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Article

Synthesis of Silver-curcumin Nanoparticles: Antioxidant Activity and Mechanistic Insights into Antimicrobial Inhibition

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Abstract: The current study aims to diagnose the possibility of synthesizing and characterizing silver-curcumin nanoparticles and studying their effects as an antioxidant and bacterial inhibitor for *Escherichia coli, Pseudomonas sp. and Staphylococcus aureus*. The diagnosis of the plasmon spectrum when scanned by electron microscope image from (300-700) nanometer showed that the silver-curcumin nanoparticles were (20-90) nm in size within the homogeneously distributed nanoscale range and the absence of agglomerations on the surface. The X-ray dispersive energy results also showed that the sample consisted of carbon, silver and oxygen, and the percentages of the elements in the sample were 67.17, 30.70 and 2.09 % respectively. The infrared spectrum of curcumin and silver-curcumin showed stretching and bending vibrations at the fingerprint area 500-1500 cm - 1, which is evidence of silver nanoparticles. The silver-curcumin nanoparticle preparation acts as an antimicrobial and pathogenic bacteria, as the highest rate of inhibition was for E. coli bacteria at a concentration of 2 mg/L and the lowest rate of inhibition at a concentration of 1 mg/L. Based on the presented results, the silver-curcumin nanoparticle has a high antimicrobial activity and can be considered a natural antioxidant.

Keywords: Silver-Curcumin, Antioxidant, Nanotechnology, Curcumin

1. Introduction

Nanotechnology and nanotechnology are among the modern and important sciences and technologies. They represent a valuable advancement in all branches of science. The nanoparticles are particles of size from (1-100) nanometers. They can import nutrients and markers between cell membranes and tissues perfectly. Also, it can be considered one of the sciences that studies the properties of local structures at the nanoscale. Nanotechnology works to change the physical properties and convert them into nanoparticles [1]. Several methods for nanoparticle synthesis of which the green method, is a preferred method to complement other processes due to the portability and reproducibility of the synthesized nanomaterials [2]. Many materials are used for the synthesis of nanoparticles such as algae and actinomycetes, sugars, biodegradable polymers such as chitosan, bacteria and plants [3],[4]. The use of plants or their extracts in the synthesis of nanoparticles is one of the best and fastest methods [5]. As plants and their extracts are natural antioxidants and can reduce the negative effects of oxidative stress, Among the plant derivatives, curcumin has great antioxidant activity. It is an excellent free radical scavenger because curcumin has an antioxidant capacity similar to that of vitamin E, Curcumin exhibits antioxidant attributes as it facilitates the removal of several reactive oxygen radicals, most notably superanions, nitrogen dioxide radicals and hydroxyl radicals, Silver nanoparticles can be produced by a range of techniques, such as chemical, biological, physical, and environmentally friendly processes, Due to their strong antibacterial properties, silver nanoparticles are a useful tool for treating infections that are resistant to many pharmaceuticals, Because silver-curcumin nanoparticles are highly poisonous to bacteria, they are especially beneficial; on the other hand, they are considered improve poultry productivity indicated that curcumin-silver nanoparticles (cAgNPs) have high efficiency in resisting bacteria [6]. It can be developed as highly effective nanomaterials for microbes, viruses and germs, especially Gram-negative *E. coli* bacteria. The increase in the concern traction of curcumin-silver nanoparticles cAgNPs leads to a decrease in the presence of bacteria because Curcumin nanoparticles have the potential to exhibit prolonged efficacy in antimicrobial situations [7]. The current study investigated the possibility of synthesizing and characterizing silver-curcumin nanoparticles and studying their effects as an antioxidant and a bacterial inhibitor for both *E. coli and Pseudomonas sp.* and *Staphylococcus aureus*.

2. Materials and Methods

1.1. Preparation of nanoparticles of a silver-curcumin preparation

The process outlined by Abood et al. is referred to as the oxidation or moist method. A silver-curcumin nanoparticle was prepared using the reduction process with minor modifications. 0.1 gram of each of the two ingredients silver nitrate and curcumin was taken, and the volume was then brought to 100 milliliters using deionized water. Once the solution's color shifts from yellow-orange to brown, indicating the creation of silver nanoparticles, place the mixture on the stirrer and heat it to 80 degrees Celsius [8].

1.2. Diagnosis using the Tyndall effect

The nanomaterial was exposed to laser radiation, and the light beam passed through the nanoparticle solution in a straight line, and this gives a preliminary inference on the composition of the nanomaterial, as shown in Figure (1).

Figure 1. Tyndall effect in the prepared nanomaterials (silver - curcumin)

2.3. Plasmon Spectrum

The plasmon spectrum of a solution of (silver-curcumin) nanoparticles prepared at a concentration of (1000) ppm was measured using a UV-Vis spectrophotometer, and by scanning at wavelengths from (300-700) nm, the plasmon spectrum appeared.

2.4. Diagnosis using a scanning electron microscope

A scanning electron microscope was used to inspect the nanocomposite's surface to ascertain its shape. Water evaporation was used to dry drops of the produced nanoparticle solution that were placed on an aluminum platform. The samples were delivered to the Baghdad-based Kak laboratory so they could be examined in the Islamic Republic of Iran.

2.5. Diagnostics using energy-dispersive X-rays

The sample prepared from silver-curcumin nanoparticles was placed on an aluminium slide, and the water was evaporated, then it was sent for examination directly with the device manufactured by Tabriz University, where a specific area of the sample was measured to find the exact elemental analysis for it.

2.6. Diagnostics using Fourier-transform infrared spectrometry

The nanomaterial prepared from silver-curcumin nanoparticles was mixed with an amount of potassium bromide salt (KBr), and dry tablets were made from the mixture consisting of three materials. The infrared spectrum was registered with a Fourier transform infrared (FT-IR) device, Model 84005, equipped by Shimadzu Company. Nanoparticle test of a silver-curcumin preparation as an antioxidant which is related to Education College for Pure Sciences- University of Basrah.

2.7. Testing of a silver-curcumin nanoparticles as an antioxidant

The antioxidant activity of the prepared nano-solution (silver-curcumin) was studied by revealing its ability to capture free radicals using Diphenylpicrylhydrazyl (DPPH). The particles prepared at three concentrations 1, 1.5, and 2 mg/L were studied. A DPPH solution was prepared at a concentration of 100 parts per million. Equal volumes of the prepared nanocomposite solution and the oxidizing agent DPPH were mixed. The effectiveness of free radical scavenging of the oxidizing agent was monitored over time using visible spectroscopy at a wavelength of 517 nm [9]. The color of the solution is dark violet and when it turns yellow, it indicates that the oxidizing agent has ended by catching free radicals. The antioxidant Butylated Hydroxytoluene (BHT) was used at concentrations of 1, 1.5 and 2 mg/litres for comparison. The effectiveness was calculated by applying the following equation:

$$\text{Antioxidant activity \%} = (AD-AS)/AD * 100$$

Where AD represents the absorbance of DPPH AS absorbance of the samples

2.8. Testing nanoparticles of silver-curcumin nanoparticles as an antimicrobial:

Pathological microbial isolates were used as microscopic test organisms to detect the effectiveness of silver curcumin nanoparticles as an antibacterial at different concentrations of 1, 1.5, and 2 mg/L. The pathogenic bacterial isolates used in this study (*E. coli*, *Pseudomonas sp.*, and *Staphylococcus aureus*) were obtained from the Department of Biology, College of Science, University of Basrah. These isolates were previously identified and confirmed to be antibiotic resistant. The test was conducted according to the method of SPSS as follows: Mueller Hinton Agar medium was prepared and then sterilized in an autoclave at a temperature of 121 °C for 15 minutes [10]. After that, the medium was poured into Petri dishes, and then the dishes containing the medium were heavily inoculated with *Pseudomonas sp.*, *Staphylococcus aureus*, and *E. coli* under CFU/ML conditions. After the inoculum dried, holes were made in the medium in each plate, and silver- curcumin nanoparticles were added using a micropipette at different concentrations (1, 1.5, 2) mg/L, and each concentration had three plates of each bacterium.

The plate was then incubated at 37°C for 24 hours, after which the silver-curcumin nanoparticles' inhibition diameter (mm) was measured.

2.9. Statistical analysis

A completely random design (One-way ANOVA) was employed in the analysis of experimental data using the [11]. Additionally, Duncan's multiple range tests were used to assess significant differences between means at the P ≤0.05 percent significance level [12].

3. Results

Diagnostics using Surface Plasmon Resonance (SPR) showed the plasmon spectrum of silver-curcumin nanoparticles prepared at 390 nm. This is consistent with what was found by Vo-Van et al. when examining the physical, chemical and optical changes of silver-curcumin nanoparticles after 12 months of storage [13]. It was observed that the plasmon spectrum was at 438 nm. The absorption occurs due to the surface plasmon resonance resulting from the electrons on the surface of the nanoparticles. The nanoparticles remained in their spherical shape as they did not change after 12 months of storage and Figure (2) represents the plasmon spectrum of silver-curcumin nanoparticles [14].

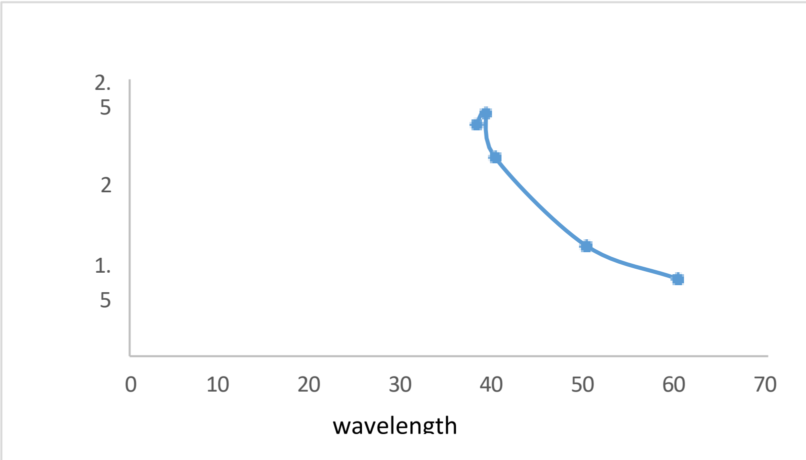

Figure 2. Plasmon spectrum of silver-curcumin nanoparticles

3.1 Diagnosis using a scanning electron microscope

Figure (3) makes it evident that all of the silver-curcumin nanoparticles were uniformly dispersed, in the nanoscale range, with an average size of between 20 and 90 nm, and free of agglomerations on the surface.

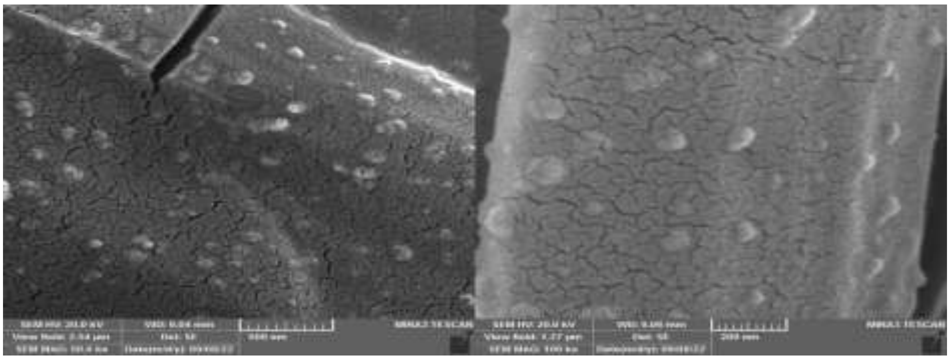

Figure 3. A scanning electron microscope image of the surface of the prepared silver-curcumin nanoparticles

3.2 Diagnostics using energy-dispersive X-ray analysis (EDX) of silver-curcumin

nanoparticles

The peaks in the results of the qualitative analysis are shown in Figure (4). It indicates the presence of carbon, oxygen and silver, and they represent the constituent elements of the sample, which are 67.17% of carbon, 30.70% of oxygen and 2.09 silver.

3.3. Diagnosis using infrared spectrum Red Fourier Infrared Spectroscopy (FTIR)

Infrared spectrum can be used in the diagnosis of nanomaterials, depending on the fingerprint area, where the nanoparticles show vibrations in the region (500-1500) cm-1, and this region is used to prove the type of the compound, Figure A-5 shows the FT-IR spectrum of curcumin, where there is an amplitude oscillation-band at 3400-3500 cm-1 belonging to the (OH) group, the curcumin spectrum showed stretching vibrations due to phenolic hydroxyl groups at 3200-3500 cm-1, the vibration stretches at 1510 cm -1 linked by an aromatic C = C bond. The bending vibration at 1246 cm -1 is attributed to the C-O phenolic group (25).

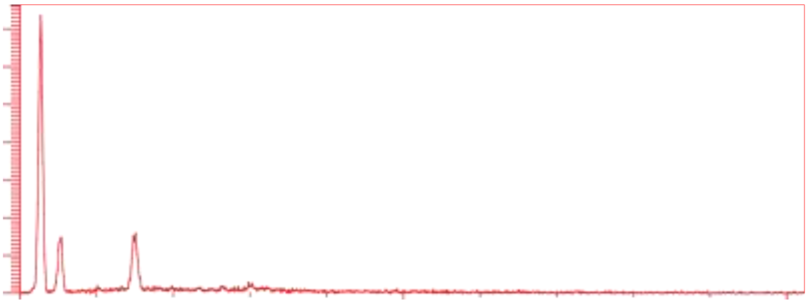

Figure 4. Qualitative analysis of a sample of silver-curcumin nanoparticles prepared using energy

The amplitude vibration at 1600 cm -1 is due to C = C and C – H for the alkyl group appearing at 2918. cm-1. The absorption band at 1627 returns to C=O. The absorption at 1184-1205 cm -1 is due to the bending vibration of the C-OH bond. The absorption at 1429-1460 cm - 1 refers to the CH2 bending. Figure (B-5) shows an infrared spectrum of the prepared silver-curcumin nanoparticles, as it notes the presence of the main peaks with the active groups in curcumin, with the appearance of a clear peak at the finger area indicating the formation of silver nanoparticles.

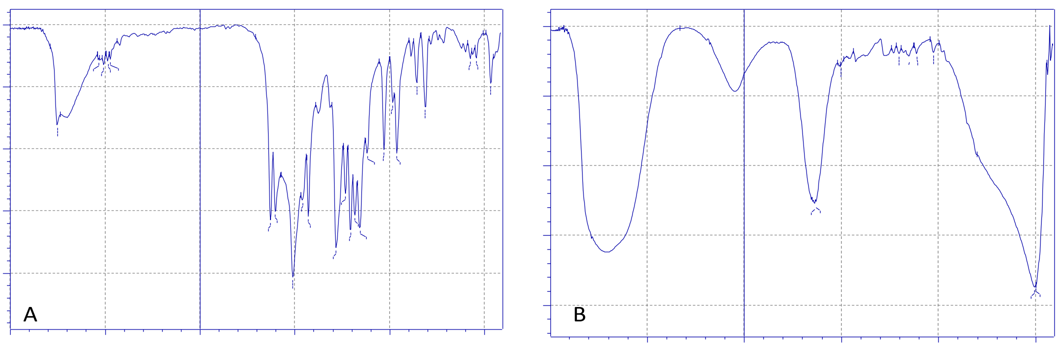

Figure 5. infrared spectrum - Fourier transform of A-curcumin B-nano-silver-curcumin

3.3 Testing of a silver-curcumin nanoparticles as an antioxidant

The results of Figure (6) indicate the effectiveness of -silver-curcumin nanoparticles as an antioxidant compared to the industrial antioxidant Butylated Hydroxytoluene (BHT) (1. 1.5, 2) mg/L, respectively, as the highest effectiveness of the silver-curcumin nano-concentration was at a concentration of 2 mg/L. A comparison was made with the compound Butylated hydroxytoluene (BHT), where the highest effectiveness reached 98.2% at a concentration of 2 mg/L. The reason for the effectiveness of the antioxidant silver-curcumin nanoparticles may be attributed to its ability to suppress free radicals, as antioxidants can protect the body from damage caused by free radicals, as curcumin works to capture free radicals. Vo-Van et al confirmed curcumin-silver nanoparticles showed antioxidant activity higher than (>90) % and anti-inflammatory activity. bacteria.12 [13].

3.4 Testing of a silver-curcumin nanoparticles as an antibacterial agent

The results showed in Figure (7) the effectiveness of -silver-curcumin nanoparticles in inhibiting bacterial isolates at concentrations of 1, 1.5, and 2 mg/L. The highest rate of inhibition at a concentration of 2 mg/L reached 4 mm, and the lowest rate at 1 mg/ L reached 2 mm for *E. coli* bacteria. The concentration exceeded 2 mg/L significantly, while there were no significant differences for *Pseudomonas sp*. and *Staphylococcus aureus* among the different study treatments. When examining the impact of curcumin nanoparticles on four bacterial isolates. Li et al discovered that Gram-positive bacteria were more negatively impacted by the antibacterial activity of the particles against the pathogenic bacteria that were examined [15]. Additionally, the investigation revealed that the antibacterial activity of nano-curcumin demonstrated a broad-spectrum inhibitory impact against all pathogens when tested against S. aureus, B. subtilis, E. coli, and P. aeruginosa.

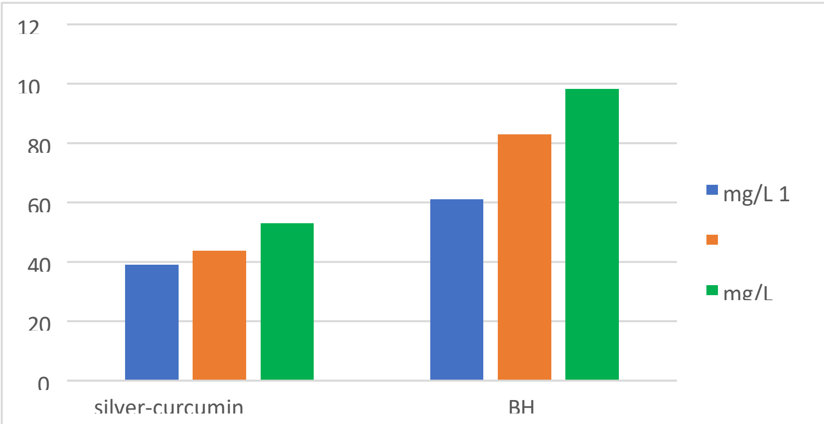

Figure 6. Antioxidant test of a nano-silver-curcumin sample

The results indicated by Negahdari that the rate of inhibition of bacteria *S. aureus, E. coli, and E. faecalis* by nano-curcumin was higher than 99% [7] .

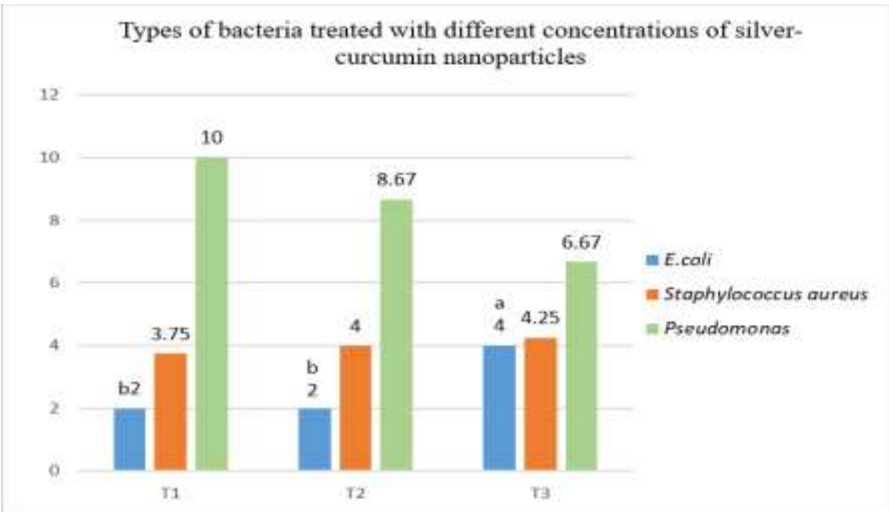

Figure 7. Test of silver-curcumin nanoparticles as antibacterial

The study shown that antibacterial plant components, like curcumin, can gradually replace chemicals in the future. This substitute will not only defeat microbe resistance but also serve as a way to lessen the usage of chemicals and all of their harmful effects. These preliminary data indicate that silver-curcumin nanoparticles (cAgNPs) have high antibacterial efficiency and may have the potential to be developed as effective antimicrobial nanomaterials. Bhawana indicate that the curcumin preparation showed more antibacterial activity (*S. pneumonia* and *E. coli*) than the antibiotic amoxicillin [16]. Silver-curcumin nanoparticles showed antimicrobial activity against three common wound pathogenic bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Pseudomonas aeruginosa* [17].

4. Conclusion

To conclude, this work effectively proved the feasibility of preparing metallic Ag–Curcumin NPs via a new approach combining a dry (neutralization) stage and a wet (oxidation–reduction) phase, and confirmed their structural–chemical and functional properties by means of exhaustive physicochemical characterization. The characterization showed that the nanoparticles were perfectly distributed in the nanoscale range (20–90 nm), had distinct surface plasmon resonance, and carbon, oxygen, and silver as major elements, confirming the stabilization of the Ag–curcumin nanocomposite. In summary, the data presented indicated silver–curcumin nanoparticles with a significant antioxidant strength, with activity increasing with concentration and nearing that of the synthetic antioxidant butylated hydroxytoluene. The nanoparticles also showed significant antimicrobial activity against Escherichia coli, Pseudomonas sp. and Staphylococcus aureus, and the greatest inhibition was seen with Escherichia coli at a higher concentrations. These findings suggest that silver–curcumin nanoparticles could be an effective natural replacement for synthetic antioxidants and other traditional antimicrobial agents for use in biomedical, agricultural and food-related applications. Further molecular work is needed to untangle the specific molecular mechanisms behind their antioxidant and antibacterial effects, additionally, their safety and cytotoxicity needs to be evaluated in biological systems, and in vivo and applied efficacy and stability studies are warranted support effective implementation of these tools into practice.

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