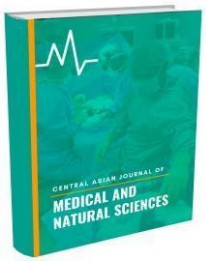

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Article

Preparation and Evaluation of the Antibacterial Activity of Prunus armeniaca Seed Extract Enhanced with Iron Oxide Nanoparticles and Chitosan Coating Against *Escherichia coli* and *Staphylococcus aureus*

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Abstract: The present study aimed to investigate the antibacterial activity of Prunus armeniaca (apricot) seed extract, both alone and in combination with iron oxide nanoparticles (Fe_3O_4 NPs) and chitosan coating, against two pathogenic bacterial strains: Escherichia coli and Staphylococcus aureus. The extract was prepared using a Soxhlet extraction method with hot water, and nanoparticles were synthesized via co-precipitation and coated with chitosan to improve stability and bioactivity. Characterization was performed using XRD, SEM, TEM, zeta potential, and zeta sizer techniques. Results revealed the formation of nanoscale composites with improved physicochemical properties. The disc diffusion method showed that the antibacterial activity increased progressively from the extract alone, to Fe_3O_4 NPs, and finally to the chitosan-coated formulation. The enhanced inhibition is attributed to the synergistic effect of bioactive compounds (e.g., amygdalin, phenolics) and the physicochemical interaction between the nanoparticles and bacterial membranes. These findings suggest that the nanoformulation has promising potential as an effective antimicrobial agent.

Keywords: : *Prunus armeniaca, Staphylococcus aureus, Escherichia coli*, Plant extraction, Fe3O4

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1. Introduction

Nanoscience is the study of matter at the nanoscale, with dimensions ranging from approximately 1 to 100 nanometers, and sometimes as small as 500 nanometers. To put these numbers into context, they are roughly 1,000 times smaller than the width of a human hair. A nanometer is roughly one billionth of a meter, or (9-10 nm). Nanoparticles possess many unique characteristics and properties due to their small size, making them promising candidates for use as drug carriers [1]. Nanoparticles are nanometer-sized particles that exist within the nanoscale in three dimensions. They include nanotubes, nanospheres, nanopores, quantum dots, nanoshells, nanocapsules, nanorings, nanobelts, nanowires, fullerenes, nanorods, nanodendrites, and liposomes. These particles have diverse applications such as drug delivery systems, targeting cancer cells, antioxidants, microbes, and others [2]. Iron oxide nanoparticles (IONPs) are nanoparticles with important properties in the medical field, which, after being chemically modified and coated with a suitable polymer, can penetrate the cell membrane walls of microbes and destroy them [3]. Medicinal plants or herbal medicine have been used since ancient times to treat many

diseases due to their active ingredients or compounds with biological activity such as terpenoids, flavonoids, alkaloids, tannins, phenolic compounds, and many others [4]. The entire plant or parts of it may be medicinally effective. One of the medicinally important plants is the apricot plant (Prunus armeniaca) [5]. Apricots have been used for centuries as an antipyretic, analgesic, antiseptic, tonic, sedative, emollient, antispasmodic, wound-healing, and detoxifying agent. Apricot juice is rich in vitamins and quenches thirst, while apricot kernel oil is used to treat respiratory ailments and coughs. All of these therapeutic benefits are due to its beneficial chemical compounds, vitamins, sugars, organic acids, and minerals. Due to its rich phenolic composition, apricots exhibit antioxidant activity and also exhibit anticancer activity due to their amygdalin (Vitamin B17), which inhibits and prevents the growth of cancer cells. Apricots have significant benefits in ophthalmology, preventing vision loss by increasing the concentration of antioxidants in the eye. They also play a major role in combating cardiovascular disease due to their flavonoid content, which acts as antioxidants [6]. Due to their small size, nanoparticles enter the body through multiple pathways and may reach sensitive organs. Therefore, this problem is solved by directing the nanoparticles' path to the desired target for delivering therapeutics, bioimaging, or other applications. Coating the nanoparticles with biological compounds is done with the aim of ensuring that the coating material does not produce any side effects. Another reason for coating is that some particles, such as iron oxide nanoparticles, are insoluble in water, causing particle aggregation. Therefore, coating is performed using steric repulsion or electrostatics to improve biocompatibility and reduce the intensity of attraction between nanoparticles to prevent their sedimentation or re-agglomeration [7]. Nanoparticles in general and iron oxide nanoparticles in particular can be coated with many biological compounds to increase their stability and direct their path towards the correct target. These materials include chitosan polymer, lipids, polyethylene glycol (PEG), proteins, and others [8]. This study aims to evaluate the inhibitory effect of Prunus armeniaca (apricot) extract after its combination with iron oxide nanoparticles (Fe_3O_4 NPs) and encapsulation with chitosan, against two pathogenic bacterial strains: Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). The research involves assessing the antibacterial efficacy of the prepared nanobiocomposites, with the goal of enhancing the bioactivity of the plant extract and reducing bacterial resistance. The study will compare the effectiveness of the extract alone, the iron oxide nanoparticles, and the chitosan-coated formulation, to determine the possible synergistic effects among these components.

2. Materials and Methods

Materials

In this study, the following materials were used: ferric chloride (FeCl2 - 2H2O , 98%), anhydrous ferric chloride (FeCl3, NH4OH), NaOH, , CH3COOH ,Sod. Citrate and Chitosan 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

The Soxhlet apparatus was used for the extraction process in the Chemistry Laboratory - College of Education for Pure Sciences, University of Kirkuk, where 12 grams of the powdered of P. armeniaca were placed inside the Thumble in the Soxhlet apparatus and using 250 ml of solvent (deionized water), at a rate of 6 hours of extraction per day, after which the extract was concentrated using a rotary evaporator at a temperature not exceeding 40°C. After evaporating all the solvent used in the extraction, a thick layer of the extract was observed to test its antibacterial effectiveness [9].

Preparation of iron oxide nanoparticles with p. armeniaca

These materials used in the preparation of iron salts by the co-precipitation method are: We measure 0.69 g of iron (II) sulfate (FeSO4.7H2O) in 10 ml of deionized water, and dissolve 1.35 g of iron (III) chloride (FeCl3.6H2O) in 10 ml of deionized water, the molar ratio is 1:2 , and add the P. armeniaca extract in a drip funnel dissolved in an alkaline solution, where we dissolve 1.77 g of sodium nitrate in 10 ml of deionized water, and we dissolve 1.22 g of sodium hydroxide NaOH in 10 ml of deionized water in an oxygen-free environment containing only nitrogen gas N2. [10]

Chitosan coating

Chitosan polymer CS from Shaanxi Sangherb Bio-Techinc was used, at concentrations of (0.05, 0.100, 0.125, 0.150, 0.250) grams, and each concentration was dissolved in 30 ml of 1% dilute acetic acid (CH3COOH) solution[11].

Effect of Hot water extract and iron oxide nanoparticles coated with chitosan on bacterial isolates

The disc diffusion method was used, as the test was conducted based on the method [12]. by transferring 3-5 pure colonies growing on the nutrient agar medium at the age of hours to the nutrient broth medium, then incubating the medium at 37°C for 18-24 hours. The bacterial suspension was then diluted with normal saline solution, compared to the standard control tube, which is equivalent to (1.5 x 108) cells/ml. After the comparison, 0.1 cm of the bacterial suspension was withdrawn and spread on the surface of the Mueller-Hinton agar medium using a glass diffuser. The plates were then incubated in the incubator for 30 minutes to obtain absorption. To study the antibacterial activity of the plant extract and nanoparticles on the growth of bacteria, 6 mm diameter filter paper discs were prepared, saturated with different concentrations of the plant extract and nanoparticles to be tested. The discs were fixed with sterile forceps, and the plates were incubated at 37°C for 18-24 hours. An hour later, the inhibition zones were measured with a standard ruler.

3. Results and Discussion

XRD analysis results

To investigate the crystalline structure and particle size of the bioactive constituents, X-ray diffraction analysis was conducted on the Prunus armeniaca Hot water extract. The resulting diffraction pattern, shown in Figure 1, displayed multiple peaks that indicate the presence of various crystalline phases. These peaks corresponded to the monoclinic phase, as referenced in the International Standard Card (No. 96-720-7324), which is often observed in natural substances rich in phenolic compounds, glycosides, and sugars—such as amygdalin commonly found in apricot seeds [13]. The relatively broad and low-intensity peaks suggest the presence of nanocrystalline or partially amorphous structures, which aligns with previous findings on the use of plant extracts as reducing and stabilizing agents in nanoparticle synthesis [14]. Following the incorporation of iron oxide nanoparticles and chitosan, a distinct shift in the XRD pattern was observed, as illustrated in Figures 2 and 3. The new diffraction peaks matched the hexagonal phase reported in the standard reference card (No. 96-723-4561). Additionally, the interaction between Fe_3O_4 nanoparticles and chitosan led to noticeable changes in peak positions and intensities, and in some cases, the appearance of new peaks—indicating potential structural modifications or composite formation [15]. These observations are consistent with similar findings reported by other researchers [16].

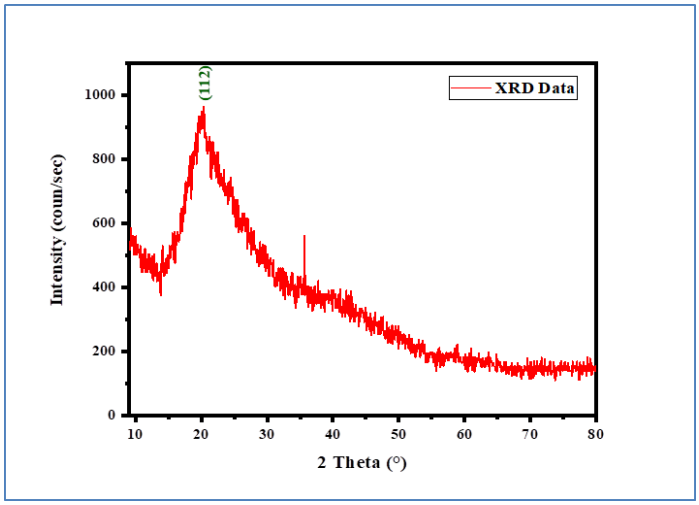

Figure 1. Results of oxygen diffraction examination of the hot plant extract of apricot seeds.

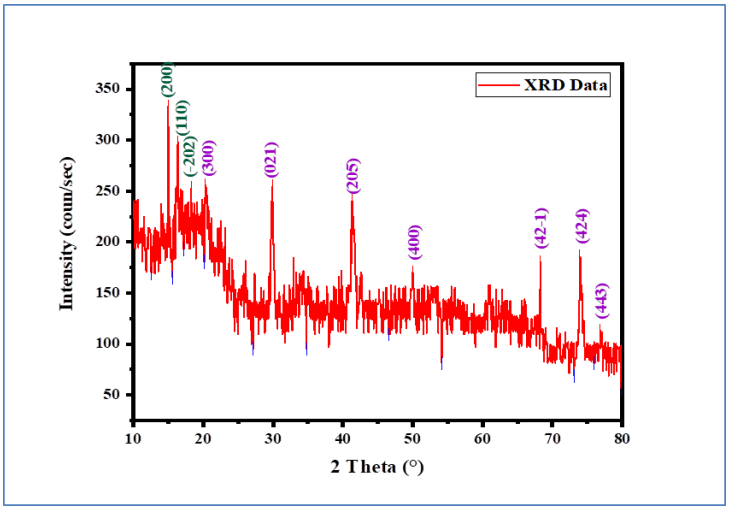

Figure 2. X-ray diffraction results of the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) nanoparticles.

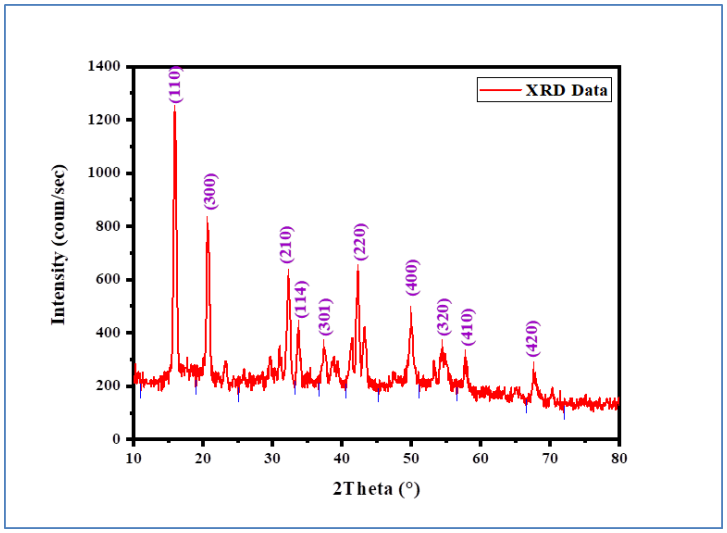

Figure 3. X-ray diffraction results of the hot aqueous extract of apricot seeds after adding iron oxide particles (Fe3O4) with chitosan nanoparticles.

Scanning Electron Microscopy (SEM)

Figure 4 shows the alcoholic apricot seed extract without the addition of nanoparticles. The particle size was approximately 73.67 nm. The particles appeared as irregular structures, porous in places, and possibly containing fine aggregates or plant fibers. These structures indicate the presence of natural components such as polysaccharides, proteins, and phenols. Figure 5 shows the apricot seed extract after the addition of Fe_3O_4 nanoparticles. Spherical or sub-spherical particles were observed with an uneven distribution, some of which were embedded in the surface structure of plant tissues. The particle sizes of the extract varied after the addition of Fe_3O_4 nanoparticles, with the average size being approximately 92.24 nm. The Fe_3O_4 nanoparticles appeared with high contrast in the SEM due to their mineral properties. The uniform distribution suggests that the plant extract played a role in stabilizing the particles and preventing their agglomeration. Plant components may contribute to particle stabilization through hydrogen bonding or coordination [17]. In Figure 6, smooth layers or shells were observed to form covering the mineral particles, with a relatively more homogeneous surface. The particle sizes varied after adding Fe_3O_4 particles to the chitosan nanoparticles, being approximately (73.25) (these results are close to the researcher's results [18].

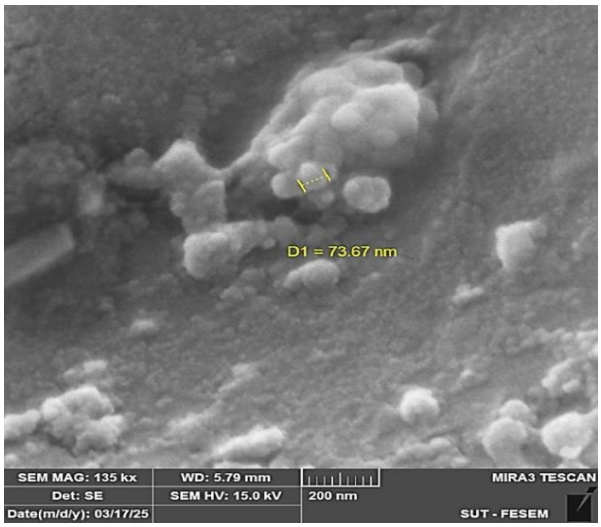

Figure 4. SEM examination results of the hot aqueous extract of apricot seeds.

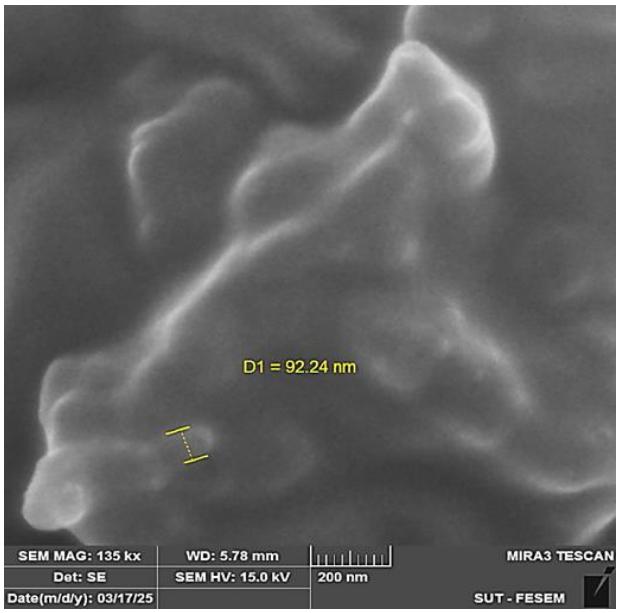

Figure 5. SEM examination results of the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) nanoparticles.

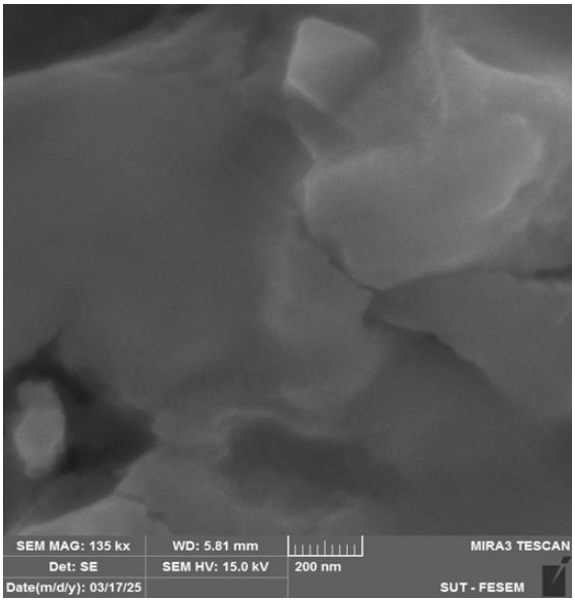

Figure 6. SEM examination results of the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) particles with chitosan nanoparticles.

Tansmission electron microscopy result TEM

Figure 7 represents P. armeniaca extract without the addition of nanoparticles. Spherical or irregular nanostructures were observed. Figure 8 shows the extract after adding Fe_3O_4 nanoparticles. The images show dense, spherical or sub-spherical nanoparticles with diameters ranging from 10–50 nm. These particles are iron oxide (Fe_3O_4) particles, which have magnetic properties and high electron density, making them clearly visible in TEM images. As for the Figure 9 they represent the nano-extract after it was coated with chitosan. The TEM images showed nanostructures coated with a thin layer, indicating that the Fe_3O_4 particles were coated with chitosan nanoparticles [19].

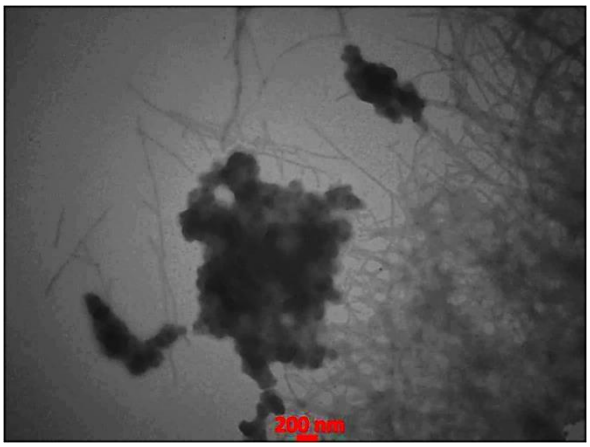

Figure 7. TEM image of the hot aqueous extract of apricot seeds.

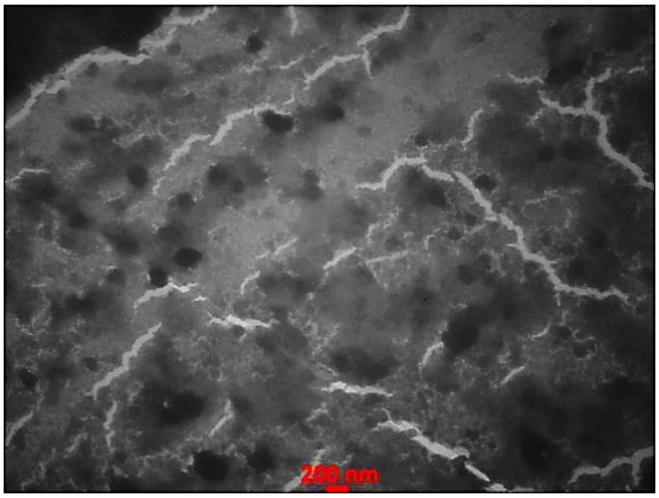

Figure 8. TEM image of the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) nanoparticles.

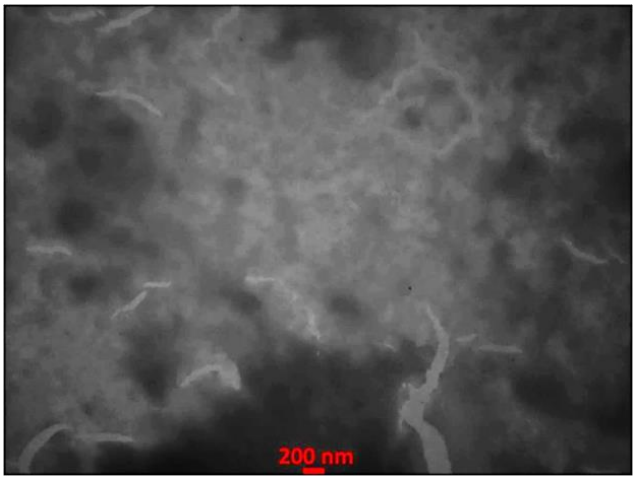

Figure 9. TEM image of the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) particles with chitosan nanoparticles.

Zeta potential results

In Figure 10, it is shown that the charge value is negative, indicating that the compounds extracted from apricot seeds (such as organic acids, phenols, proteins) have a negative charge on their surface. The figure11 shows an increase in the negative charge after adding the Fe3O4 nanoparticles. This indicates that the nanoparticles interact with the extract components, adding a negative charge resulting from the functional groups attached to them (such as -COOH and -OH). The magnetic particles themselves may also contribute to a negative surface charge. The figure 12 shows that although chitosan typically carries a positive charge in acidic media, the overall charge remained significantly negative. This suggests that chitosan may interact with the negatively charged compounds in the extract, modifying the surface of the nanoparticles to increase stability without changing the charge polarity. This Result is Compatible with [20].

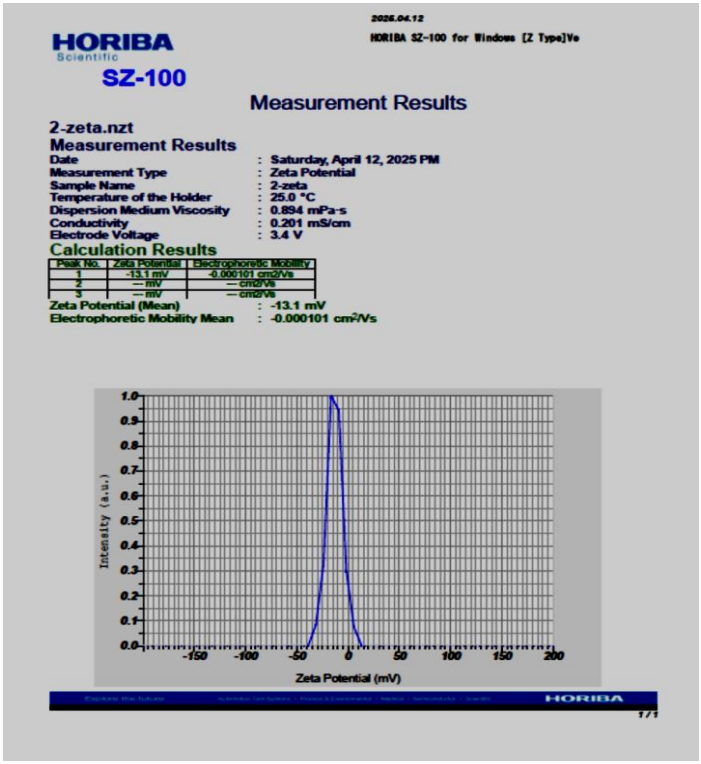

Figure 10. Zeta potential test results for the hot aqueous extract of apricot seeds.

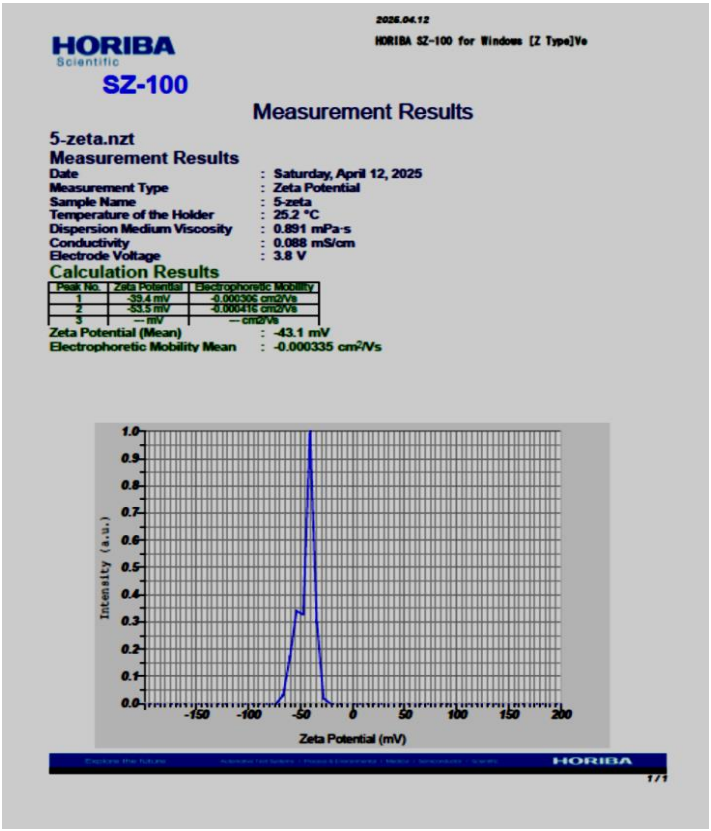

Figure 11. Zeta potential test results for the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) nanoparticles.

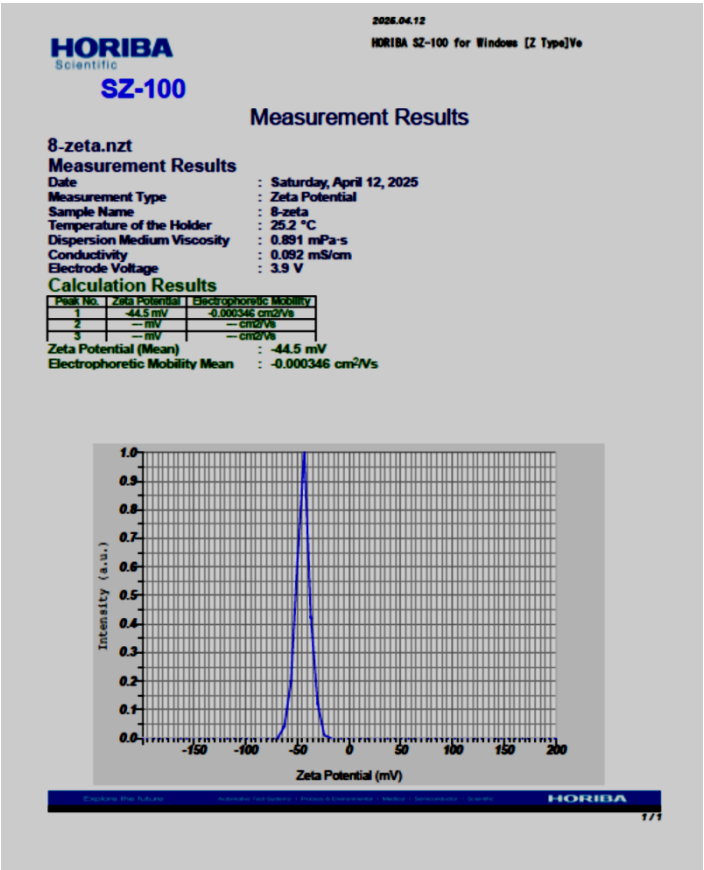

Figure 12. Zeta potential test results for the hot aqueous extract of apricot seeds after adding iron oxide particles (Fe3O4) with chitosan nanoparticles.

Zeta seizer Result

Figure 13 represents the hot aqueous extract of apricot seeds. The large size observed in the hot aqueous extract (3536.1 nm) indicates aggregation of molecules, probably due to heat stimulating interactions between extract components (e.g., proteins, polyphenols). Figure 14 shows the apricot seed extract after adding Fe_3O_4 nanoparticles. A significant size decrease was observed in the hot aqueous extract from (3536 → 556.7) nm after adding Fe_3O_4, indicating that the nanoparticles helped reduce agglomeration due to surface stabilization. Figure 15 shows the extract after adding Fe_3O_4 + chitosan nanoparticles. In the hot aqueous extract, the size decreased to 273.9 nm, indicating that chitosan acted as a good encapsulating and stabilizing agent with Fe_3O_4, resulting in more uniform and less agglomerated particles.

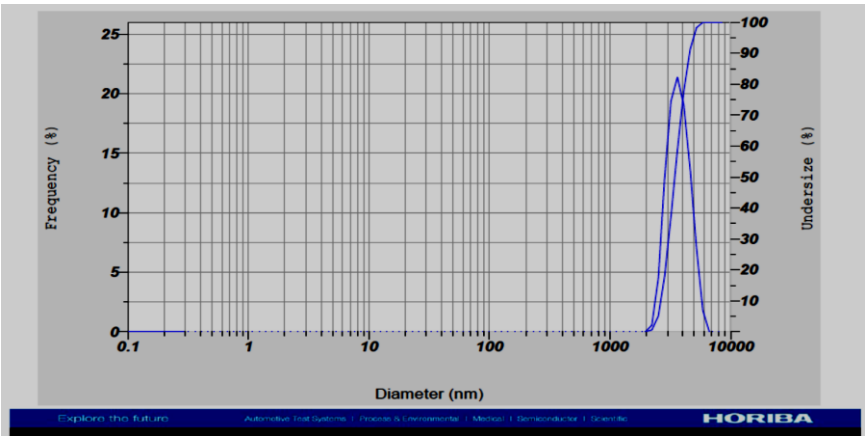

Figure 13. Zeta-sizer test results for the hot aqueous extract of apricot seeds.

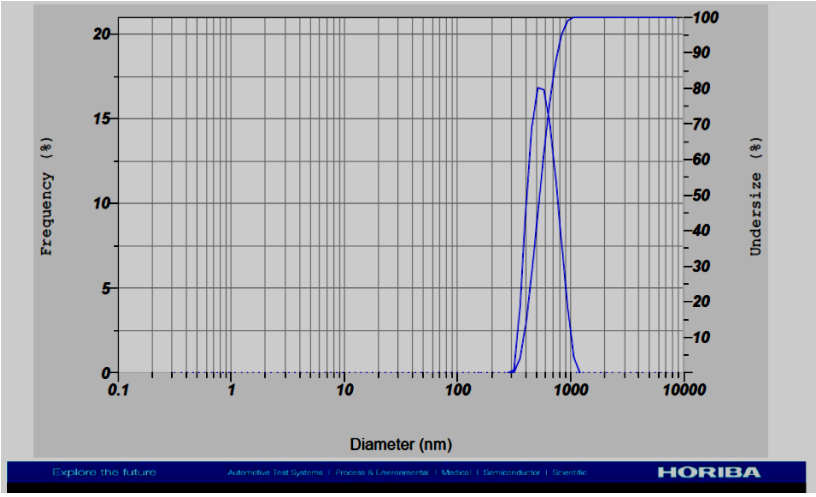

Figure 14. Zeta-sizer test results for the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) nanoparticles.

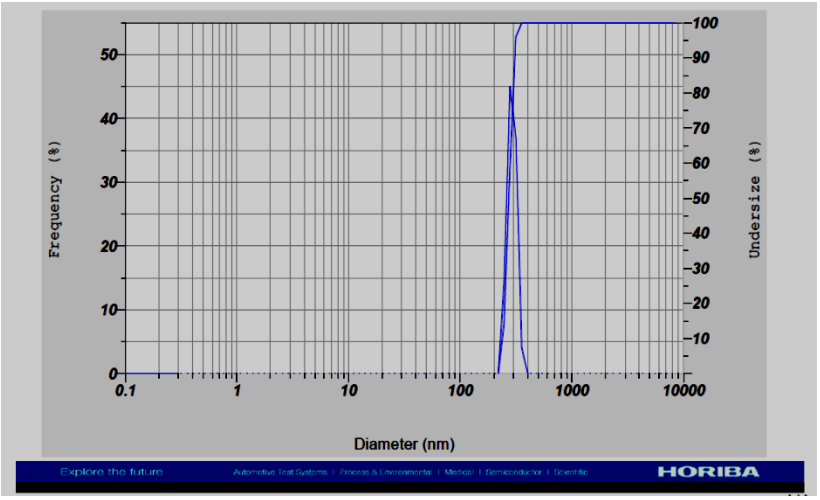

Figure 15. Zeta-sizer test results for the hot aqueous extract of apricot seeds after adding iron oxide (Fe3O4) particles with chitosan nanoparticles.

The effectiveness of plant extract and nano iron oxide coated with chitosan

The inhibitory effect of the hot aqueous extract, as the average diameters of inhibition at concentrations of 350, 300, 250, and 200 micrograms/ml were (18, 21, 23, 24) mm for E. coli, while the results increased to (21, 23, 26, 25) mm when adding nano iron oxide Fe3O4. After coating with chitosan, the average diameters of inhibition increased to (24, 26, 28, 33), while the results for S. aureus were (19, 22, 24-25) mm. After adding nano iron, the results increased to (20, 24, 25, 27) mm, and after coating it, it increased to (22, 24, 25, 27).

Apricot seed extract contains a group of biologically active compounds, most notably amygdalin, phenolic compounds, and flavonoids, which play a key role in its antibacterial effect. Its mechanism of action is attributed to a set of integrated mechanisms, including damage to the bacterial cell wall and membrane and inhibition of vital bacterial enzymes. Chitosan-coated iron oxide nanoparticles are promising nanomaterials for combating pathogenic bacteria, thanks to their unique physical and chemical properties. Their mechanism of action against bacteria is through binding to the cell membrane. The positive charge of chitosan facilitates the attachment of nanoparticles to the negatively charged bacterial cell walls, disrupting the permeability of the cell membrane. Cell wall penetration: Due to their nanoscale size, the particles are able to penetrate the bacterial cell wall, causing damage to the cellular structure. In addition to producing reactive oxygen

species (ROS), nanoparticles stimulate the production of ROS within the bacterial cell, leading to the oxidation of vital components such as proteins and DNA, resulting in cell death. Chitosan's bioinhibition impedes the synthesis of DNA and proteins within the bacterial cell, inhibiting their growth and reproduction. Studies have shown that chitosan-coated nanoparticles have antibacterial efficacy superior to that of iron oxide or chitosan alone. For example, one study showed that these particles were effective against various types of bacteria, including Escherichia coli and S. aureus [21].

4. Conclusion

Prunus armeniaca seed extract exhibits antibacterial properties due to its active compounds such as amygdalin and phenolics , The addition of iron oxide nanoparticles enhanced the antibacterial activity through a synergistic effect with the plant extract, Coating the nanoparticles with chitosan further improved their biological stability and increased their inhibitory effectiveness, Physical analyses confirmed the formation of a stable and effective nanocomposite, The resulting nanostructure represents a promising approach for developing improved plant-based antibacterial agents.

REFERENCES

[1] H. D. Beyene, A. A. Werkneh, H. K. Bezabh, and T. G. Ambaye, "Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review," *Sustain. Mater. Technol.*, vol. 13, pp. 18–23, 2017.

[2] S. S. Mirsasaani, M. Hemati, E. S. Dehkord, G. T. Yazdi, and D. A. Poshtiri, "Nanotechnology and nanobiomaterials in dentistry," in *Elsevier eBooks*, pp. 19–37, 2019.

[3] S. Ashrafi-Saiedlou, M. Rasouli-Sadaghiani, M. Fattahi, and Y. Ghosta, "Biosynthesis and characterization of iron oxide nanoparticles fabricated using cell-free supernatant of Pseudomonas fluorescens for antibacterial, antifungal, antioxidant, and photocatalytic applications," *Sci. Rep.*, vol. 15, no. 1, 2025.

[4] A. Abubakar and M. Haque, "Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes," *J. Pharm. Bioall. Sci.*, vol. 12, no. 1, p. 1, 2020.

[5] N. S. Gupta, N. M. Chhajed, N. S. Arora, G. Thakur, and R. Gupta, "Medicinal value of apricot: a review," *Indian J. Pharm. Sci.*, vol. 80, no. 5, 2018.

[6] S. Z. Hussain, B. Naseer, T. Qadri, T. Fatima, and T. A. Bhat, "Apricots (Prunus Armeniaca)—Morphology, taxonomy, composition and health benefits," in *Springer eBooks*, pp. 91–102, 2021.

[7] M. Nikzamir, A. Akbarzadeh, and Y. Panahi, "An overview on nanoparticles used in biomedicine and their cytotoxicity," *J. Drug Deliv. Sci. Technol.*, vol. 61, p. 102316, 2020.

[8] M. González-Béjar, L. Francés-Soriano, and J. Pérez-Prieto, "Upconversion nanoparticles for bioimaging and regenerative medicine," *Front. Bioeng. Biotechnol.*, vol. 4, 2016.

[9] K. P. Ingle et al., "Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts," *J. Pharmacogn. Phytochem.*, vol. 6, no. 1, pp. 32–36, 2017.

[10] A. Gupta and M. Gupta, "Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications," *Biomaterials*, vol. 26, pp. 3995–4021, 2005.

[11] X. Jiang, L. Li, Y. Zhu, and Z. Tang, "Synthesis and magnetic characterizations of uniform iron oxide nanoparticles," *Phys. B Condens. Matter.*, vol. 443, pp. 1–5, 2014.

[12] E. Matuschek, D. F. J. Brown, and G. Kahlmeter, "Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories," *Clin. Microbiol. Infect.*, vol. 20, pp. O255–O266, 2014.

[13] V. J. Sundar, C. Muralidharan, and A. B. Mandal, "Eco-benign stabilization of skin protein—role of Jatropha curcas oil as a co-tanning agent," *Ind. Crops Prod.*, vol. 47, pp. 227–231, 2013.

[14] P. Dutta, K. Rinki, and J. Dutta, "Chitosan: A promising biomaterial for tissue engineering scaffolds," in *Chitosan for Biomaterials II*, pp. 45–79, 2011.

[15] S. O. Ogunyemi et al., "Green synthesis of zinc oxide nanoparticles using different plant extracts and their antibacterial activity against Xanthomonas oryzae pv. oryzae," *Artif. Cells Nanomed. Biotechnol.*, vol. 47, no. 1, pp. 341–352, 2019.

[16] S. M. Ahmed, H. S. Akbar, and A. H. Al-Mashhadani, "Antioxidant and antibacterial activities of bio-synthesized Al-Hanaa nanoparticles," *Iraqi J. Appl. Phys.*, vol. 20, no. 2A, pp. 291–296, 2024.

[17] S. Bhattacharjee, "DLS and zeta potential–what they are and what they are not?," *J. Control. Release*, vol. 235, pp. 337–351, 2016.

[18] A. H. Fathallah, H. S. Akbar, and F. M. N. Al-Deen, "Preparation and characterization of superparamagnetic iron oxide nanoparticles (Fe3O4) for biological applications," *Tikrit J. Pure Sci.*, vol. 26, no. 1, 2021.

[19] M. Arakha et al., "Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface," *Sci. Rep.*, vol. 5, p. 14813, 2015.

[20] S. Ashrafi-Saiedlou, M. Rasouli-Sadaghiani, M. Fattahi, et al., "Biosynthesis and characterization of iron oxide nanoparticles fabricated using cell-free supernatant of Pseudomonas fluorescens for antibacterial, antifungal, antioxidant, and photocatalytic applications," *Sci. Rep.*, vol. 15, p. 1018, 2025.

[21] S. Faisal et al., "Tailoring the antibacterial and antioxidant activities of iron nanoparticles with amino benzoic acid," Dept. of Chemistry, Univ. of Malakand, 2022. [Online]. Available: https://doi.org/10.1039/D2RA06756G