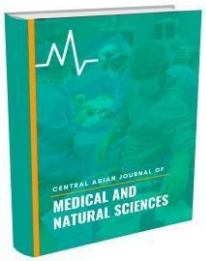

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Article

Green Preparation of Nano-Curcumin Extract and its Applications on Klebseilla Pneumoneiae and Staphylococcus Aureus

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Abstract: This study aims to identify the inhibitory effect of curcumin extract and iron oxide nanoparticles synthesized by co-precipitation method from curcumin extract as a reducing agent, and to test their antimicrobial activity against Klsensiella pneumoniae and Staphylococcus aureus using disk diffusion method. The nanoparticles were characterized using (XRD, SEM, Zeta sizer, zeta potentioal, VSM) techniques, where the crystalline size of the nanoparticles was . 26.8nm and the surface morphology of the particle tends to form spherical clusters with an average hydrodynamic size of 27.58 nm and has a negative charge of about -29.7mV and the zeta size examination showed about 256.4 nm and the particles showed a saturation magnetization of about. The results indicated that the aqueous curcumin extract did not show any inhibitory effect on the bacterial isolates, while the alcoholic extract gave the highest inhibition diameter at a concentration of 500 mg/ml 8 mm for K. pneumoniae. As for the curcumin extract with nano iron oxide, the inhibition diameters for K. pneumoniae at concentrations (600, 500, 400, 300, 200, 100 µg/ml) were (15, 13, 11. , 9, 7, 5) mm, respectively. As for S. aureus, the average inhibition diameters at the same concentrations were (18, 17, 14, 13) mm.

Keywords: Green Synthisis , Fe3O4, K.Pneumoneiae , S.Aureus , Curcumin

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1. Introduction

The increasing demand for metal oxide nanoparticles has led to their increased production using high-energy methods and toxic solvents, causing significant environmental pollution [1] .This conventional process creates harmful effects on the environment and human health, making the search for environmentally friendly alternatives an urgent necessity. Therefore, "green" preparation methods have become necessary, relying on bio-resources such as plants, plant products, bacteria, fungi, yeast, and algae to manufacture metal nanoparticles [2] These methods are characterized by low toxicity and are safe for human health and the environment compared to conventional methods. Moreover, these sustainable methods are cost-effective and reduce chemical waste [3] making them one of the best methods for obtaining metal oxide nanoparticles in an environmentally friendly and sustainable manner. In addition, nanoparticles are used as an alternative treatment for a number of diseases, including diseases caused by multidrug-resistant microorganisms such as bacteria, fungi, parasites, and malaria, including leishmaniasis, and parasitic worms [4]. This interest is due to their unique properties such as high magnetic susceptibility, non-toxicity, chemical stability, high magnetic saturation, and biocompatibility [5]. Green nanoparticle manufacturing is a sustainable method that combines efficiency and environmental compatibility. This method is based on the use of medicinal herbs extracts to reduce metal ions and convert them into nanoparticles. Medicinal herbs contain biologically active compounds such as

phenols and flavonoids, which contribute to the reduction processes and formation of nanoparticles [6].

Curcumin, extracted from turmeric (Curcuma longa) plant, is a prominent example of the use of medicinal herbs in the synthesis of nanoparticles. A study published by indicated that curcumin possesses antioxidant properties that enable it to reduce metal ions such as silver nitrate into stable nanoparticles. When curcumin extract was used to synthesize silver nanoparticles, the particles exhibited strong antibacterial properties against Klebsiella pneumoniae and Staphylococcus aureus [7]. Nanoparticles synthesized using medicinal herbs have been shown to be effective in disrupting bacterial cell functions. According to a study by [8] nanoparticles disrupt the bacterial cell membrane, leading to leakage of their internal contents and death. For example, nanoparticles prepared from ginger (Zingiber officinale) extract showed strong activity against multi-resistant pathogenic bacteria. In addition to medical applications, green-synthesized nanoparticles have shown great potential in water purification and heavy metal removal. According to [9] nanoparticles prepared from hibiscus extract are able to adsorb organic and inorganic pollutants with high efficiency. These studies demonstrate that the manufacture of nanoparticles using medicinal herbs represents a safe and sustainable alternative to conventional methods, with broad potential in medicine, industry and environment [10]. The present study aims to use curcumin extract in the preparation of iron oxide nanoparticles and combine the therapeutic properties of curcumin extract with those of SPIONs, and evaluate their antimicrobial efficacy against K. pneumoniae and S.aureus.

2. Materials and Methods

Materials

In this study, the following materials were used: ferric chloride (FeCl2 - 2H2O , 98%), anhydrous ferric chloride (FeCl3, NH4OH), NaOH, and Sod. Citrate was obtained from BDH, England. The media used for culture were (Mannitol Salt Agar, MacConkey Agar, Nutrient Broth, and Mueller-Hinton). Obtained from Liofilchem, USA

Extraction Process:

Turmeric was collected from local markets in Kirkuk city, dried and then ground. Soxhlet extraction method was used for the extraction process, where 50 g of the ground plant material was placed inside the Soxhlet extraction device using 500 ml of solvent (99% ethyl alcohol and DM water). The extraction process was carried out for 48 hours. The extract was then concentrated using a rotary evaporator, where 5 g of the concentrated extract was obtained. 2 g was dissolved in 10 ml of distilled water to obtain a concentration of 200 ml. It was sterilized using a Millipore filtration unit (0.22μm). The remaining concentrations were prepared [11].

Preparation Of Iron Oxide Nanoparticles With Curcumin

Nano-iron oxide was prepared from ethanolic curcumin extract by the system, and this work was conducted in the graduate studies laboratory in the Department of Physics, College of Education for Pure Sciences. By dissolving (0.69) grams of iron sulfate (FeSO4) (II) (7H20) and (1.35 grams) of iron chloride (III) (FeCl3) (6H20) in (20) ml of deionized water, adding (10) ml of curcumin extract and placing them in a distillation funnel, dissolving (1.2) grams of sodium hydroxide (NaOH), and in an alkaline solution, dissolving (1.77) grams of sodium citrate (NaCH) in (10) ml of deionized water and placing them in a three-necked flask, and adjusting the temperature to (80) Celsius and a rotation speed of 900 rpm for 60 minutes in an oxygen-free environment containing nitrogen gas N2 only [12] (as in the following chemical equation), $Fe^{+2} + 2Fe^{+3} + 8OH^{-1} \rightarrow Fe_3O_4 + 4H_2O$

The Effect of Curcumin Alcoholic Extract and Nano Iron Oxide on The Growth of Bacterial Isolates

The effectiveness of plant extracts and nanoparticles was tested by spreading 0.1 ml of the previously prepared bacterial suspension on the surface of Mueller Hinton Agar using a sterile cotton swab and brushing it well. Then, the culture medium was pierced using a sterile cork piercer with a diameter of 6 mm at regular distances. After that, 0.1 ml of each plant dilution was added into the respective hole and the plates were left to soak with the extract after which they were incubated at 37°C for 24 hours. The diameters of inhibition were measured using the unit of measurement in millimeters [13].

3. Results and Discussion

Characterization Of Fe3o4 Using Xrd

Fig: 1 shows the structural study of the nanoparticles using X-ray diffraction (XRD) in that the crystal size of Fe3O4 nanoparticles prepared from curcumin extract is 26.8nm and this result is consistent with the results of the research [14].

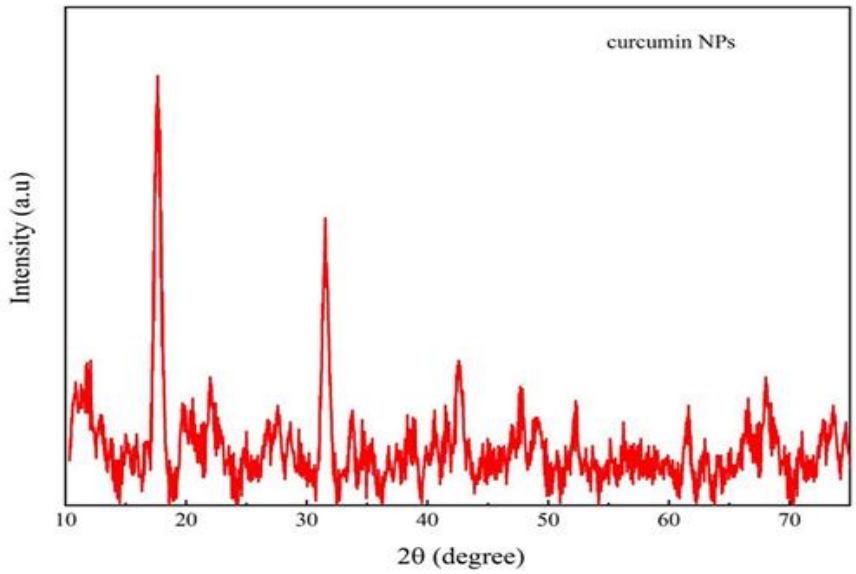

Figure 1. XRD Test Result.

Scanning Electron Microscope (Sem)

Fig: 2 shows that the surface morphology of the molecule tends to form spherical clusters with an average hydrodynamic volume of 27.58, which is consistent with the researcher's study.

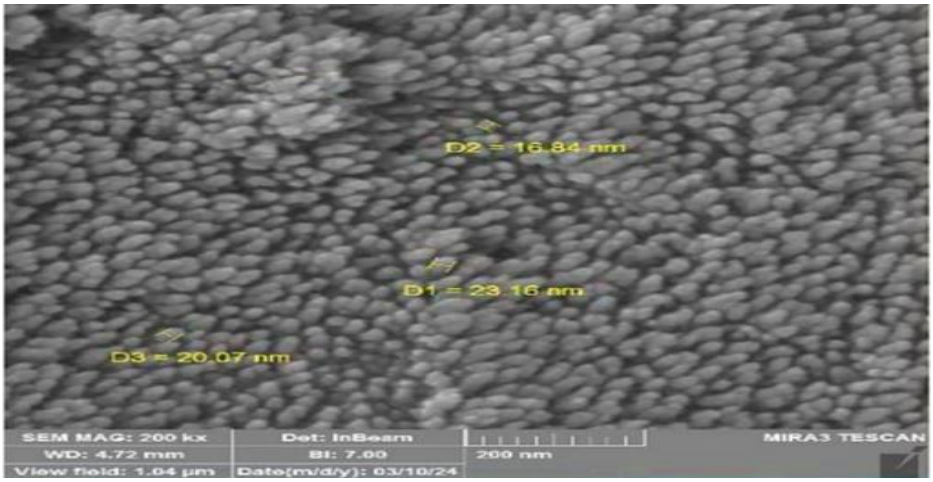

Figure 2. SEM Test Result.

Zeta Potential

measurement Fig: 3 shows the surface charge of Fe3O4 nanoparticles about (-29.7mV) which is consistent with the results of the researcher [15].

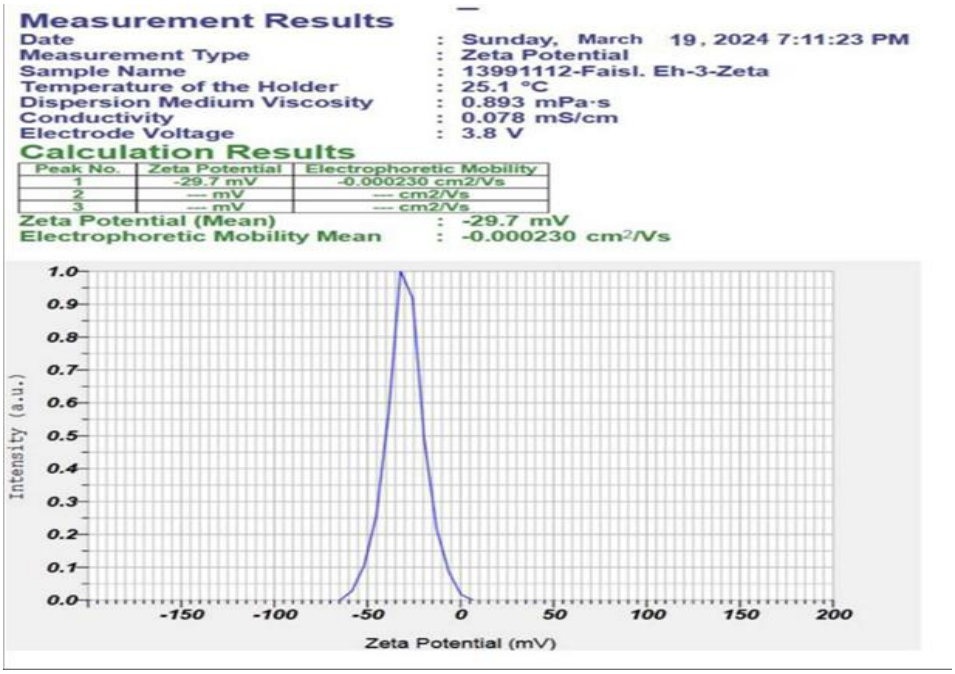

Figure 3. Zeta Potential Result.

Zeta Size

In our current study, the results of the zeta size analysis of the nanoparticles prepared from curcumin extract showed that the zeta size was equal to (256.4 nm) as shown in the figure 4.

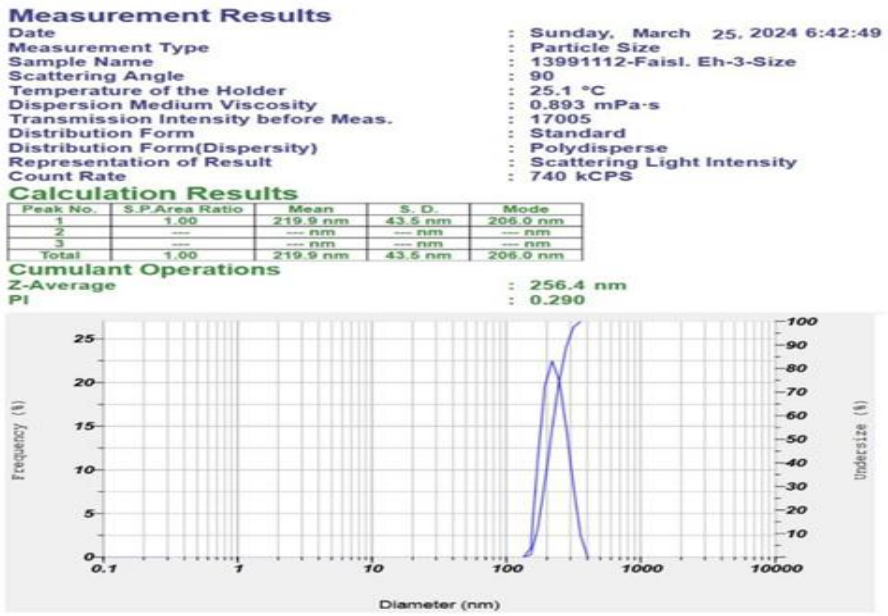

Figure 4. Zeta Size Result

VSM

Fig.5 show Nanoparticles prepared from curcumin extract showed a saturation magnetization of about 5.2 emu/g, which is in agreement with the results of [15].

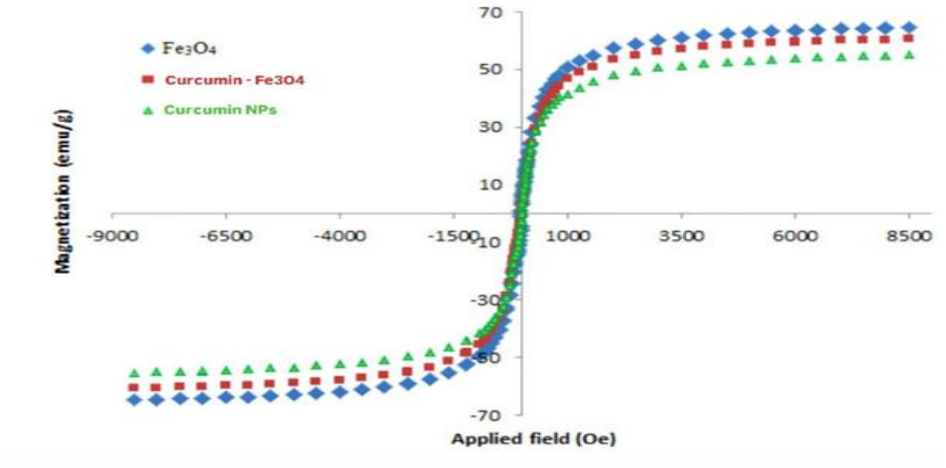

Figure 5. VSM Result,

The Antibacterial Efficacy Of Curcumin

the inhibitory ratios of the alcoholic extract of curcumin gave inhibitory results with an inhibitory diameter of 8 mm for K.pneumoniae, while for S.aureus bacteria, the inhibition ratio of the isolates was 4 mm at a concentration of 500 mg/ml. The concentrations of 400, 300, and 200 mg/ml did not give any inhibitory effect. As shown in the Table. 1 and fig.6. While for the Aqueous extract that did not give any inhibition result for bacterial isolates . These results are comparable to the researcher's results [16].

Table 1. Results of the inhibition diameters of curcumin alcoholic extract on bacterial isolates.

Bacteria species	500 mg/ml	400 mg/ml	300 mg/ml	200 mg/ml
K.pnemoniae	8 ملم	-	-	-
S.aureus	4ملم	-	-	-

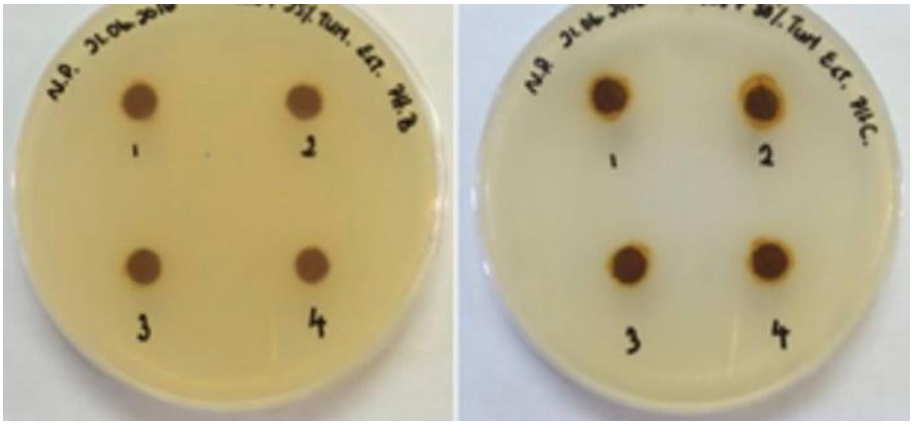

Figure 6. Inhibitory diameters of curcumin extract on bacterial isolates

Anti-Curcumin Activity In The Presence Of Nano-Oxide

The results show that the average diameter of inhibition of the nano-alcoholic curcumin extract on K. pneumoniae at concentrations (600, 500, 400, 300 µg/ml) was (30, 24, 21, 14 mm) respectively, and for S.aureus the average diameter of inhibition at the same concentrations was (33, 27, 23, 17 mm) respectively. As shown in Fig. 7. These results are comparable to the researcher's results [17].

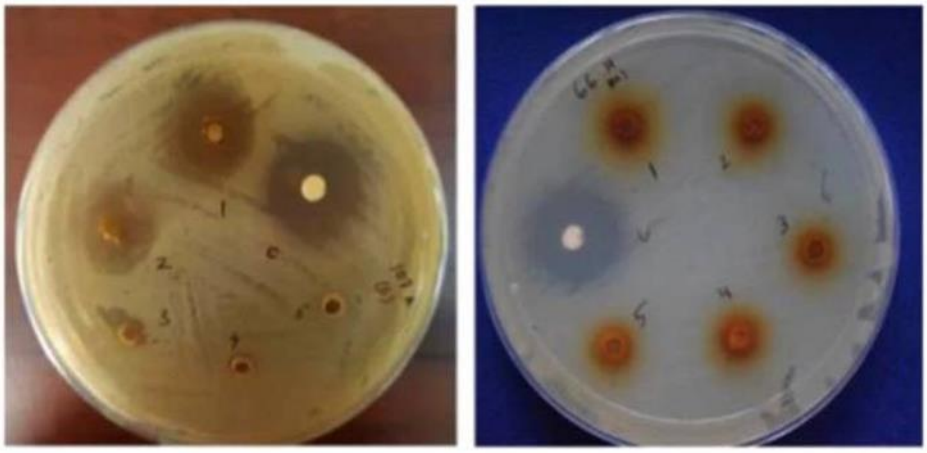

Figure 7. The effect of Fe3O4Nanoparticles prepared from curcumin on K.pneumoniae and S.aureus.

It is clear that with increasing concentrations, the inhibition rate also increases. Iron oxide nanoparticles (Fe3O4) prepared by co-precipitation method showed antibacterial effects against both Gram-positive and Gram-negative bacteria, indicating that these nanoparticles are effective antibacterial agents [18]. The possible mechanism of action is that the metal secondary particles carry positive charges while the microbes carry negative charges, which creates an electromagnetic attraction between the nanoparticles and the microbes. Upon attraction, the microbes are oxidized and die immediately. In general, the nanomaterials release ions that react with the thiol (SH) groups of proteins on the bacterial cell surface, leading to cell lysis [19]. The iron ions generate oxygen radicals. Radicals by converting hydrogen peroxide to reactive hydroxyl radicals through Fenton reaction. Hydroxyl radicals generated by iron ions can damage bacterial polysaccharides, due to breaking DNA strands and inactivating enzymes. Furthermore, magnetic nanoparticles bind to cell membrane or membrane proteins through electrostatic interactions, which can disrupt bacterial function and cause death. The main mechanism that caused the antibacterial activity via the particles may be oxidative stress caused by ROS, which includes radicals such as superoxide (02), hydroxyl (-(OH) and hydrogen peroxide (H2O2). Singlet oxygen (O) can be responsible for damaging proteins and DNA in bacteria. It is possible that reactive oxygen species are produced by the present metal oxide (iron oxide), which inhibits most pathogens such as K.pneumoniae and S.aureus [20].

4. Conclusion

Through this study, the following conclusions were reached: It was found that there is a clear inhibitory effect of the alcoholic extract of curcumin, while the aqueous extract did not give any result on both types of bacteria. The iron oxide nanoparticles prepared from the alcoholic extract of curcumin showed a higher inhibitory effect than the plant extract itself. Characterization evidence indicates that the prepared particles have crystalline sizes within the levels of nanoparticles, are spherical in shape, have a negative surface charge, and possess a super magnetic property.

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