes, pH = 9, and temperature = 60 °C) <sup>14</sup> used the bioreduction approach to synthesize  $\text{Al}_2\text{O}_3$  NPs in the green treatment by using the green synthesis of  $\text{Al}_2\text{O}_3$  NPs with a leaf extract (*prunus x Yedoensis*) (PYLE). The results showed a reduction of nitrate by (94%) and to enhance the catalytic activity of  $\text{Al}_2\text{O}_3$  NPs, the researchers proposed improving the size of the nanoparticles and the pH to achieve effective and vital medical drugs in a clean environment, so plant extracts were used, such as clove extract for the first time. To synthesize the  $\text{Al}_2\text{O}_3$  NPs which can be used to replace many commercial drugs on the market in addition it has shown effective antimicrobial activities.

The present paper highlights the current scenario and knowledge concerning the capability of clove extract for the eco-benevolent synthesis of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs with particle size (28-32) nm, and also provides optimization strategies and fresh perspective and their impact on the novel preparation of plant extract.

## 2. MATERIALS AND METHODS

### Materials

The materials used are aluminum nitrate nonahydrate  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (Merck - GER), sodium hydroxide from (chem alpha- India), EtOH (RBL-pain),  $\text{CoSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and Nickel sulfate heptahydrate ( $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$ ). All chemicals are employed without additional purification. Clove (*Syzygium aromaticum*) was taken from a nearby source local market in Baghdad, as shown in **Figure 1(a,b)**.

### Preparation of Clove Extract

Cloves must be prepared first by removing contaminants by rinsing them with water and then drying them at 37 °C. Add (20 g) of clove to (200 mL) of deionized water, then heat the mixture for (30 min) with continuous stirring at a temperature of 90 °C. The product will be reddish-purple. It will be cooled to room temperature and then filtered using (Whatman type filter paper), then separated in a tube with a capacity of (1.5 mL using a centrifuge at a speed of (4000 rpm/min) for (15 min) to obtain clove aqueous extract product.

### Preparation of $\gamma$ - $\text{Al}_2\text{O}_3$ -NPs

7.06 g of  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  is dissolved in deionized water 30 mL, then add to it an aqueous extract of cloves 100 ml; after that, a drop of 1 M NaOH was added to the mixture with stirring and heating until the value pH = 12, the mixture is left for (48 h) forming a pale green precipitate, then separated

in a centrifuge, washed (with hot deionized water), the operation is returned a second time with (hot ethanol) after that, dried the product in an electric oven at 300

°C for (10 h) to obtain a white powder of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs as depicted in **Figure 2**.



**Figure 1.** (a) Clove tree, (b) clove (*Syzygium aromaticum*).



**Figure 2.** The preparation steps of  $\gamma$ - Al<sub>2</sub>O<sub>3</sub>NP powder using clove extract and aluminum nitrate. Nona hydrate in alkaline media.

### Instrumentation techniques

Various approaches were used to synthesize and identify of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs. as below: A Balance electronic, Mod/As 220/C/1 kind (RADWAG). Centrifuge (4000–4500) rpm – Centrifuge, Kind PLC.FAITHFUL-Electric oven, model–25AB–kind WHL, (SCL FINETEDI–shaking water bath / kind SCL) at the lab of (Uno-Baghdad-College of edu-Ibn/Al- Hiatham) (Shimadzu-UV-Vis) /(160/UV) spectroscopy. (Shimadzu FT-IRKind -S8500 from (Uno Baghdad / College of Science). XRD/ kind (Holland–XRD-Phillips), from (Baghdad Center/ Lab). (SEM). Type (FE) (Ziess-HV 300 / GER) operating at 10 kV. (EDX). (TEM) 100Kv–Kind GER/ EM10C- 100Kv , at Kashan /Uno - Iran. (AFM) , Kind Liestal - AG nanosurf (Swiss) at (Uno -Baghdad / College of Science). The antimicrobial activity of the synthesized  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> NPs was examined with two bacterial strains, (G-), *Escherichia coli* (G+) *Staphylococcus aureus*, and (*Candida Albicans*) fungal (nutrient medium of jellose medium – kind Muller Hinton agar), in a disc using (diffusion method), and (Potato dextrose- PDA – in a nutrient medium) for antifungal activity nutrient. Adsorption

measurements were carried out by using (UV-Vis) spectrum type (160/UV) Shimadzu spectroscopy at (Uno - Al-Mustansiriya / College of Science).

### Antimicrobial Method

This study shows that two pathogenic bacterial strains are Gram-negative (-ev) and Gram-positive (+ev) bacteria, namely, *E. Coli* and *S. aureus*, and *Candida albicans* fungal. The antimicrobial activity of ammonium oxide nanoparticles was evaluated by the agar diffusion method, using sterile Petri dishes into which nutrient media and seeds of L.B were poured (Loria/Agar Bertani). These clinical isolates are placed in an incubator for a period of (24 h), Separately all these bacterial strains are spread on the agar medium, and a sterile cork drill is used to make a well or wells (6 mm) under sterilization conditions and with special care. In the presence of a Mueller Hinton agar nutrient medium, the disk diffusion method was used to treat *Candida albicans* fungi. As for measuring the effectiveness of the fungi, the same method of nutrient medium (agar) type (potato dextrose) PDA was used in various concentrations of nanoparticles (i.e., 2,1,0.5, and 0.25

mg/mL) applied to study and evaluate the effectiveness of ( $\gamma$ - $\text{Al}_2\text{O}_3$ NPs), where these particles are placed in sterile water and dispersed using special high-precision, sterile pipettes, then added to the wells, with a direct test, against pathogens. Then, the dishes are placed in an incubator at 37 °C (36 h). The inhibition zone for each well is then measured, and the results are recorded. The ultimate antimicrobial activity values were estimated in millimeters (mm).

### Adsorption Method

Varying selected metal ions solutions with a concentration of 0.00005 gm/mL were made by proper dilution of stock solution, which was agitated for half

an hour with pH = 6. This study concluded the effect of the concentrations of (M (II): Co, Ni, Cu) using different metal ions concentrations from 1 to 10 mg /L and 0.5 gm of adsorbent at contact time (1-30) min.

### 3. RESULTS AND DISCUSSION

An aqueous extract of clove was used with  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ . The changing color solutions are from brown at pH = 6 to peggy at pH = 9, and then to dark brown at pH =12 after adding heating drops of 1 M NaOH. The final product was a white powder of  $\gamma$ -aluminum oxide nanoparticles, as depicted in (Figures. 3a, b, c, and d).



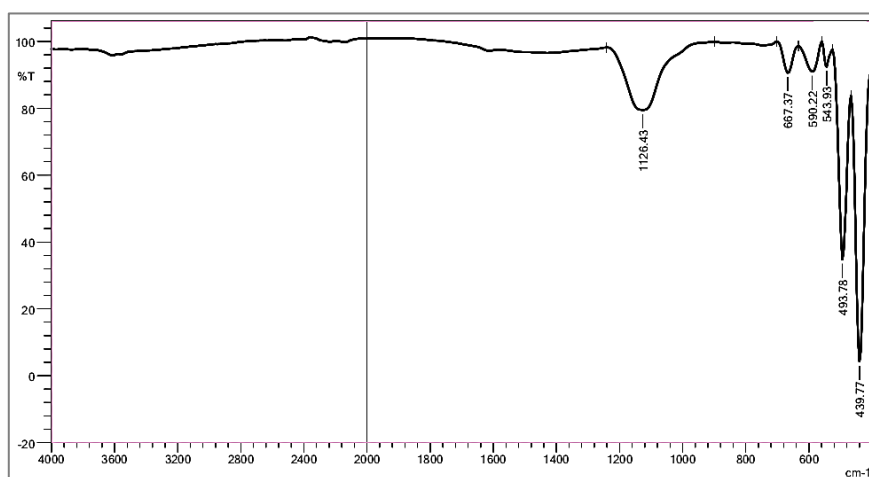
**Figure 3.** Color change of solutions in the alkaline media with  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and, bio-reductors of clove extract (a) brown, (b) Peggy, (c) dark brown, and (d) the final product of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs powder.

### Characterization

#### Fourier Transform Infrared (FTIR) of $\gamma$ - $\text{Al}_2\text{O}_3$ NPs

Typically, the FTIR spectrum of  $\gamma$ - $\text{Al}_2\text{O}_3$ NPs has multiple significant peaks indicative of distinct functional groups within the material. These peaks can reveal information about the structure and chemical composition. Two adsorption bands at (590 and 667 $\text{cm}^{-1}$ ) are among the most noticeable peaks in the

FTIR spectra of gamma alumina Nps and were attributed to the stretching vibration mode of Al-O. The peak at 3640  $\text{cm}^{-1}$  is due to the stretching vibration of OH. Another peek at 1126  $\text{cm}^{-1}$  is due to the  $\text{CH}_2$ , C-O bond of clove vibrations<sup>15</sup>. Peaks were identified at 543  $\text{cm}^{-1}$ , 493  $\text{cm}^{-1}$ , and 439  $\text{cm}^{-1}$ , which were ascribed to the Al-OH groups' bending vibration<sup>16</sup>, as depicted in **Figure 4**.



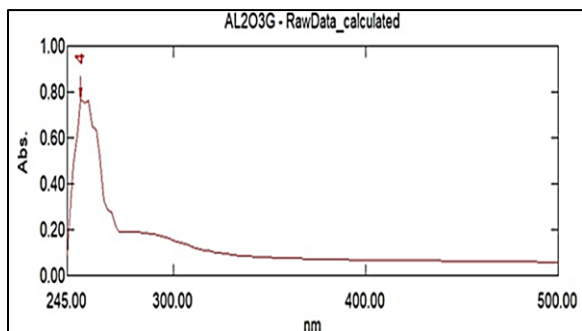
**Figure 4.** FT-IR of  $\gamma$  -  $\text{Al}_2\text{O}_3$ NPs powder from aqueous clove extract.

#### UV-Vis spectrum of $\gamma$ - $\text{Al}_2\text{O}_3$ NPs

It has been shown that UV-vis spectroscopy is a reasonably sensitive technique for verifying the synthesis of  $\text{Al}_2\text{O}_3$ -NPs (**Figure 5**). The synthesized

$\text{Al}_2\text{O}_3$ -NP aliquots were examined at different wavelengths between 245 and 500 nm and exposed to UV-visible analysis. According to the literature, the absorption position varies depending on the sources

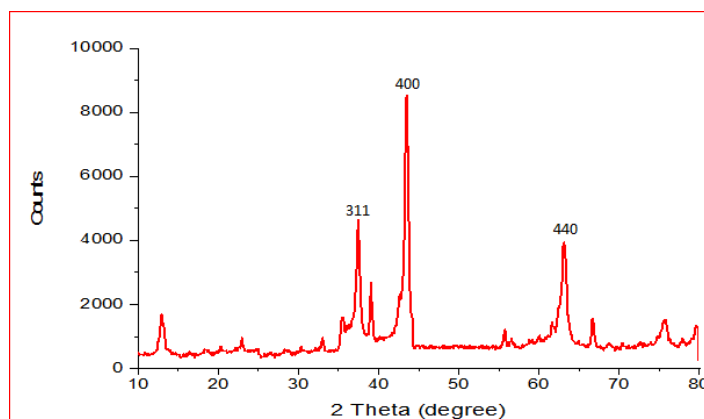
employed to synthesize Al<sub>2</sub>O<sub>3</sub>-NPs, such as the seed extract of Phoenix dactylifera (264 nm)<sup>17</sup>, the macroalgae Sargassum ilicifolium extract (227 nm)<sup>18</sup>, and Citrus aurantium L extract (322 nm)<sup>19</sup>. The UV-vis (Figure .5) showed absorbance peak (λ max) at 252. The energy band gap (E<sub>g</sub>) was calculated by 1239.83 / 252 = (4.91 eV)<sup>20</sup>.



**Figure 5.** U.V- vis spectrum of γ-Al<sub>2</sub>O<sub>3</sub> NPs from aqueous extract of clove.

### X-Ray Diffraction (XRD) of γ-Al<sub>2</sub>O<sub>3</sub> NPs

**Figure 6** illustrates the unique diffraction peaks for γ-Al<sub>2</sub>O<sub>3</sub> nanoparticles that are seen at



**Figure 6.** XRD of γ - Al<sub>2</sub>O<sub>3</sub> NPs powder from aqueous extract of clove.

**Table 1.** Represented XRD data of γ-Al<sub>2</sub>O<sub>3</sub> NPs

Pos. [° 2Th.]	hkl	FWHM [° 2Th.]	Particle size (nm)	Average particle size
37.4014	311	0.2952	29.69	35.04 nm
43.5194	400	0.3444	25.92	
63.0645	440	0.1968	49.49	

### Morphological analysis of γ-Al<sub>2</sub>O<sub>3</sub>NPs

The nanoparticles' size, shape, and surface characteristics are among the morphological details the SEM may reveal. The form and size of γ-Al<sub>2</sub>O<sub>3</sub>NPs may be seen by transmission electron microscopy (TEM) images, as depicted in Figures 7(a and b). Different sizes of Np formed ranged from (28 to 37 nm) with agglomerated cubic lattice<sup>24</sup>. The as-prepared nanoparticles' minimal agglomeration nature is evident from TEM investigation, which contributes to

particular 2θ values, indicating that the aluminum oxide nanoparticles are in the single-crystalline phase and belong to the cubic crystal lattice of the aluminum oxide phase, exhibit peaks at 37.70°, 43.51°, and 63.06°, which are referred to Miller indices (311), (400), and (440) as shown in **Table 1**. Even so, the existence of broad peaks and additional minors peaks indicates crystallinity and lower phase purity of γ-Al<sub>2</sub>O<sub>3</sub> NPs<sup>21</sup>, and also the absence of impurity peaks in the XRD spectra underscores the high purity of the γ- Al<sub>2</sub>O<sub>3</sub> nanoparticle sample. According to<sup>22</sup>, these findings were consistent with (JCPDS card No: 00-029-0063). Debye Scherrer's formula provided an estimate of the crystallite size of around 28.53 nm.  $D = K \lambda / \beta \cos \theta$ , K represents the Scherrer constant (0.9) where λ is the x-ray wavelength, β is full width at half-maximum (FWHM) of the reflections, and θ stands for the Bragg's angle in radian<sup>23</sup>. The average particle size is 35.04 nm, depicted in the XRD data of γ - Al<sub>2</sub>O<sub>3</sub> NPs. According to these results, X-ray diffraction was used to determine the crystallite size of Al<sub>2</sub>O<sub>3</sub> nanoparticles. The XRD investigation verifies that gamma alumina is present.

their increased surface area and, as a result, their increased antimicrobial activity. One can ascertain the qualities of the material by examining the size and distribution of these particles.

### Energy Dispersive X-Ray of γ -Al<sub>2</sub>O<sub>3</sub>NPs

The EDX results for γ -Al<sub>2</sub>O<sub>3</sub>NPs are illustrated in **Figure 8**. It was shown that facilitated the γ -Al<sub>2</sub>O<sub>3</sub>NPs sample elemental composition to be

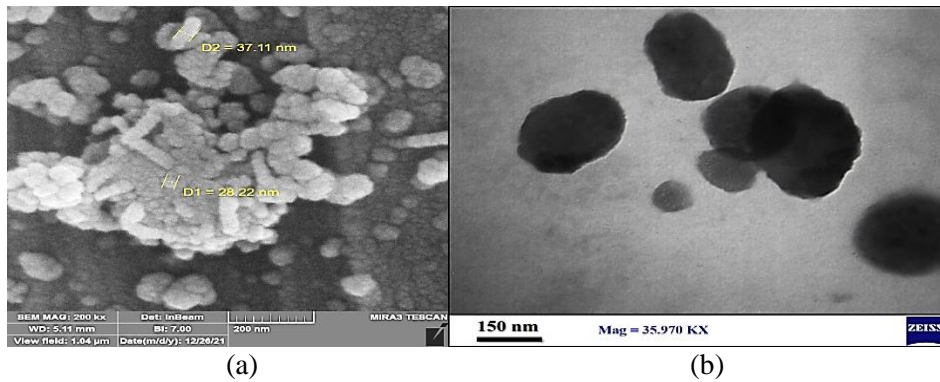


identified. It was found to consist of A% (35.83% Al and 64.17 % O), confirming the Al<sub>2</sub>O<sub>3</sub>-NPs structure, that means both Al and O in identical proportions, which indicates that it is chemically pure and compatible with (XRD) <sup>25</sup>.

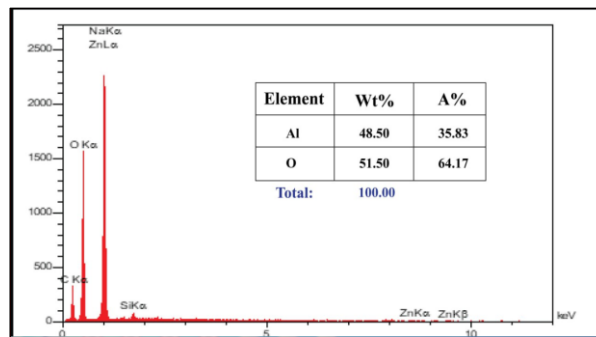
**Atomic Force Microscope of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs**

AFM technique is used to investigate the dispersion and aggregation of NPs with size, shape, and sorption too <sup>26</sup>. **Figure 9** (a,b) and **Table 2**,

showed particle (analysis, image ), with threshold detection, and information of individual results with global statistics of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs powder. The histogram and statistical particle analysis of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs are shown in **Figure 10** <sup>27</sup>. The particle analysis's length, height, and width are shown in **Figures 11(a,b)**. **Figures 12 (a,b) and 13 (a, b)** show other particle analysis and topography parameters. The 3D AFM surface structure image of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs powder is depicted in **Figure 14** <sup>28</sup>.



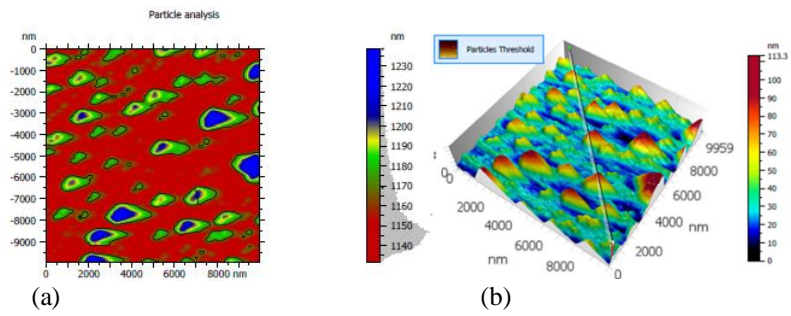
**Figure 7.** (a) Images of SEM and (b) TEM of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> NPs powder.



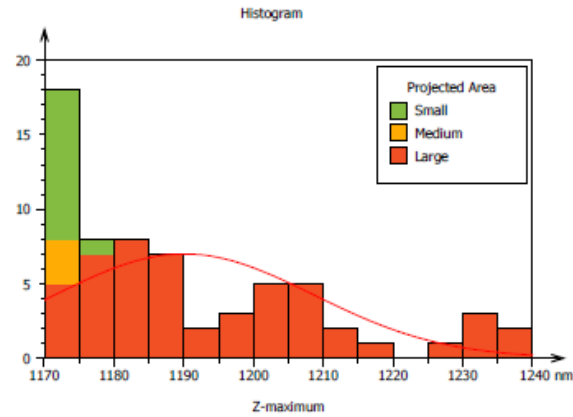
**Figure 8.** EDX of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> NPs powder from aqueous extract of clove.

**Table 2.** AFM information and individual results with global statistics of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs powder.

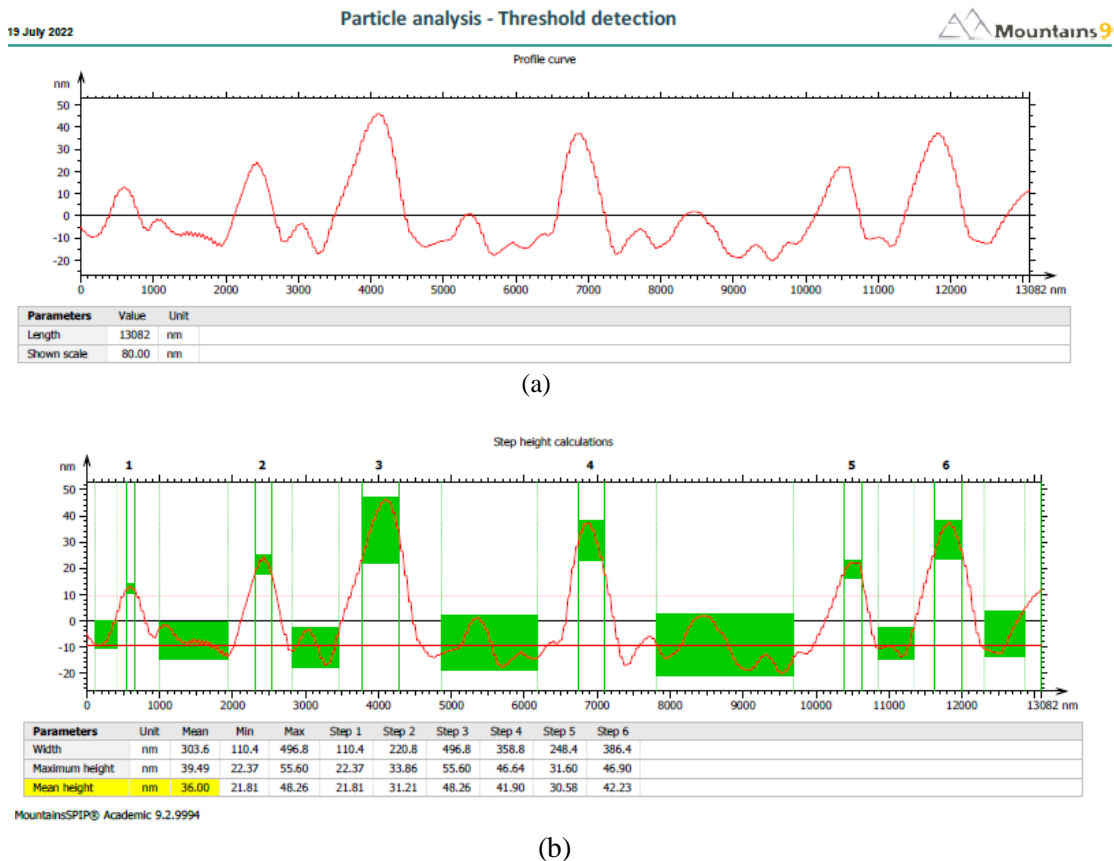
Information				
Method 1		Threshold detection		
Threshold 1		1169 nm		
Number of particles		65		
Coverage		21.54 %		
Density		655348 particles / mm <sup>2</sup>		
Individual results				
Parameters	Project Area	Project Area	Mean diameter	Z-maxim
Unit		Nm <sup>2</sup>	nm	nm
Particle N1	small	1525	22.22	1170
Particle N1	small	429.0	16.80	1170
Particle N1	small	5005	65.76	1171
Particle N1	small	3265	54.65	1171
Particle N1	small	3265	54.65	1171
Particle N1	small	4886	67.08	1171
Global Statistics				
Mean		331323	486.2	1190
Min		429.0	16.80	1170
Max		162254	1361	1239



**Figure 9.** (a) AFM particle analysis and threshold detection of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder, (b) Image of particle threshold of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder



**Figure 10.** AFM histogram and statistical particle analysis of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder.



**Figure 11.** (a) AFM curve of particle analysis length of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder, (b) AFM height and width calculations parameters of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder.

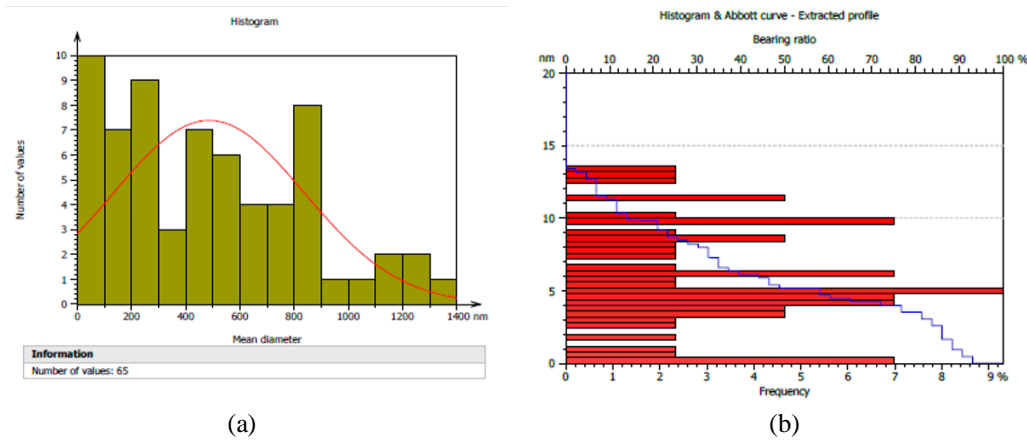


Figure 12. AFM (a) histogram Number of values against mean diameter, (b) Abbott curve – extracted profile explains the bearing ratio against frequency of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder.

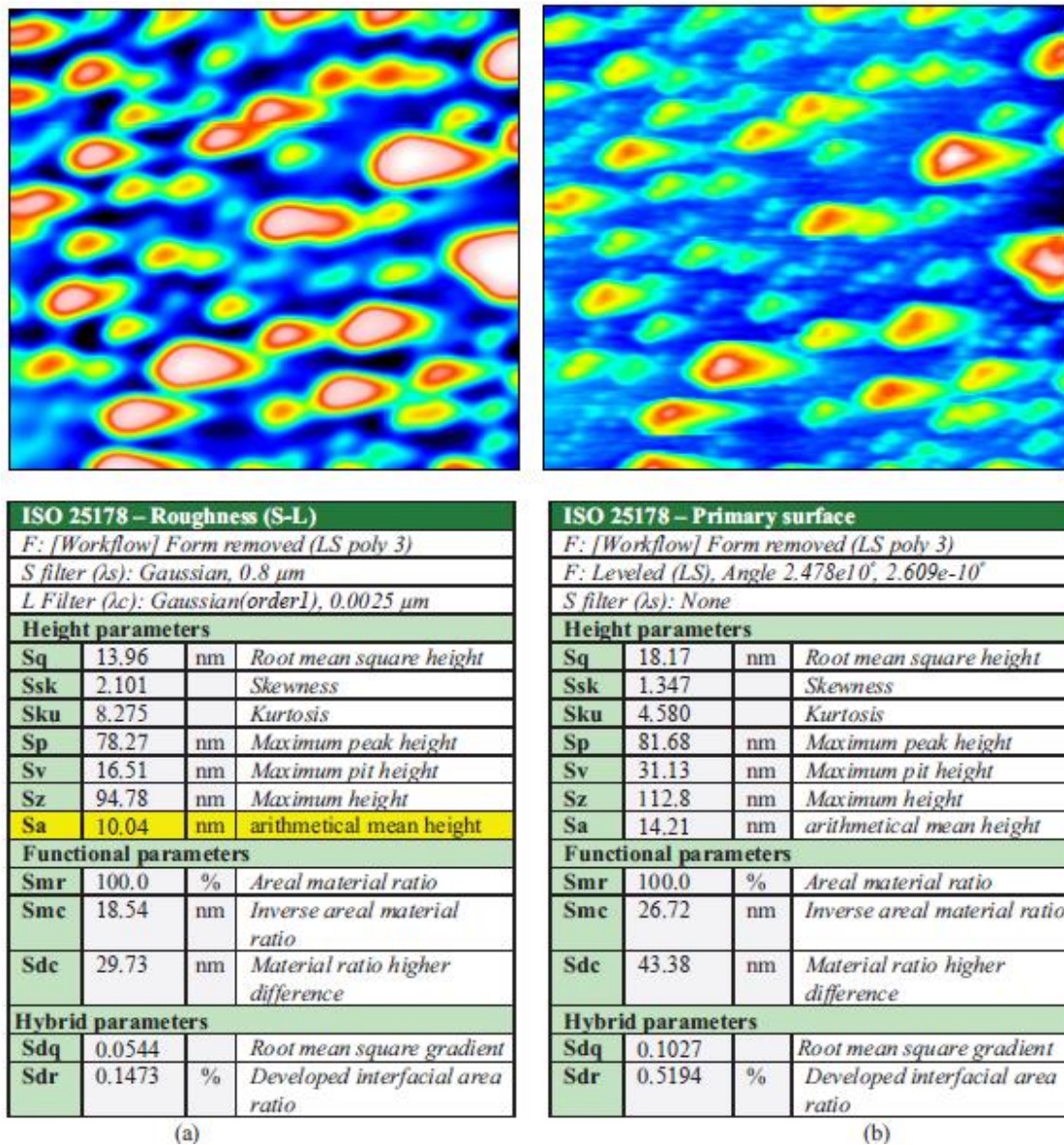
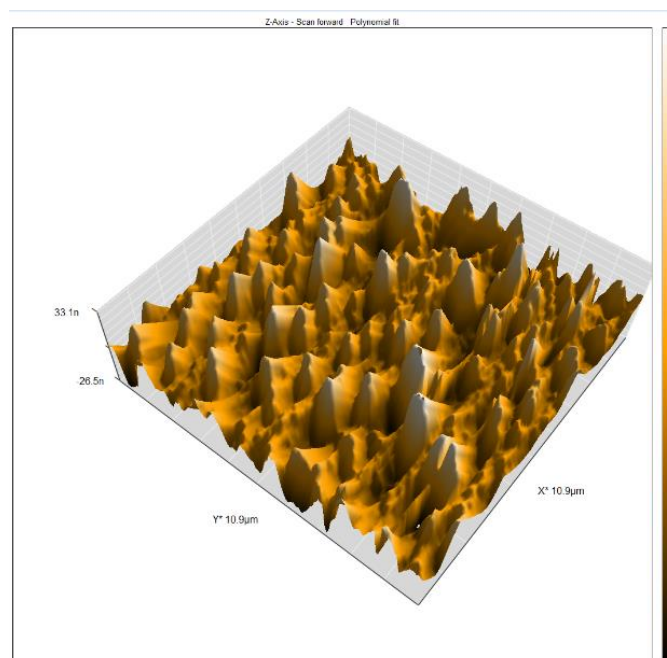


Figure 13. AFM images: (a) Roughness (S-L), (b) Primary surface and their topography parameters of  $\gamma$ -  $\text{Al}_2\text{O}_3$ NPs powder.





**Figure 14.** 3D AFM surface structure image of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs powder.

### Antimicrobial studies

This study used the agar diffusion method to evaluate the antimicrobial activity against *E. coli*, *S. aureus*, *E. coli* bacteria, and *Candida albicans* fungi. The results showed that using (diluted hydrochloric acid) or a negative control without using ( $\gamma$ - $\text{Al}_2\text{O}_3$  NPs) did not show any zone of inhibition. Notably, the antimicrobial *E. Coli* is substantially more prevalent, as depicted in the data below, followed by *C. albicans*, *S. aureus*, etc. The antimicrobial activity against *C. albicans* is found to range between (20–25) mm; this finding is nearly in agreement with other studies that have been reported<sup>29</sup>. In contrast, the inhibition activity against *E. Coli* and *S. aureus* was found to range between (20–31) mm and (15–25) mm at concentrations of (2, 1, 0.5, and 0.25). On the other hand, the presence of the control alone does not give any effectiveness against bacteria when the leading of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs were (2, 1, 0.5, and 0.25) mg /ml.

It was found that at all these concentrations, of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs caused a growth delay of all bacteria and *Candida albicans* fungal as shown in **Table 3**<sup>30</sup>. It showed higher antimicrobial activity at 2 mg /ml for *E. coli*, while the higher effect in *Candida albicans* activity occurred at 0.25 mg/mL. The statistical diameter inhibition zones of B samples of  $\gamma$ - $\text{Al}_2\text{O}_3$  and the inhibition diameter zones of the antimicrobial of sample (B) in different concentrations are depicted in **Figure 15 (a and b)**<sup>31</sup>. The main mechanisms of realization of the bacteriostatic effect of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs are the electrostatic interaction of these nanoparticles

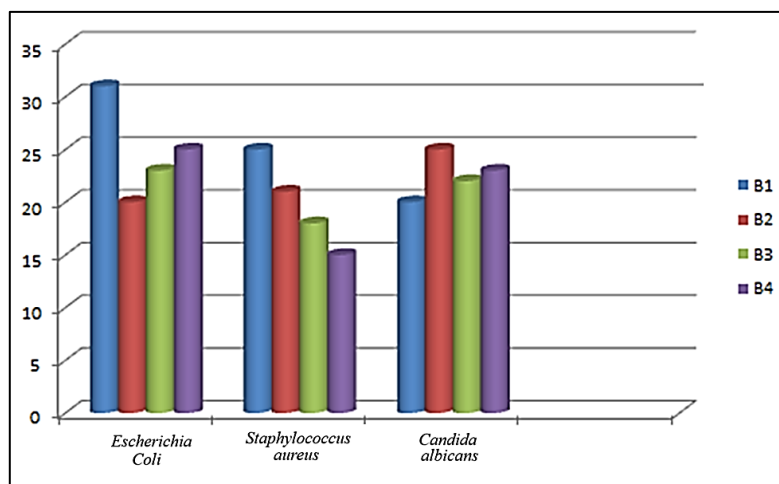
with the bacterial outer membrane/cell wall and the formation of aluminum cations initiating the reactive oxygen species (ROS) generation and oxidizing biopolymers.

The direct interaction of  $\text{Al}_2\text{O}_3$  NPs nanoparticles with the exterior membrane surface may be one of the causes of the damage. Therefore, more research is required to validate these findings. Also, the relationship between oxide nanoparticles and the bacterial cell wall and the potential for NP penetration into the bacterial cells remains unclear.  $\text{Al}_2\text{O}_3$  NPs neutralize the bacterial surface charge and form big aggregates because they are considerably smaller than the bacterial cells and coat them with electrostatic attractive forces. Large NP-bacterial complex aggregates would swiftly settle in the suspension.

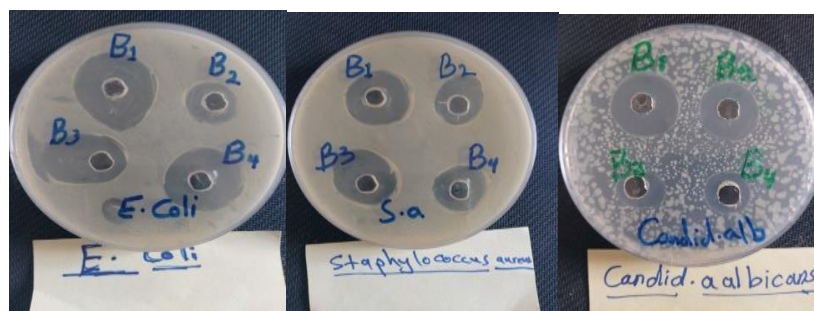
It suggests a phenomenon that is depending on particle size. While it would be challenging to attract bulk particles, negatively charged bacterial cells (a few micrometers in size) may easily trap NPs through electrostatic attractive forces. In light of the results obtained, we can conclude that the highest effect against bacteria is achieved by  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs formed from the green synthesis, as depicted in Figure 16 below, where contact between bacteria and nanoparticles results in toxicity to the bacteria and the destruction of diseased cells. It is due to the high antimicrobial activity that has been reported. Finally,  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs can be used as a powerful antimicrobial agent and treat a wide range of diseases affecting humans.

**Table 3.** Antimicrobial activity of the  $\gamma$  -  $\text{Al}_2\text{O}_3$ NPs at different concentrations of (B) samples against three pathogenic microbes and their diameter inhibition zoon in (mm).

Samples	B1	B2	B3	B4
Concentrations	2	1	0.5	0.25
	(mg/mL)	(mg/mL)	(mg/mL)	(mg/mL)
Microorganisms	Inhibition area diameter in mm for sample (B) $\gamma$ - $\text{Al}_2\text{O}_3$ NPs			
<i>Escherichia Coli</i>	31 mm	20 mm	23 mm	25 mm
<i>Staphylococcus aureus</i>	25 mm	21 mm	18 mm	15 mm
<i>Candida albicans</i>	20 mm	25 mm	22 mm	23 mm



(a)



(b)

**Figure 15.** (a) Statistical diameter inhibition zones in mm of B samples of  $\gamma$ - $\text{Al}_2\text{O}_3$  NPs, (b) Antimicrobial activity expressed the inhibition of diameter zones of B samples of  $\gamma$ - $\text{Al}_2\text{O}_3$ NPs at different concentrations against three pathogenic microbes.

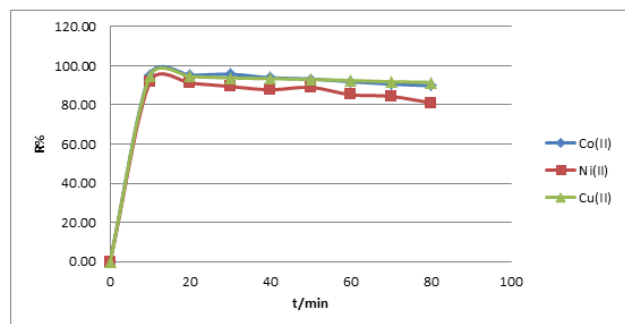
### Adsorption study

The aim of the biosynthesized of  $\gamma$ - $\text{Al}_2\text{O}_3$ NPs to generates a good adsorbent for the removal of different divalent metal ions (Cobalt, Nickel, and Copper) from a solution of distilled water was depicted in **Figure 16**<sup>32</sup>. The percentage of cobalt ions removed decreased for  $\gamma$ - $\text{Al}_2\text{O}_3$ NPs from 95.49 after 10 min to 89.84 after 80 min<sup>33</sup>. In the case of nickel ion removal also decreased from 91.92 after 10 min to 81.08 after 80 min, and the same thing happened from 94.85 to 91.52 for the copper metal ion<sup>34</sup>. The only explanation for that is ( $\gamma$ - $\text{Al}_2\text{O}_3$ NPs) contain many effective and active sites that prepare for adsorption. When the adsorption process began, these sites were effective. But as time passed, these sites

gradually saturated. On this basis, the percentage of displacement efficiency of binary metal ions for each (cobalt, nickel, and copper) is measured was 93.22 %, 87.49 %, and 93.17 %, respectively<sup>35</sup>.

The adsorption of Co (II), Ni (II), and Cu (II) ions on the surface of  $\gamma$ - $\text{Al}_2\text{O}_3$ NPs is based on the interaction of the magnetic attraction between the positive charged of (Co, Ni, and Cu) metal ions and the negatively charged of  $\gamma$ - $\text{Al}_2\text{O}_3$ NPs by electrostatic forces. Once adsorbed on the surface, the Co (II), Ni (II), and Cu (II) ions can form chemical bonds with surface functional groups such as hydroxyl (-OH) and carboxylate (-COO-) groups<sup>23</sup>. Therefore, heavy ions can be captured from wastewater quickly, so when there is a need to remove pollutants quickly, the

increased adsorption efficiency is of special value and importance, as is the case in conditions where pollutants are in high concentration, so improving and designing systems that have applications such as removing heavy metal ions, wastewater with water purification and environmental treatment depend on such results. Much research deals with improving the practical methods of adsorption techniques and making them more active and effective<sup>36,37</sup>.



**Figure 16.** Show the adsorption efficiency curve of some metal ions [Co(II), Ni(II), and Cu (II)].

#### 4. CONCLUSION

$\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs were obtained from the green synthesized method using an aqueous clove extract. FT-IR exhibited bands at (590 and 667) cm<sup>-1</sup>, which is attributed to the stretching mode of Al-O. The bands at the range (543-439 cm<sup>-1</sup>) were due to Al-O, the bending vibration of Al-OH groups. The XRD and the morphology study by SEM & TEM indicate that the aluminum oxide nanoparticles are in the single-crystalline phase and belong to the cubic crystal lattice of the aluminum oxide phase. The structure matched the reference pattern (JCPDS card No:00-029-0063), and the morphology and the size of synthesized  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs formed particles have different sizes ranging from 28 nm to 37 nm with cubic agglomerated particles. AFM showed particle analysis-threshold detection and the topography parameter.

The measurement of the energy band gap (Eg) mediated UV-vis spectroscopy, which is about 1239.83 / 252 = 4.91eV. EDX revealed the existence of Al and O. It was found that  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> was chemically pure. The antimicrobial activity of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs was evaluated against *staphylococcus auras*, *E. coli*, and *Candida albicans* fungal when the leading of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs were (2, 1, 0.5, and 0.25) mg/ml, it was found that at the higher concentration, the nanoparticles  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> caused increasing of the inhibition area diameter of the sample (B) of all bacteria and at the lower concentration gave the same effect on the *Candida albicans* fungal. Adsorption studies show a decrease in the percentage of bioabsorption. The aluminum oxide nanoparticles' active sites are first effective in adsorption; as time goes on, these sites gradually fill and become

saturated, decreasing the percentage of adsorbed ions. Co (II), Ni (II), and Cu (II) ions can form chemical bonds with surface functional groups such as hydroxyl (-OH) and carboxylate (-COO-) groups.

The outcome of this work is that the aqueous plant extract clove and monitoring of reaction conditions, temperature, and pH play an important role in enhancing the properties and applications of  $\gamma$ -Al<sub>2</sub>O<sub>3</sub>NPs. The particle size is a crucial factor in the inhibition activity of bacteria. Also, it contributes to the development of adsorption and increasing their efficiency by removing different kinds of metal ions. That will open the future to researchers for many studies and applications.

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