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Published Online First: March, 2023

Baghdad Science Journal
2023, 20(5 Suppl.): 1840-1857

P-ISSN: 2078-8665
E-ISSN: 2411-7986

DOI: https://dx.doi.org/10.21123/bsj.2023.7526

Effect of Green-biosynthesis Aluminum Nanoparticles (Al NPs) on *Salmonella enterica* Isolated from Baghdad City

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Received 9/6/2022, Revised 3/10/2022, Accepted 4/10/2022, Published Online First 20/3/2023, Published 28/10/2023

Abstract:

This study is aimed to Green-synthesize and characterize Al NPs from Clove (*Syzygium aromaticum* L.) buds plant extract and to investigate their effect on isolated and characterized *Salmonella enterica* growth. *S. aromaticum* buds aqueous extract was prepared from local market clove, then mixed with Aluminum nitrate $Al(NO_3)_3$. 9 H_2O, 99.9% in ¼ ratio for green-synthesizing of Al NPs. Color change was a primary confirmation of Al NPs biosynthesis. The biosynthesized nanoparticles were identified and characterized by AFM, SEM, EDX and UV–Visible spectrophotometer. AFM data recorded 122nm particles size and the surface roughness RMs) of the pure *S. aromaticum* buds aqueous extract recorded 17.5nm particles size, while the results of Al NPs in the tested sample recorded 21nm particles size with surface roughness RMs about 2.35nm. SEM images revealed the presence of Al NPs with diameters ranged from 33.5-70.4nm with regular spiracles shape particles in the prepared biosynthesized nanoparticle sample. The EDX spectrum analysis showed that the Aluminium weight ratio was 1.75, while it was 50.498 in the Al NPs sample prepared from aqueous extract. UV-Visible spectroscopy data revealed that biosynthesized Al NPs were absorbed at 213nm while Aluminum nitrate was absorbed at 258nm. These results indicate the formation of Al NPs. The antibacterial activity showed that Al NPs exhibited having high antibacterial activity on *Salmonella* spp. isolates compared to the effect of the control agent (imipenem) in this experiment. We conclude that biosynthesized Al NPs from clove aqueous extract can be exploited as natural antibacterial compounds to inhibit the growth of *Salmonella*.

Keywords: Al NPs, Biosynthesized Al NPs, Antimicrobial effect of Al NPs, Foodborne disease, Salmonellosis.

Introduction:

Nanotechnology is a modern field of research. Richard Feynman who is an American physicist, Nobel Prize laureate, presented the nanotechnology concept in 1959 in his famous lecture “There's Plenty of Room at the Bottom”, which caused various revolutionary expansions in nanotechnology field. Nanoscience development is traced to the time of the Greeks and Democritus B.C. in the 5th century, when scientists regarded the question of whether the matter is constant and therefore infinitely divisible into tinier pieces, or comprised of small, indivisible and indestructible particles, which experts now call atoms [1]. All science fields are involved in nanotechnology, including physics, materials science, chemistry, biology, computer science and engineering. Recently, these nanotechnologies have been employed in human health with hopeful results, notably in the scope of cancer treatment. [2] Nano-particles (NPs) can be synthesized from various materials, metal oxide ceramics such as; titanium, zinc, aluminum and iron oxides are commonly used, as well as gold and silver metal, which are other forms of NPs widely used. These chemical methods, such as; chemical reduction, electrochemical techniques and photochemical reduction, suffer from different limitations such as; low yield, cost ineffectiveness, toxicity, instability manifold and harmful effects on health and the environment [3-5]. Therefore, there is an increasing necessity to generate nontoxic, high-yielding, environment-friendly and biocompatible methods for NPs synthesis. In sense of this, biological procedures (green synthesis) have excited a great appointment of interest. [3]. Nanomaterials are categorized into one dimension (1D) nanomaterials, two-dimensional (2D) nanomaterials and three Dimension (3D) nanomaterials. For the latest 10 years, 3D nanomaterials have shown attention in research and medical science. These nanomaterials applications are widely involved in the field of catalysis, batteries, magnetic materials carrier of reactant and product. An example of 3D NPs includes Fullerenes, Dendrimers and Quantum dots [6]. Methods used for manufacturing nanomaterials can be ordinarily classified into two groups: top-down method, a destructive approach is applied, by breaking down large molecules and decomposed them into a Nano-size unit. The top-down method has been employed for creating micron-sized products [7] by slicing or continuously cutting off the large molecules to produce Nano-sized particles as well as construct their structure with fitting/appropriate properties. This technique is beneficial to generate Nano-sized particles at a broad scale by utilizing mechanical force, while in the bottom-up methods which is the opposite direction of a top-down approach, the nanomaterial, such as; Nano-coating is realized beginning from the atomic or molecular precursors and gradually collected until the aspired structure is built [8]. Self-assembly is a bottom-up method, in which atoms/ molecules regulate themselves into aligned nanostructures through chemical-physical interactions that occur between them. These single atoms, molecules, or clusters are positioned freely one-by-one, only via the positional assembly technique [9]. The main advantages of this bottom-up approach repose in the synthesis of nanostructures with few defects, the chemical composition more homogeneous, better control of shape and size [10]. Green biosynthesis of NPs-based plant extract has been observed that plant extract products and microorganisms such as yeast, bacteria, actinomycetes, fungi, algae, can be used to synthesize NPs. The green NPs are manufactured using biomass filtrate from these distinct biological systems. Plant nanotechnology has several advantages, including scalability, biocompatibility and the capacity to synthesize NPs using universal solvents (water) as reducing agents [11]. Toxic or dangerous ingredients are not required in the process of synthesizing NPs with phytonanotechnology since it uses plants to synthesize NPs at mild pH, pressure and temperature at a significantly low cost, eco-friendly, simple as well as avoids the addition of external reducing, capping and stabilizing agents [12]. To decrease and stabilize, a variety of plants can be utilized. Many researchers have been using a different section of a plant-like; leaf, stem, root and fruit to synthesize metal/ MO NPs [11]. Plants produce NPs by reducing the metal salt into NPs through the main compounds that affect the reduction and the NPs capping are biomolecules such as; phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids and alcoholic compounds. However, quinol and chlorophyll pigments, linalool, methyl chavicol, eugenol, caffeine, theophylline, ascorbic acid and many other vitamins were reported [13]. The non-toxic phytochemicals containing the aforementioned flavonoids and phenols have novel chemical power to reduce and also completely wrap NPs, thus preventing their agglomeration. Phenolic compounds hold hydroxyl and carboxyl groups, which are capable to bind with metals [13]. Environment friendly manufacturing of NPs by using diverse biological entities can help to mitigate the harmful impacts of physical and chemical processes [12]. *Syzygium* (S.) *aromaticum*, is a dried flower bud from the *Myrtaceae* family, its leaves and buds (the tree commercial part) begin to bloom four years after planting. Afterward, they are harvested either manually or with the aid of a natural phytohormone during the pre-flowering period. This plant is a rich source of phenolic compounds like eugenol, eugenol acetate and Gallic acid and it has a lot of potential for medicinal products, cosmetic, food and agricultural uses [14, 15]. Plant sources are emerging as a preferred choice as it avoids the pathogenic and time-consumes procedures in laborious for maintenance of cell cultures and offers fast synthesis. With the plant sources, cloves, or *S. aromaticum* as it's generally known, are readily available year-round and have strong reducing properties, as well as quick synthesis times of only min [3]. Furthermore, to its widespread use as a food flavor enhancer, clove oil has been used as a topical pain reliever in dentistry for ancient times [16]. Al_2O_3 is a chemical formula of Aluminum oxide or alumina, which is composed of aluminum plus oxygen and characterized with an electronic insulator or having high thermal conductivity, as well as unique chemical and physical properties such as high hardness, high insulation, high transparency and as a result it can be employed in many applications [17]. Bacterial growth effected by Al NPs, several studies reported that normal biological, chemical and nutrient cycles of bacteria communities were being attracted by nanoparticles [18]. Metal NPs antibacterial action can be explained by three distinct mechanisms: First, the cell walls and cell membrane damaging; second or after cell well penetration, the intracellular microbial components are damaged; and finally, the oxidative stress process is triggered. Metallic NPs penetrate into the cell wall leading to irreversible DNA and protein damage, depending on

Open Access
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Baghdad Science Journal
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P-ISSN: 2078-8665
E-ISSN: 2411-7986

the degree of the cell wall damage. The interaction occurring in the bacterial cell between the NPs and DNA causes the DNA to be converted from a normal state to a condensed state and therefore, the ability of DNA replication is lost. Moreover, a thiol group contained within cysteine amino acid reacts with NPs to inactivate enzymes [19]. *Salmonella enterica* spp. is the second most common cause of food poisoning and a leading cause of diarrheal complications around the world. Contaminated food products such as poultry, eggs, vegetables, nuts, contaminated drinking water, ruminants, as well as direct contact with infected animals, are the most common sources of *Salmonella* infections [20]. The research estimated that about 93.8 million infections and 155,000 fatalities which occur globally each year are caused by *Salmonella* infection [21]. In Iraq, poultry and egg production has increased in recent years, and rudimentary slaughterhouses have sprouted in places with no hygienic conditions, potentially causing a *Salmonella enterica* poisoning problem. According to the Iraqi (COSQC, number 2270), the percentage of *Salmonella* development in chicken cuts (thighs, breast, wings) should be zero. The goals of this study is to Green-synthesize and characterize Al NPs from Clove (*Syzygium aromaticum* L.) buds plant extract and to examine their effect on isolated and characterized *Salmonella enterica* growth.

Materials and Method:

1. Collection and identification of the plant materials.

Buds of the Clove *Syzygium aromaticum* L. plant were collected from the local markets in Baghdad. The plant was identified as *S. aromaticum* at the Dept. of Biology, College of Science, University of Baghdad, Iraq. *S. aromaticum* buds were washed then cleanly chopped into small pieces, air-dried for three days at room temperature and in the shade. Then they were pulverized with an electric blender till obtaining 250g fine powder.

2. Preparing of *S. aromaticum* aqueous extract.

Sterilized ddH_2O was used to prepare *S. aromaticum* extract, then chopped and dried, to get powdered clove (*Syzygium aromaticum* L.). Fifty grams were taken and macerated with 250ml of sterilized ddH_2O for 24hrs at room temperature using hot plate magnetic stirrer (Gallenkamp/ Japan). The maceration liquid product was filtered using Whatman No.1 filter paper. The macerated and filtrated liquid was collected and dried using a rotary evaporator (Daihan/ Korea) [22].

3. Biosynthesis (Green synthesis) of Al NPs

In order to prepare stock mentioned in table 1 of plant aqueous extract for green synthesis of NPs, each 1g of dried plant extract was dissolved in 1ml sterilized ddH_2O. Aluminum nitrate $Al(NO_3)_3 \cdot 9H_2O$, Sigma-Aldrich/ American) was 99.9% concentration pH=3.5 mixed with plant extract pH=5.7 in 1:4 ratios, then stirred on a hot plate magnetic stirrer (Gallenkamp/ japan) for 6hrs at 45°C, the mixture pH was 3.7 that adjusted to pH=5.7 through adding drops of NaOH, irradiated in the microwave (Built-in Grill Microwave, 22L/ American) for 5min. It was centrifuged (Daihan/ Korean) at 2500rpm twice to discard the supernatant and collected the pellets to wash with sterilized ddH_2O for 20min two times. The collected sample was dissolved in sterilized ddH_2O and treated with ultrasonic vibration (KMD-28100 Ultrasonic vibration in 50W power/ Russia) for 5min to reduce the agglomeration [23] to get stock concentration of Al NPs mentioned in table 1. In general, the plant-based green synthesis of NPs is carried out by mixing the plant biomass (extract) with the metal salt solution at the optimum conditions Temperature and pH. The primary indicator for the NPs synthesis was by looking at the changes in the color of the solution. The experimental procedure for the green synthesis of NPs using plant extracts is shown in Fig 1.

Table 1. Preparation of plant aqueous extract and Al NPs concentration mg/ml and µg/ml

Concentrations no.	mg/ml	µg/ml
1	0.0005	0.5
2	0.0015	1.5
3	0.003	3.0

Figure 1. The experimental procedure for the green-synthesis of NPs using plant extracts [24].

4. Characterization of the plant-based green synthesized Al NPs

The plant-based green synthesized Al NPs characterization was carried out using the following techniques. All the examinations were carried out in the Dept. of Physics and Dept. of Chemistry laboratories/ College of Science/ Al-Nahrain

University and the Dept. of Chemistry laboratory/ College of Science/ Baghdad University.

Atomic Force Microscope AFM.

Atomic Force Microscope analysis was used to determine the surface morphology, diameters and size of the biosynthesized Al NPs using scanning prop microscopy Angstrom advanced AA2000, USA. The AFM pattern used was in contact under normal atmospheric conditions, a small drop of solution samples was placed on a 1x1cm glass slide, and allowed to dry at room temperature to be ready for examination [25].

Scanning Electron Microscopic SEM

Scanning electron microscope (type Japan Meiji) was used to determine the shape and size of plant-based green synthesized Al NPs according to Selvi and Sivakumar [26], by placing approximately 5 microliters of solutions ready for examination on an electron microscope holder consisting of a gold and carbon clip, then examination the sample using different magnification powers.

UV-Visible Nanoparticle Analysis.

UV–Visible spectrophotometer (UV-1700 Shimadzu) from 200nm to 1100nm was used to analyze the surface plasmon resonance, sterile deionized water was used as blank. UV-Vis spectrophotometer was used to measures the extinction (scatter+absorption) of the light that passed through the prepared sample.

Energy-dispersive X-ray analysis EDX

Energy-dispersive X-ray analysis EDX (Bruker Nano GmbH, Germany) with an acceleration voltage of 20kV was used to determine the purity of plant-based green synthesized Al NPs.

5. Bacterial isolates

Bacteria *Salmonella enterica* isolates were isolated and identified previously at Biotechnology Research Center - Al-Nahrain University.

6. Investigation the Antibacterial Activity of Biosynthesized NPs

Disk diffusion method as described by Hudzicki [27] was chosen to determine the antibacterial effect of green synthesized Al NPs and plant extract by using a different prepared concentration of each one 0.5, 1.5, and 3.0μg/ml[28] against *Salmonella* isolates. Firstly, 3-5 isolated pure colonies were picked by sterilizing wire loop to be inoculated into 5 ml of nutrient broth and incubated at 37°C for 18 h. The culture turbidity was compared with standard 0.5 McFarland turbidity to get an equivalent suspension. On Mueller Hinton agar inoculated last prepared suspension by streaking the plate using a sterile cotton swab, twist it four times to ensure that it is fully inoculated and the plate was left for 3-5 min at room temperature to ensure absorbed the excess moisture. After that, eight microliters from each concentration of plant extract and NPs solution were disposed on sterilized blank disk filter paper (Whatman No.1/ Germany) that was sterilized by autoclave (Karl kolb/ Germany) at 15lbs/in2 pressure, 121°C for 15 min. Using sterile forceps, the disks were applied on MH surface and Imipenem 10μg/ml was carried out in each dish with each concentration of Al NPs and plant extract, then incubated also at 37ºC for 18hrs. Imipenem is a wide-spectrum antibiotic that was used as a control antimicrobial drug to compare its effect with the effect of our treatment prepared (synthesized Al NPs and plant extract). Eventually, after incubation, the inhibition zone diameter was measured nearest mm by ruler and classified as susceptible, intermediate, or resistant according to NCCLs standardized table. These green synthesized Al NPs and plant extract were also applied on *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* subsp. *aureus* ATCC 25923 strains (was employed as a quality control microorganisms) using well diffusion method was done in the Iraqi Ministry of Science and Technology.

7. Statistical and Experimental Design

The statistical program IBM SPSS Statistics Base was used in analyzing the data according to the Factorial experiment. The experimental design was Completely Randomized Blocks Design (CRBD) with three replications for each concentration to study the effect of different Al NPs conc. on bacterial growth, and the significant differences between means were compared at p=0.05.

Results and Discussion:

The data in Fig. 2 displays the 3-Dimentinal image of the surface morphology for *S. aromaticum* buds aqueous extract and *S. aromaticum* buds biosynthesized Al NPs using AFM, it showed the measurements of the size, surface morphology and diameter of the biosynthesized Al NPs. AFM data in Fig. 2 indicates the absence of Al NPs in the pure *S. aromaticum* buds aqueous extract recording 122nm particles size and the RMs of the pure *S. aromaticum* buds aqueous extract recording 17.5nm particles size which was with obvious heterogeneity of distribution granular aggregation. While results in Figs. 3 exhibit the presence of biosynthesized Al NPs in the tested sample recording 21nm particles size with RMs about 2.35nm and the homogeneity of the granulated accumulation distribution which is very clear.

AFM data record 122nm particles size and the RMs of the pure *S. aromaticum* buds aqueous extract recording 17.5nm particles size, while the results of biosynthesized Al NPs in the tested sample record 21nm particles size with RMs about 2.35nm.

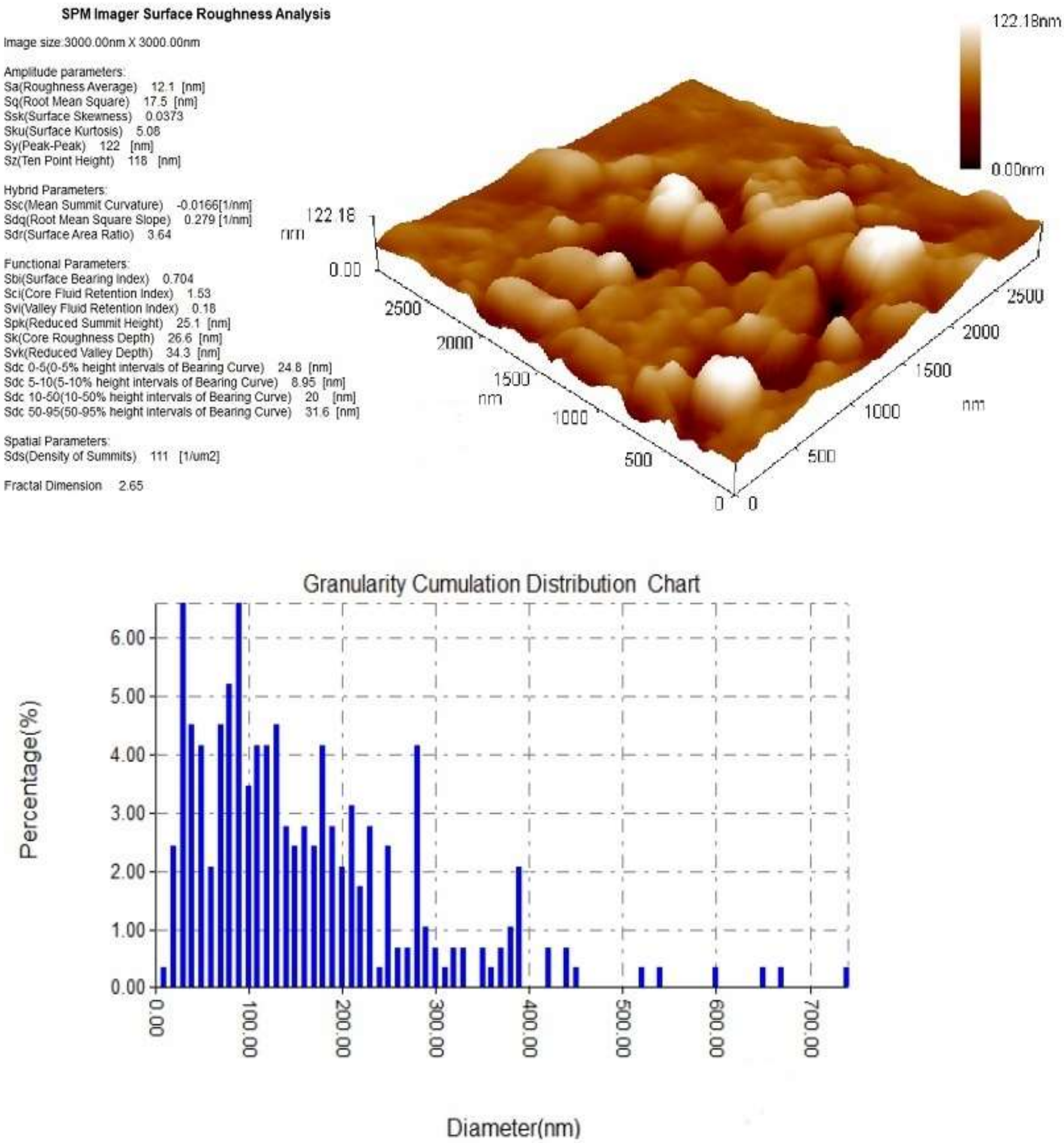

Figure 2. The 3-Dimentional image of the surface morphology for the *S. aromaticum* buds aqueous extract using Atomic Force Microscopy AFM.

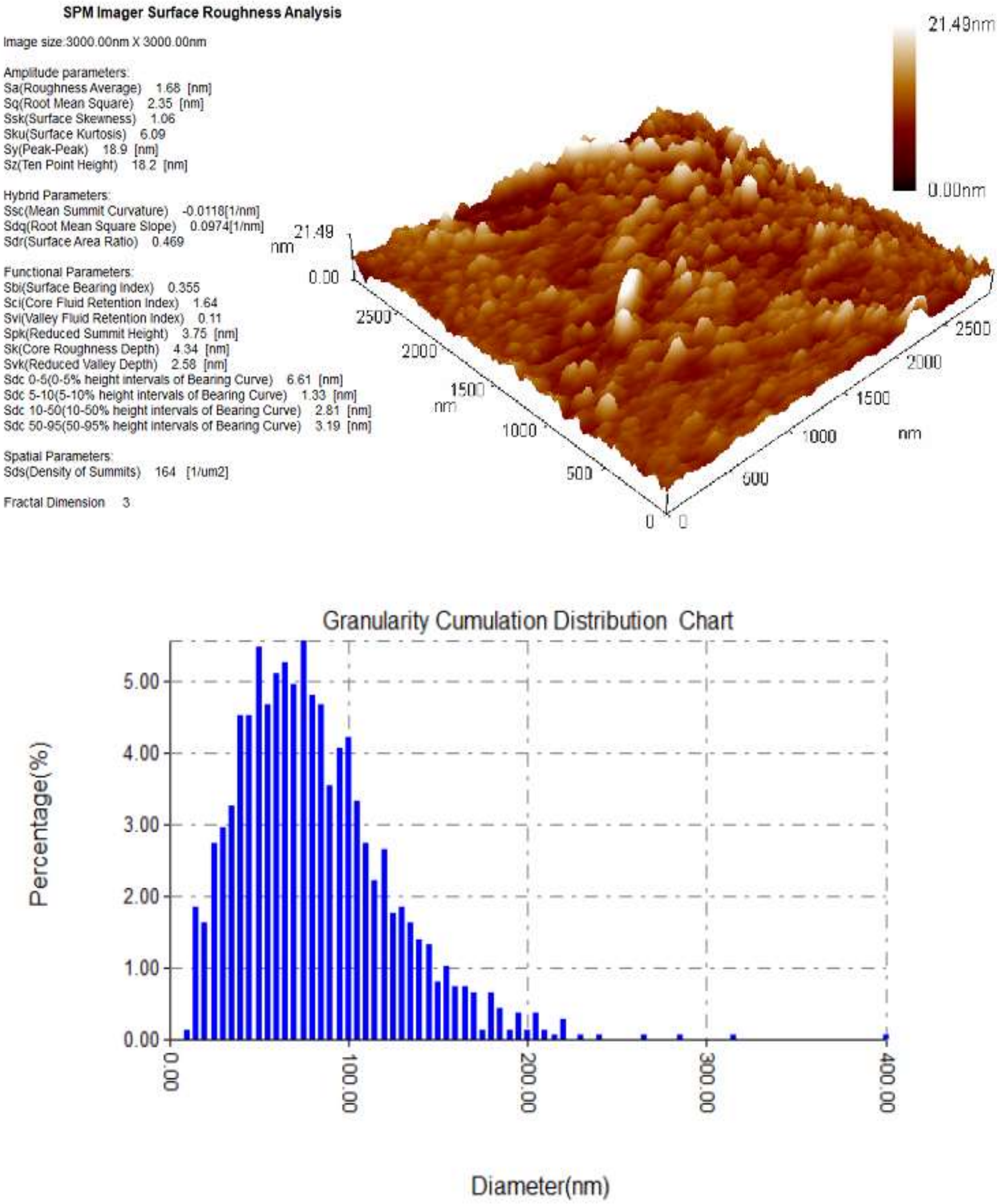

Figure 3. The 3-Dimentional image of the surface morphology for the *S. aromaticum* buds biosynthesized Al NPs using Atomic Force Microscopy AFM.

Results in Fig. 4 show the color change of *S. aromaticum* buds aqueous extract after the addition of $Al(NO_3)_3 . 9 H_2O$. The changes of the solution color from brown to yellowish brown were a primary confirmation of NPs synthesis. This method of NPs synthesis is more suitable compared to the intracellular method due to easy scale-up and downstream processing, non-toxic, renewable, eco-friendly, and biocompatible [12]. Furthermore, because of the green synthesized NPs biocompatible nature, they are known to have various biological applications [3]. The plant-based green synthesis NPs are initiated by mixing the plant extract with the metal precursor solution containing the salts of respective metals $Al(NO_3)_3 . 9 H_2O$. The plant-based green synthesis NPs are categorized into three stages, in the first stage the reduction of metal ions M^+ or M^{2+} to metal atoms (Mθ) occur as well as successive nucleation of the reduced metal atoms [24]. In the second stage, the fusion of neighboring small NPs into larger particles occurs with a simultaneous increase in thermodynamic stability. The process is terminated at the final stage by producing the final shape of the biosynthesized NPs. The presence of many bioactive molecules in the plant extract plays an important role in reducing and stabilizing metal ions in the solution and due to the presence of a large number of phytochemicals in the plant extract, it is difficult to ascertain the exact reduction and stabilizing agents involved in the reaction for the biosynthesis of NPs [13].

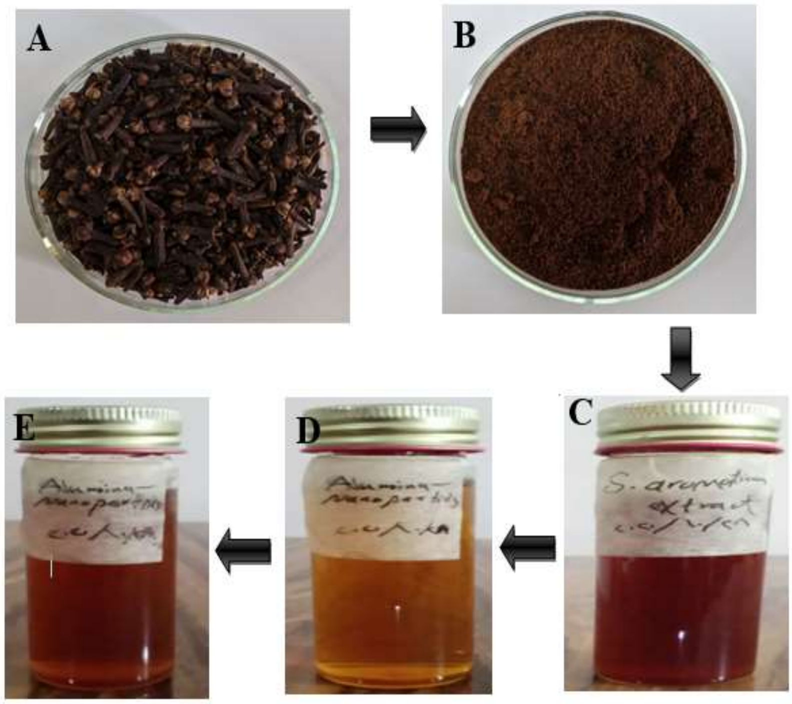

Figure 4. The Color change of *S. aromaticum buds aqueous extract* after addition of $Al(NO_3)_3 \cdot 9H_2O$; A: Plant buds; B: Powdered bud Clove; C: *S. aromaticum* buds aqueous extract; D: Biosynthesized Al NPs after 6hrs and E: Biosynthesized Al NPs after 12hrs

Figure. 5 illustrates the SEM images of *S. aromaticum* buds biosynthesized Al NPs 5A and its aqueous extract 5B at 100nm scan area. It determines the surface features, structural form, and composition of both the *S. aromaticum* buds biosynthesized Al NPs and *S. aromaticum* buds aqueous extract. It reveals the presence of Al NPs. Fig. 5A with diameters ranging from 33.5-70.4nm with regular spiracles shape particles, while Fig. 5B presents the irregular particles' shape and recorded 302.2-396.4nm particle size for the *S. aromaticum* buds aqueous extract.

Baghdad Science Journal
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P-ISSN: 2078-8665
E-ISSN: 2411-7986

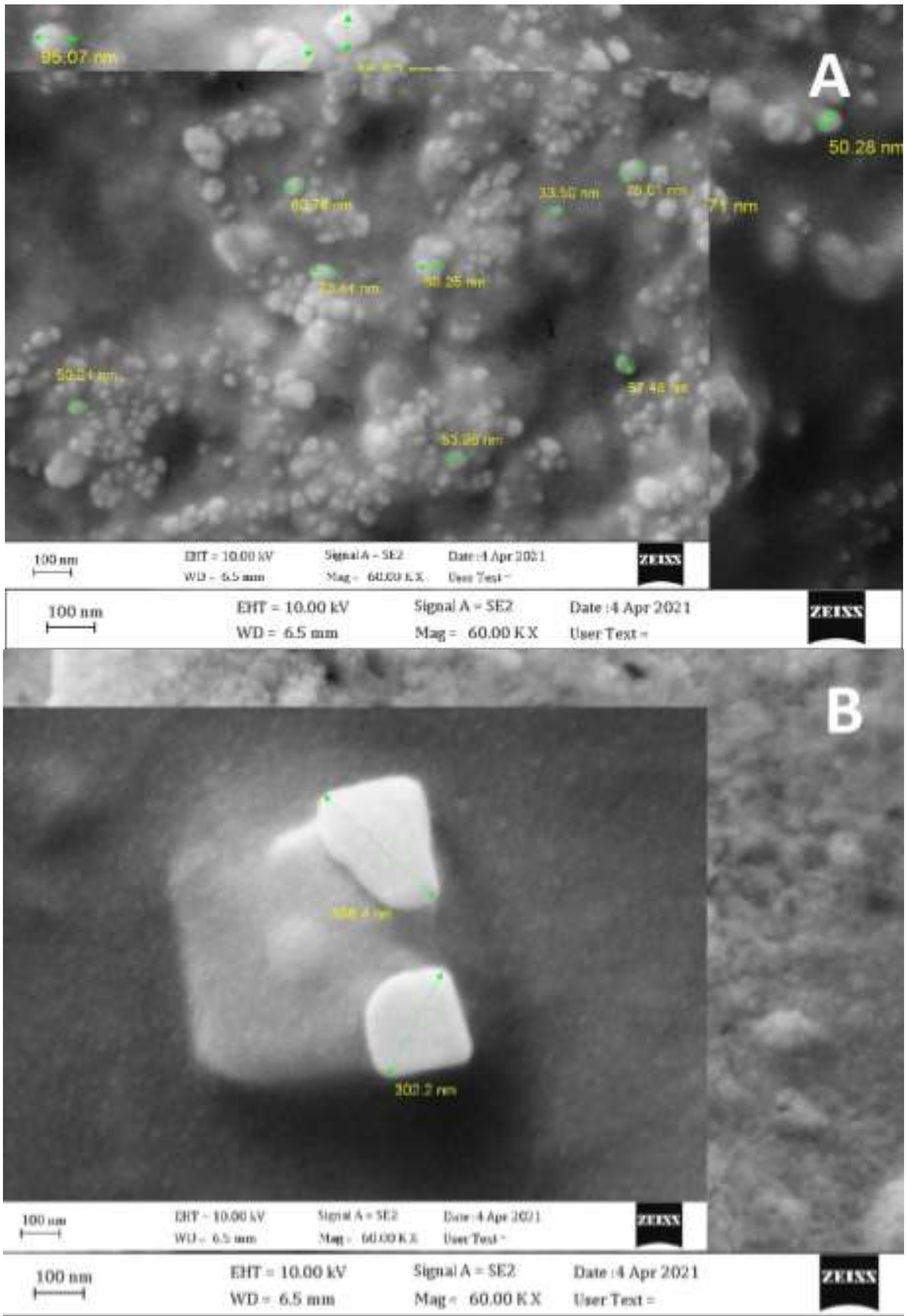

Figure 5. SEM images of *S. aromaticum* buds biosynthesized Al NPs (A) and its aqueous extract (B) at 100nm scan area.

Data in Table. 2 and Fig. 6A exhibit the EDX spectrum analysis of *S. aromaticum* buds aqueous extract and reveal that the Aluminium weight ratio was 1.75, while it was 50.498 in the EDX spectrum analysis of the biosynthesized Al NPs from *S. aromaticum* buds aqueous extract with the disappearance of some elements after the manufacturing process of Al NPs as shown in Table. 3, and Fig. 6B.

Table 2. EDX spectrum analysis of *S. aromaticum* buds aqueous extract.

Elements	Atomic number	Weight ratio
Silicon	14	35.483
Sodium	11	18.381
Carbon	6	12.627
Antimony	51	11.167
Tungsten	74	7.101
Niobium	41	4.222
Magnesium	12	3.928
Calcium	20	3.601
Aluminium	13	2.715
Potassium	19	0.775
Sum:	261	100

Baghdad Science Journal
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E-ISSN: 2411-7986

Table 3. EDX spectrum analysis of the biosynthesized Al NPs from *S. aromaticum* buds aqueous extract.

Elements	Atomic number	Weight ratio
Aluminium	13	50.498
Silicon	14	12.4547
Sodium	11	11.565
Carbon	6	9.2913
Magnesium	12	7.5
Calcium	20	4.285
Potassium	19	4.406
Sum:	95	100

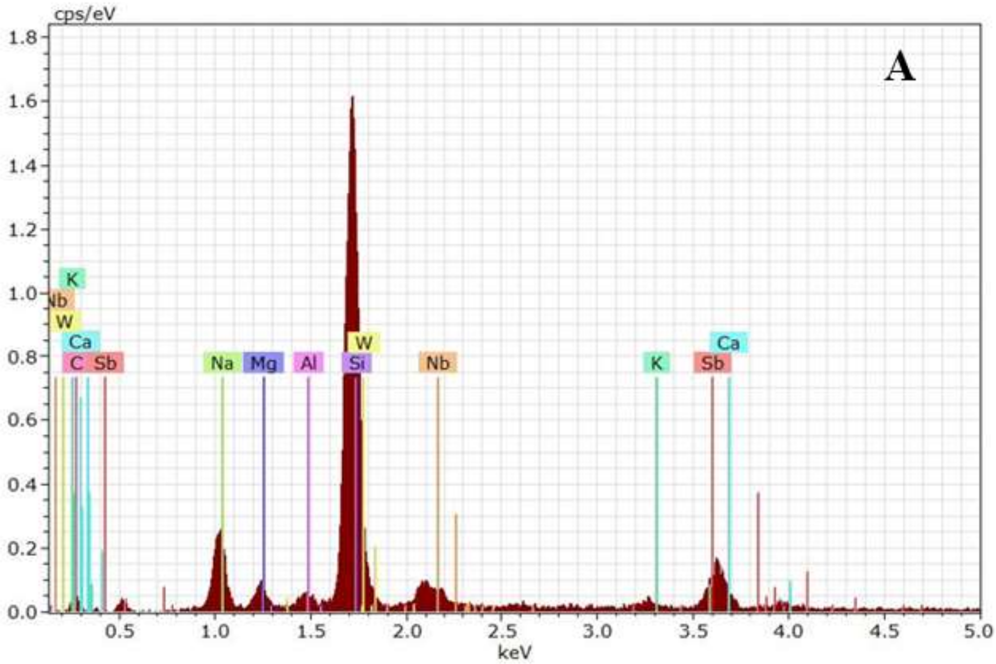

Figure 6. A: EDX spectrum analysis of *S. aromaticum* buds aqueous extract.

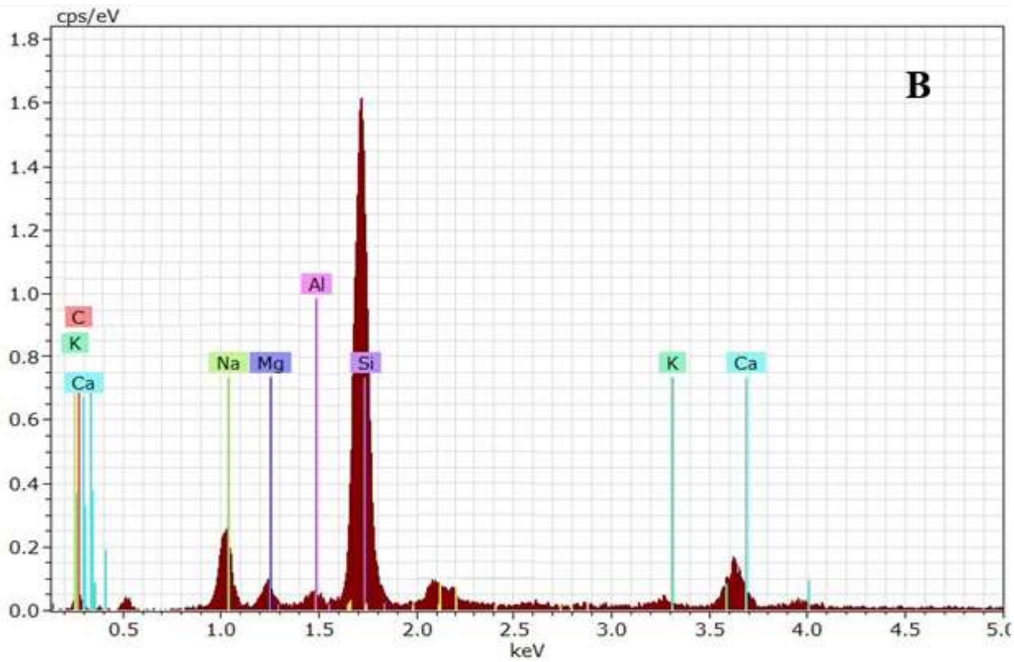

Figure 6. B: EDX spectrum analysis of the biosynthesized Al NPs from *S. aromaticum* buds aqueous extract.

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2023, 20(5 Suppl.): 1840-1857
P-ISSN: 2078-8665
E-ISSN: 2411-7986

Results in Fig. 7 show the UV-Visible spectroscopy of the biosynthesized Al NPs from *S. aromaticum* buds aqueous extract and reveal that the biosynthesized Al NPs was absorbed at 213nm while Aluminum nitrate was absorbed at 258nm. These results indicated the formation of Al NPs in which the absorption peaks of solutions were around 213nm wavelength discloses, and these products were alumina particles.

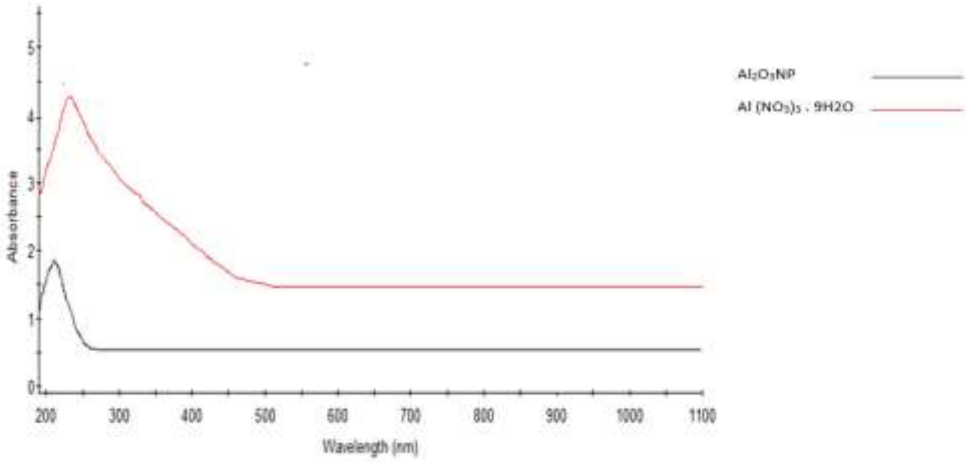

Figure 7. UV-Visible spectroscopy of the biosynthesized Al NPs from *S. aromaticum* buds aqueous extract.

In recent years, the biogenic reduction of elements such as Au, Ag, Cd, Cu, Se, Al, Zn, Ti, Ce, or Fe with plant extracts green-synthesis has become one of the most acceptable techniques for manufacturing of NPs. It's considered a cost-effective and ecological process without using the chemical contaminants [29]. MO NPs were used in numerous fields. They could be prepared using different methods such as green-synthesis and the conventional chemical-synthesis methods. The majority of plants have features such as renewable suppliers and sustainability compared with that of enzymes and microbes, because they can pick up about 75 % of light energy and transform it into chemical energy, contain chemicals such as sugars and antioxidants, that play fundamental roles in the manufacturing of the NPs. Plants are considered the main factory for the green synthesis of metal NPs (M NPs) or MO NPs [30].

Three different plants sources; *Ziziphus Spina—Christi*, *Eucalyptus globulus*, and *Piper nigrum* were used for the bio-synthesis of Ag NPs. The average particle size distribution ranged between 8–35 nm also decreased with the increase in the concentration of plant extract [31]. While Dhar [32], reported the bio-synthesis of Ag NPs from the fruit extract of *Phyllanthus Emblica* and the fabricated Ag NPs were spherical with an average size ranging between 60 to 80nm. Additionally, Adewale [33] exhibited that the extraction methods used in preparing the plant extract using leaves of *Crassocephalum Rubens*, which are then used for biosynthesizing the NPs, play an important role and influence the antioxidant properties of the prepared Ag and Au NPs.

AFM allows for 3D characterization of the synthesized NPs with sub-nanometer resolution. This technique has several advantages over electron microscopy, dynamic light scattering, and optical characterization methods. Also, the results of this article were in line with Hammodi [34] who used the AFM data for characterizing the biosynthesized NPs and reported that the AFM data showed Ag NPs, Zn NPs, or extract of the pure ivy were respectively of 41.70nm, 90.07nm, and 21.00nm size; 27.5, 45.00nm, and 12.5nm size range; 21.23%, 6.88%, and 38.38% size ratio; 1.61nm, 15.9nm, and 6.14nm surface roughness; 85nm, 60nm, and 50.00nm diameter range and 8.04%, 9.69%, and 10.75% distribution ratio. The conducted results of the AFM photogrammetry were clear and can be inferred from their compatibility with Zn and Ag NPs of the Ivy survey data by using SEM scanner, and other data of FTIR Spectroscopy and UV-vis spectroscopy describing the formal properties of the bio-synthesized Ag and Zn NPs. This is evidenced by the success of the process of synthesizing Ag and Zn NPs from the Ivy plant water extract.

The Accurate morphology of Al NPs can be characterized using SEM and this article result is in agreement with those obtained by Ghotekar [35] who reported the research work in the area of green-synthesis of Al NPs using distinctive plant parts extract and special attention of scientific community is required for developing the swift, efficient, noxious, sustainable, environmentally gracious and affordable method of the biosynthesis of Al NPs through the green-chemistry bottom to top approach. Also, different plant species could be used in the future towards completely rapid and facile biosynthesis of MO NPs, which were manufactured through green chemistry approaches receiving great attention because of their significant physical and chemical characteristics and their remarkable uses in the area of nanotechnology. The sustainable improvement of the bio-synthesizing NPs by the extract of different parts of plants has become a major focus of researchers and scientists because they have minimum noxiousness on human health and minimum effect on the ecosystem. Among MO NPs; Al NPs draw great attention due to their significant applications in textiles, ceramics, catalysis, drug delivery, biosensor wastewater treatment. Many natural bioactive-compound in the plant extracts such as alkaloids, saponins, amino acids, tannins, proteins, enzymes, polysaccharides, coumarins, steroids, vitamins, and polyphenols could have participated in Al NPs bio-reduction and stabilization. On the other hand, SEM depends on the signals from the surface of the samples. Thus, SEM can visualize the samples surface structures. The normal resolution of SEM is not enough to characterize and observe the NPs or nanomaterials with sizes ranging between 10 to 50nm. Therefore, recent developments including the low-landing electron energy methods that improved the SEM spatial resolution, also the use of holder for liquid sample enabled the observation of nanocrystals in water solutions, high-resolution SEM images provide an excellent tool for determining the morphology and size of the green-synthesized NPs [36].

The results are in line with Sagadevan and Koteeswari [37] who reported that the elemental composition of Cu NPs was analyzed using the EDX spectrum. EDX analysis was used for the identification and characterization of metal and salt aggregates at a specific point within the prepared sample. It’s an analytical technique used for the characterization of the sample material elemental composition and the data obtained using the EDX analysis consists of spectra with peaks corresponding to the different elements that were present in the prepared sample that every element has specific peaks with unique energy. EDX analysis could be also used for the type of elements qualitative as well as for the percentage of the concentration of each element of the sample quantitative analysis [38]. Also, Iqbal [39] reported that Energy dispersive x-ray spectroscopy and a SEM are employed to check the composition of the elements and the surface morphology.

Raveendraa [40] reported that UV–vis measurements were used to characterize the synthesized NPs, which have unique optical properties that are sensitive to the shape, size, agglomeration state, refractive index, and concentration near nanoparticle surface that makes the UV-Vis a valuable tool for the identification and characterization of the nanomaterials. Also, Agudelo [41] reported that SHIMADZU UV-1800 UV-visible spectrophotometer was used for analyzing the synthesized gold and silver NPs in a range of 300 to 800nm; the reducing agent solutions without the precursor agent were used as a control. Also, UV–vis spectroscopy represents a convenient technique for characterizing the nanomaterials, as it allows fast data acquisition, and it's available in most chemistry laboratories. This technique can, in theory, be used for the characterization of plasmonic nanomaterials synthesis kinetics [42]. While Al-Nassar [43] reported that the absorption peak of synthesized sample in water was 225nm, whereas the one that synthesized using ethanol was 210 nm and they found that the absorption spectrum of samples produced in ethanol is lower than those produced using water and the absorption peak of colloidal solutions on around 210nm wavelength discloses that produces alumina particles and its oxides are not formed due to prohibition of ethanol surrounding media form oxidation.

The quality control *Escherichia coli* ATCC 25922 and ATCC 25923 *Staphylococcus aureus* subsp. *aureus* strains susceptibility test results were shown a susceptible effect 100% against all concentration of Al NPs antimicrobials and 100% resist against concentrations of aqueous plant extract as shown in the Figs. 8a, 8b, 9a and 9b respectively.

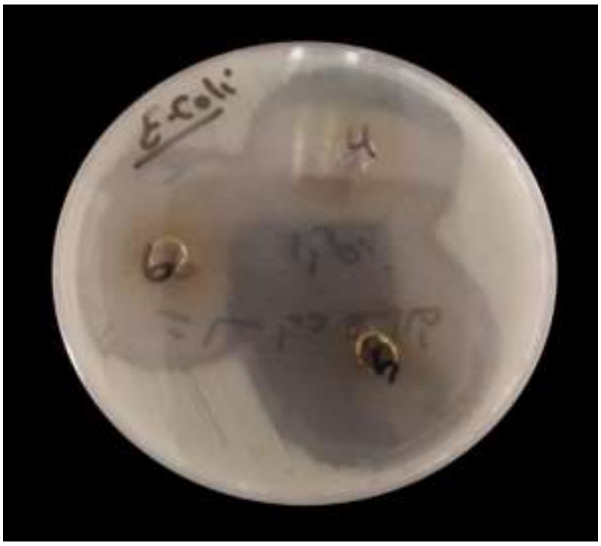

Figure 8. a: Effect of Al NPs concentrations 0.5µg/ml, 1.5µg/ml and 3.0 /ml numbered with 4, 5, 6 respectively on ATCC 25922 *E. coli* strain.

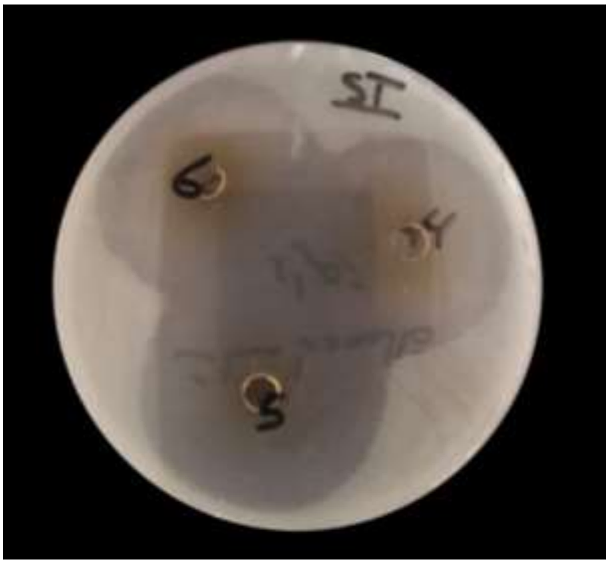

Figure 8. b: Effect of Al NPs concentrations 0.5µg/ml, 1.5µg/ml and 3.0µg/ml numbered with 4, 5, 6 respectively on ATCC 25923 *Staphylococcus aureus* subsp. *aureus* strain.

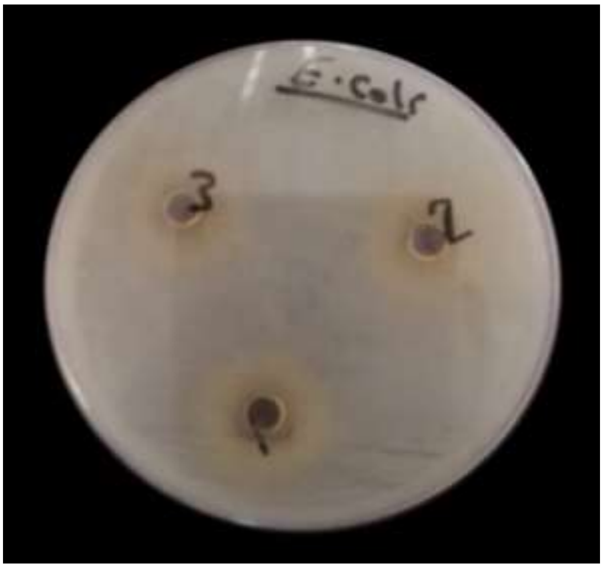

Figure 9. a: Effect of aqueous plant extract concentrations 0.5µg/ml, 1.5µg/ml and 3.0µg/ml numbered with 1, 2, 3 respectively on ATCC25922 *E. coli* strain.

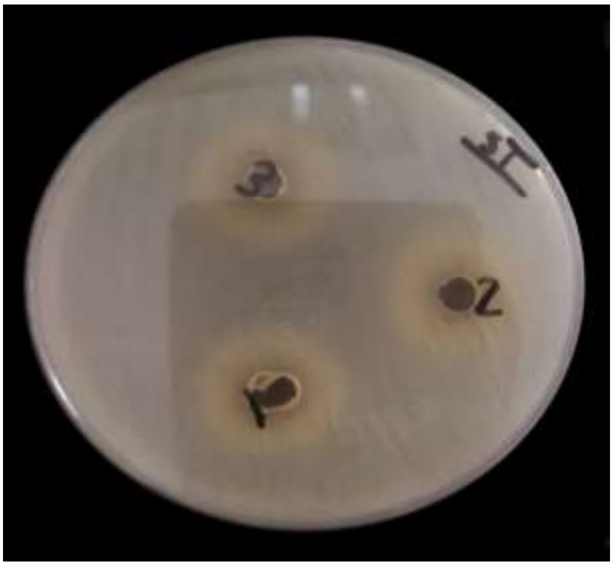

Figure 9. b: Effect of aqueous plant extract concentrations 0.5µg/ml, 1.5µg/ml and 3.0µg/ml numbered with 1, 2, 3 respectively on ATCC 25923 *Staphylococcus aureus* subsp. *aureus* strain.

The data in Table. 4 indicate a significant increase in the inhibition zone (mm) obtained in *S. enterica* serovar *Typhimurium* 14 isolate recording 29.57 mm compared with all other bacterial isolates. Also, there was a significant increase in the inhibition zone occurred in *S. enterica* serovar *Typhimurium* 2, *S. enterica* serovar *Typhimurium* 5, *S. enterica* serovar *Typhimurium* 7, *S. enterica* serovar *Typhimurium* 8, *S. enterica* serovar *Typhimurium* 9, *S. enterica* serovar *Typhimurium* 10, *S. enterica* serovar *Typhimurium* 11, *S. enterica* serovar *Typhimurium* 12, *S. enterica* serovar *Typhimurium* 13, *S. enterica* serovar *Typhi* 1, *S. enterica* serovar *Typhi* 2, *S. enterica* serovar *Typhi* 3, *S. enterica* serovar *Typhi* 4, *S. enterica* serovar *Diarizonae*, and *S. enterica* serovar *Enterica* recording 15.7788 mm, 18.01 mm, 16.63 mm, 15.65 mm, 17.65 mm, 17.35 mm, 19.08 mm, 18.63 mm, 19.26 mm, 17.62 mm, 17.98 mm, 13.60 mm, 17.71 mm, 18.8 mm, and 19.15 mm inhibition zone respectively in comparison with *S. enterica* serovar *Typhimurium* 3, *S. enterica* serovar *Typhimurium* 4, and *S. enterica* serovar *Typhimurium* 6 that recorded 8.97125 mm, 7.255 mm, and 9.89 mm respectively and shown a significant decrease in the inhibition zone. Furthermore, *S. enterica* serovar *Typhimurium* 1 isolate showed a significant increase in the inhibition zone compared with *S. enterica* serovar *Typhimurium* 5, *S. enterica* serovar *Typhimurium* 9, *S. enterica* serovar *Typhimurium* 10, *S. enterica* serovar *Typhimurium* 11, *S. enterica* serovar *Typhimurium* 12, *S. enterica* serovar *Typhimurium* 13, *S. enterica* serovar *Typhimurium* 14, *S. enterica* serovar *Typhi* 1, *S. enterica* serovar *Typhi* 2, *S. enterica* serovar *Typhi* 4, *S. enterica* serovar *Diarizonae*, and *S. enterica* serovar *Enterica* that recorded 18.01 mm, 17.65 mm, 17.35 mm, 19.08 mm, 18.63 mm, 19.26 mm, 29.57 mm, 17.62 mm, 17.98 mm, 17.71 mm, 18.8 mm, and 19.15 mm inhibition zone respectively. The data also revealed that there was a significant decrease in the inhibition zones in the negative control, 0.5µg/ml, 1.5µg/ml, and 3.0µg/ml of water extract shown no Inhibition zone compared with the positive control 40.35 mm as shown in Fig. 10. Results also indicated that there was a significant decrease in the inhibition zone obtained in 0.5µg/ml of biosynthesized Al NPs 26.38 mm compared with +control (imipenem). Whereas no significant differences were obtained between imipenem 10µg/ml and 1.5µg/ml, 3.0µg/ml Al NPs that recorded 30.55 mm and 34.61 mm respectively.

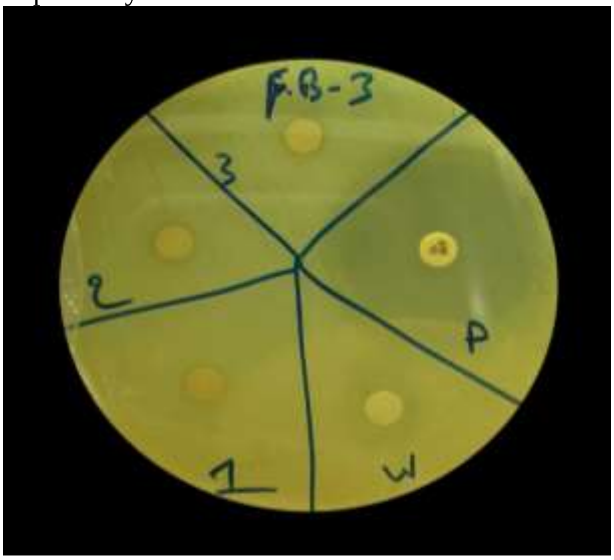

Figure 10. Effect of aqueous plant extract concentrations 0.5µg/ml, 1.5µg/ml and 3.0µg/ml numbered with 1, 2, 3 respectively and P referred to positive control imipenim antibiotic drug 10µg/ml while W referred to negative control water on *S. enterica* spp. strain.

The interaction between the type of treatment and bacterial isolates indicated a significant increase in the inhibition zone obtained in two bacterial isolates only, that *S. enterica* serovar *Typhi* 4 isolate recorded increasing in all the Al NPs concentrations 0.5µg/ml= 35 mm, 1.5µg/ml= 40 mm, and 3.0µg/ml= 38.33 mm compared with the positive control 28.33 mm. While the other isolate, *S. enterica* serovar *Typhimurium* 13, revealed a significant increase in the inhibition zone in 1.5µg/ml and 3.0µg/ml concentration of Al NPs with 40 mm, 40 mm respectively and no significant differences were recorded in 0.5µg/ml Al NPs 38.33 mm in comparison with 35.77 mm of positively control. Moreover, no significant differences were exhibited in other isolates. Furthermore, 33.66 mm, 38 mm, and 37 mm inhibition zone were recorded in *S. enterica* serovar *Typhi* 2 isolates in all the Al NPs concentrations 0.5µg/ml, 1.5µg/ml, and 3.0µg/ml respectively compared with the positive control 35.22 mm. Whilst *S. enterica* serovar *Typhimurium* 11 isolates which were investigated displayed no significant differences except in 1.5µg/ml Al NPs, recording 38.33 mm inhibition zone compared with the positive control 41 mm. However, *S. enterica* serovar *Diarizonae* isolate showed no significant differences in two concentrations of Al NPs 1.5µg/ml and 3.0µg/ml recording 38.33 mm and 38 mm inhibition zone respectively in comparing with imipenem 41.22 mm. Furthermore, *S. enterica* serovar *Typhimurium* 14 isolate showed no significant differences in all Al NPs concentrations 0.5µg/ml, 1.5µg/ml, and 3.0µg/ml were denoted 31.66 mm, 34.66 mm, and 33.3 mm mean value of inhibition zone respectively in comparison with imipenem 36.94 mm.

S. enterica serovar *Typhimurium* 13 isolate revealed 38.33 mm, 40 mm, and 40 mm mean inhibition zone in 0.5µg/ml, 1.5µg/ml, and 3.0µg/ml Al NPs respectively in compared to the positive control 28.33 mm which indicated that it was the most affected bacterial isolate with Al NPs treatment. Lastly, the other remaining bacterial isolates recorded a significant decrease in the mean inhibition zone compared with the positive control treatment. This antibacterial biosynthesized NPs activity showed that all concentrations 0.5µg/ml, 1.5µg/ml, and 3.0µg/ml of Al NPs had the maximum antibacterial activity on the isolated and characterized *Salmonella* spp. that when the Al NPs effects were compared to the effect of the antibiotic drug used as a control agent in this experiment imipenem 10µg/ml, it was discovered that the Al NPs were more effective, despite their concentration being 0.5µg/ml, 1.5µg/ml, 3.0µg/ml is much lower than those of standard antibiotic concentration and as shown in Fig. 11. This is an indicator that employing bio-manufactured NPs to fight microbial diseases may be preferable to using a medical antibiotic, which is so overused and abused nowadays that most bacteria types had developed resistance against it [44].

Table 4. The antibacterial effects of different concentrations of *S. aromaticum* aqueous extract and biosynthesized Al NPs against isolated and characterized *Salmonella* spp.

Type of treatment / Bacterial Isolates	Imipenem (+ve control)	Water (-ve control)	Water extract (crud) concentrations (µg/ml)			Al NPs concentrations (µg/ml)			Mean
			0.5	1.5	3.0	0.5	1.5	3.0	
S. enterica* serovar *Typhimurium 1	**37.22**	**0**	**0**	**0**	**0**	**3.33**	**31.66**	**16.66**	**11.1088**
S. enterica serovar Typhimurium 2	**42.27**	**0**	**0**	**0**	**0**	**33.63**	**16**	**34.33**	**15.7788**
S. enterica serovar Typhimurium 3	**39.11**	**0**	**0**	**0**	**0**	**8.33**	**10.33**	**14**	**8.97125**
S. enterica serovar Typhimurium 4	**37.38**	**0**	**0**	**0**	**0**	**0**	**11.66**	**9**	**7.255**
S. enterica serovar Typhimurium 5	**44.05**	**0**	**0**	**0**	**0**	**31.66**	**33.33**	**35**	**18.01**
S. enterica serovar Typhimurium 6	**37.11**	**0**	**0**	**0**	**0**	**0**	**10**	**32**	**9.89**
S. enterica serovar Typhimurium 7	**45.72**	**0**	**0**	**0**	**0**	**29.66**	**32.66**	**25**	**16.63**
S. enterica serovar Typhimurium 8	**44.22**	**0**	**0**	**0**	**0**	**20.66**	**24**	**36.33**	**15.65**
S. enterica serovar Typhimurium 9	**43.5**	**0**	**0**	**0**	**0**	**30**	**33.33**	**34.33**	**17.65**
S. enterica serovar Typhimurium 10	**42.83**	**0**	**0**	**0**	**0**	**30**	**35**	**31**	**17.35**
S. enterica serovar Typhimurium 11	**41**	**0**	**0**	**0**	**0**	**37**	**38.33**	**36.33**	**19.08**
S. enterica serovar Typhimurium 12	**45.05**	**0**	**0**	**0**	**0**	**33.66**	**36**	**34.33**	**18.63**
S. enterica serovar Typhimurium 13	**35.77**	**0**	**0**	**0**	**0**	**38.33**	**40**	**40**	**19.26**
S. enterica serovar Typhimurium 14	**36.94**	**0**	**0**	**0**	**0**	**31.66**	**34.66**	**33.3**	**29.57**
S. enterica* serovar *Typhi 1	**43.61**	**0**	**0**	**0**	**0**	**32.33**	**34.33**	**30.66**	**17.62**
S. enterica* serovar *Typhi 2	**35.22**	**0**	**0**	**0**	**0**	**33.66**	**38**	**37**	**17.98**
S. enterica* serovar *Typhi 3	**41.11**	**0**	**0**	**0**	**0**	**31**	**36.66**	**0**	**13.60**
S.enterica* serovar *Typhi 4	**28.33**	**0**	**0**	**0**	**0**	**35**	**40**	**38.33**	**17.71**
S. enterica* serovar *Enterica	**45.44**	**0**	**0**	**0**	**0**	**32**	**36.66**	**36.66**	**18.8**
S. enterica* serovar *Diarizonae	**41.22**	**0**	**0**	**0**	**0**	**35.66**	**38.33**	**38**	**19.15**
Mean	**40.35**	**0**	**0**	**0**	**0**	**26.38**	**30.55**	**34.61**	
L. S. D=0.05	**Type of treatment=12.71; Bacterial isolate=6.13 Type of treatment*Bacterial isolate=4.21**								

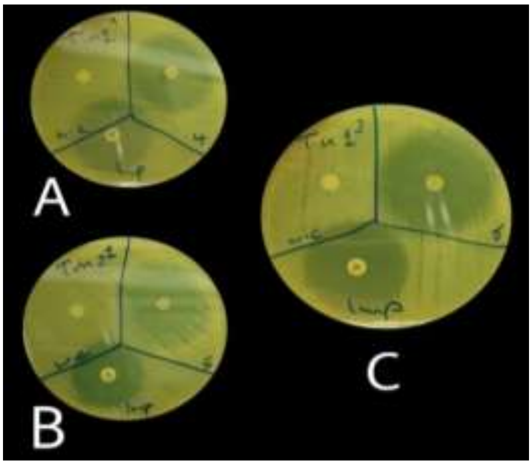

Figure 11. Effect of Al NPs concentrations 0.5µg/ml, 1.5µg/ml and 3.0µg/ml numbered with A/4, B/5, C/6 respectively and Imp referred to positive control imipenim antibiotic drug 10µg/ml while W.C referred to negative control water on *S. enterica* spp. strain.

The results of NPs in this study are in line with those obtained by Bansal [45] that was conducted in India and reported the antimicrobial effects of clove Ag NPs suspensions against *Bacillus cereus* (MTCC 1272), *Salmonella typhi* (MTCC 734), *Pseudomonas aeruginosa* (MTCC PAO1) and *Escherichia coli* (MTCC 9537). In contrast with our results, they found that clove extract has substantial inhibitory effects on the examined pathogens at various doses. In contrast, Indian researchers in Kavitha [46] indicated that only *Syzygium aromaticum* bud clove displayed an inhibition zone against *Staphylococcus aureus, Escherichia coli, Salmonella enteritidis* and *Clostridium perfringens*. Similarly, Afanyibo [47] reported that *S. aromanticism* aqueous extract has been inhibiting approximately 66.67% of tested germs (*E. coli, S. aureus, S. enterica serovar Typhi, P. aeruginosa, S. flexneri, C. albicans*). Hence, this may be due to the nature of bacterial isolates and the possibility of mutations occurring that could increase their resistance. While in another Egyptian study [48], they reported that antimicrobial activity was estimated against bacterial strains (*Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633) and yeasts (*Candida parapsilosis* AUMC 8909 and *Trichosporon domesticum* AUMC 8918). Silver NPs displayed a good antibacterial impact and the clove had the best antimicrobial effect among the spices tested. Another recent study [49] used *Syzygium aromaticum* extract to biosynthesize CuO NPs and showed that CuO NPs displayed resistance to the three types of bacteria with the greatest resistance to *E. coli* followed by *Salmonella* and then *Enterobacter*. In addition, a recent Russian study [50] investigated examples of toxicity of NPs where many microorganisms are available: *Proteus spp., Salmonella spp., Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, etc. It is a potential trend to employ these NPs as anticancer agents, antifungal and antibacterial medications against pathogenic and drug-resistant microorganisms. For the green-synthesis of NPs, the most important issue is that the solvent medium combined with a selection of ecologically nontoxic, reducing, and stabilizing agents. The use of the biosynthesized NPs may be one of the promising approaches to overcome bacterial and fungi resistance and could also play a new key role in pharma-co-therapeutics [51].

Conclusion:

In our study, we conclude that it is possible to employ biosynthesized Al NPs from the clove plant aqueous extract as an alternative to conventional antibiotics in order to inhibit the growth of food born bacteria due to their beneficial effects of increased bacterial growth inhibition, compared to the concentrations of NPs and the antibiotic drugs and lack of resistance when used in excess. It can be exploited as a possible source of natural antibacterial compounds if it is confirmed that it does not show a toxic effect on cells in the laboratory, therefore, we proceed to study the toxic effect of these NPs *invtro* and *invivo* study.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in Al-Nahrain University.

Authors' contributions statement:

Z. A. A. Laboratory analysis, writing original graph, investigation methodology. A. G. O. Supervised the aspects related to the preparation and diagnosis, reviewing and editing the writing and analyzing of the plant and nanoparticles product. Z. A. Th. Formal analysis, writing review and editing, investigation methodology. All authors have read and agreed to the published version of the manuscript. Ethics Approval: Ethics approval (Ref. No. E. B. 12) was got from the Biotechnology Research Center/ Al-Nahrain University.

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تأثير جزيئات الألمنيوم النانوية المصنعة الخضراء Al NPs على *السالمونيلا* المعوية المعزولة من مدينة بغداد

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الخلاصة:

هدفت هذه الدراسة إلى التوليف الأخضر وتوصيف جسيمات الألومينا النانوية Al NPs من مستخلص نبات القرنفل (*Syzygium aromaticum* L.) وللتحقق من تأثيرها على نمو السالمونيلا المعوية المعزولة والمشخصة. تم تحضير المستخلص المائي لبراعم القرنفل من نبات القرنفل المحلي، ثم خلط مع نترات الألومنيوم $Al(NO_3)_3.9H_2O$، 99.9٪ المحضر بنسبة ¼ لتخليق أخضر لـ Al NPs. تغير اللون كان تأكيدًا أوليًا للتخليق الحيوي لـ Al NPs. تم التعرف على الجسيمات النانوية المركبة حيويا وتشخيصها عن طريق AFM و SEM و EDX ومقياس الطيف الضوئي المرئي للأشعة فوق البنفسجية. أظهرت بيانات AFM حجم جسيمات 122 نانومتر وخشونة السطح RMs للمستخلص المائي لبراعم *S. aromaticum* النقية التي تسجل حجم جسيمات 121 نانومتر، بينما سجلت نتائج Al NPs في العينة المختبرة حجم جسيمات 21 نانومتر مع خشونة السطح (RMs) حوالي 21 نانومتر. وقد حددت صور SEM وجود Al NPsبأقطار تتراوح من 33.5-70.4 نانومتر مع جزيئات منتظمة الشكل في عينة الجسيمات النانوية المحضرة حيوياً. أظهر تحليل طيف EDX أن نسبة وزن الألمنيوم كانت 1.75، وكانت 50.498 في عينة Al NPsالمُصنَّعة حيوياً المحضرة من المستخلص المائي لبراعم *S. aromaticum*. كشفت بيانات التحليل الطيفي المرئي للأشعة فوق البنفسجية أن Al NPsالمركب حيوياً قد تم امتصاصه عند 213 نانومتر بينما تم امتصاص نترات الألومنيوم عند 258 نانومتر. تشير هذه النتائج إلى تكوين Al NPs. أظهر النشاط المضاد للبكتيريا أن Al NPs أظهر نشاطًا عاليًا مضادًا للبكتيريا على *Salmonella* spp. مقارنة بتأثير عامل التحكم (imipenem) في هذه التجربة. نستنتج، بأنه يمكن استخدام Al NPs المركب حيويًا من مستخلص القرنفل المائي كمركبات طبيعية مضادة للجراثيم لمنع نمو بكتيريا *Salmonella*.

الكلمات المفتاحية: جزيئات الألومينا النانوية، Al NPs المركب حيوياً، التأثير المضاد للميكروبات لـ Al NPs، الأمراض المنقولة بالغذاء، السالمونيلا.