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RESEARCH PAPER

Sustainable-Green Synthesis and Characterization of ZnO Nanoparticles and Evaluation of Their Antibacterial and Antioxidant Activities

Alyaa Salah Rashid [1], Ishrak Khalil Ibraheem [1], Juman Ahmed Nasser [2], Sahar Salim Mohammad [1]*

[1] *Department of Basic Sciences, Collage of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Iraq*

[2] *Ibn al-Haitham Department of Chemistry, College of Education for Pure Sciences, University of Baghdad, Iraq*

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ABSTRACT

Nanoparticles (NPs) display unique characteristics in contrast to conventional physio-chemical synthesis methods, they are utilized in a diverse array of life science fields. Metal nanoparticles synthesized using green methods, primarily derived from plants and herbs, have attracted significant interest because of their inherent properties such as environmental friendliness, quick production, and cost efficiency. In this study, zinc oxide nanoparticles (ZnO-NPs) were synthesized through an aqueous extraction of *Achillea fragrantissima*, which served as a reducing agent. Following synthesis, the ZnO-NPs were characterized using a combination of techniques: ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). The antioxidant potential of these synthesized ZnO-NPs was assessed using the DPPH assay, with varying concentrations of ZnO-NPs employed. The results demonstrated significant scavenging activity, with an IC_{50} value of 1.09 ± 0.09 mg/ml^{-1}. Furthermore, the disk diffusion method was employed to assess the anti-bacterial efficacy of ZnO-NPs synthesized using green techniques against two pathogenic bacterial strains. The findings revealed that *Staphylococcus aureus* exhibited the highest susceptibility to the biosynthesized zinc oxide nanoparticles (ZnO-NPs), in contrast, *Escherichia coli* demonstrated the lowest sensitivity among the tested microorganisms. Overall, this research offers biologically synthesized ZnO-NPs as a viable substitute for artificial compounds, showcasing their potential applications as antioxidants and antibacterial agents within the biomedical and pharmaceutical sectors.

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INTRODUCTION

The use of nanotechnology and green synthesis of nanoparticles in various fields is a novel trend. The creation and engineering of novel materials with unique properties can be achieved through the use of nanoparticles, which are a multidisciplinary arena. [1]. When reduced to nanosized levels, nanomaterial structures have unique functionalities such as large surface area and size-dependent properties that enhance their catalytic, biological, physical, and chemical properties compared to bulk counterparts. [2].

* *Corresponding Author Email: alyaa.alsalihi@ibnsina.edu.iq*

Thus, the utilization of nanoparticles in various industrial applications, as well as chemical and medical fields, has been made possible by their unique properties [3,4]. Through this, consumers are able to choose a green and environmentally-safe method for synthesizing nanoparticles [5]. Zinc oxide (ZnO) has sparked the interest of numerous scientists due to its possible application in the biosynthesis of NPs, because of its special characteristics and various applications, including solar cells, drug delivery, photocatalytic breakdown, and self-care products such as cosmetics and sunscreens [6,7,8].

More than 100 species are included in the genus *Achillea* (Asteraceae) and they are chemically distinguished by the accumulation of sesquiterpene lactones and flavonoids [9]. Volatile oils, tannins, monoterpene ketones, and sesquiterpene lactones have been identified by previous phytochemical investigations of *Achillea fragrantissima* [10]. In addition, *A. Fragrantissima*, like many other plants, has been previously investigated for the presence of cirsimaritin, chrysoplenol and other phytochemical components, it also contains essential fatty acids lauric, myristic, palmitic, stearic, linoleic, linolenic and oleic acid [11].

Therefore, in recent years, there has been an increase in the number of studies focusing on using plant extracts through employing a green synthesis method, thus nanoparticles of metal and metal oxide can be produced in controlled sizes and shapes [12]. According to many reports, biosynthetic pathways can create nanoparticles with a more accurate size and morphology than certain physicochemical methods of production [13].

The development of antimicrobial agents and surface coatings has been increasingly popular in recent years due to the serious issue of microbiological contamination in the healthcare and food industries [14]. In consequence, controlling pathogenic microbes can be achieved by using green synthesized nanoparticles, which are environmentally friendly and cost-effective [15]. Moreover, the antibacterial efficacy of NPs synthesized from plant extract can be improved by the presence of diverse biomolecules that cap these nanomaterials [16]. The alteration of the protein structure in bacteria has been confirmed to occur as a result of the direct interaction between nanoparticles and bacterial cells, causing an increase in cell membrane permeability [17]. Thus, ZnO-NPs synthesized through biological processes exhibit stronger inhibitory properties against pathogenic bacteria and fungi compared to NPs manufactured through chemical means [18].

Antioxidants are crucial in biosystems as they work to eliminate the free radicals produced as a result of biological processes [19]. Due to the toxicities associated with synthetic antioxidants, there has been a shift in research focus towards antioxidants derived from natural sources [20]. Plant extracts are composed of diverse concentrations and combinations of bioactive compounds, enabling them to function as both reducing and capping agents in the nanoparticle synthesis process. Prior reports have demonstrated that aqueous extracts derived from *A. fragrantissima* displayed antioxidant properties by effectively scavenging 1,1-Diphenyl-2-picrylhydrazyl [DPPH) free radicals, alongside their capacity to hinder bacterial proliferation. [21,22]. Moreover, it was found that phytochemicals present in the *A. fragrantissima* extract have shown promising neuroprotective properties, including the ability to reduce oxidative stress, inflammation, and neuronal damage in the brain, these factors are crucial for the growth and advancement of neurodegenerative disorders like Alzheimer's and Parkinson's [23]. Thus, this research aims to achieve two main goals: the green synthesis and characterization of ZnO-NPs using *A. fragrantissima* extract, and the assessment of ZnO-NPs' antibacterial and antioxidant properties.

MATERIALS AND METHODS

Materials

The study utilized several chemical compounds, including gallic acid, rutin, and 1,1-diphenyl-2-picrylhydrazyl (DPPH, ≥99%). Additionally, Folin–Ciocalteu's reagent and L-ascorbic acid were sourced from Sigma-Aldrich, located in St. Louis, MO 63103, USA. Anhydrous aluminum chloride was obtained from Fluka in Buchs, Switzerland, while sodium carbonate (>99%) and zinc acetate dihydrate were procured from Advent Chembio PVT. LTD in Mumbai, India. The culture media employed in this research comprised Luria-Bertani (LB) broth medium from Himedia, also based in Mumbai, India, along with nutritional broth. All chemicals utilized in this investigation were of analytical grade.

Preparation and extraction of A. fragrantissima Extract

Freshly collected *Achillea fragrantissima* were washed, and dried in the oven for two days at 50 °C and then grinded into a fine powder. The process of extracting took place by mixing 5 g of the powdered substance with 100 mL of distilled water in a 250 mL conical flask and then mixing for 30 minutes at 60 °C. After that, the solution was cooled and filtered through a Whatman No.1 filter paper. The water-based extract was utilized as soon as the filtration step was completed.

Estimation of Total Phenolic and Flavonoid Contents (TPC and TFC)

The quantification of total phenolic content (TPC), expressed as mg GAE/g extract, and total flavonoid content (TFC), represented as mg RE/g extract, was conducted using spectrophotometric techniques. The Folin-Ciocalteu reagent was employed for TPC analysis [24], while the aluminum chloride method was utilized for TFC assessment [25].

Green Synthesis of ZnO Nanoparticles

About 50 mL of 0.1 M zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) (1.095 g of zinc acetate dehydrate salt was dissolved in 50 mL of deionized H_2O). A mixture was prepared by combining 25 ml of *A. fragrantissima* extract with the other components, followed by vigorous stirring for a duration of two hours. Upon completion of the reaction, the resulting precipitate, which exhibited a dirty coloration, was allowed to settle for 24 hours. The precipitate was subsequently isolated from the reaction mixture through centrifugation at 6000 rpm for 15 minutes. It was then washed multiple times with deionized water to eliminate impurities and dried in an oven at 80 °C. The resulting powdered sample was subjected to calcination in a muffle furnace at 350 °C for three hours. The powder obtained was utilized for further characterization.

Characterization Methods of ZnO-NPs

UV-Vis Spectroscopy

In order to investigate the light-absorption characteristics of ZnO nanoparticles synthesized via green methods, a specific mass of ZnO nanoparticles (0.05 g) was uniformly dispersed in 5 mL of 96% ethanol. The absorption spectrum was obtained using a UV-Vis (U-2900) spectrophotometer featuring a double beam configuration (Hitachi, Tokyo, Japan), covering a wavelength range from 200 to 800 nanometers.

Fourier Transform Infra-Red Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy from Bruker in Berlin, Germany, was utilized to pinpoint the chemical functional groups (FGs) engaged in the production of zinc oxide nanoparticles (ZnO-NPs). The spectra were examined at a range of 4000 to 400 cm^{-1} with a spectral resolution of 4.0 cm^{-1}.

Scanning Electron Microscopy (SEM)

The morphology of biosynthesized ZnO-NPs was identified by using a scanning electron microscope (SU3500, Hitachi), operating at a voltage of 10 kV, a working distance of 12.3 mm, and a pressure of 42 Pa. These solid ZnO-NPs were uniformly coated over an aluminium plate, secured through a contact adhesive layer on the aluminium surface.

Energy-dispersive X-ray Spectroscopy (EDX)

The purity of the biosynthesized zinc oxide nanoparticles (ZnO-NPs) was assessed using energy-dispersive X-ray spectroscopy (EDX) (JOEL 6390LA instrument from Japan). To prepare the ZnO-NPs for analysis, they were diluted in ethanol and subjected to sonication in an ultrasonic cleaner (Elma, Germany) for a duration of 30 minutes. Subsequently, a 4 µl aliquot of the ZnO-NPs sample was placed onto an aluminum plate prior to EDX analysis.

Estimation of Antioxidant Activity of ZnO-NPs

The antioxidant activity of the ZnO-NPs was determined using a slightly modified version of a previously described technique by Brand-Williams (26]. The antioxidant capacity of ZnO-NPs was tested using five varying concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) against DPPH. As a means of control, ascorbic acid was utilized. Briefly, a test tube was used to mix 1 mL of DPPH with 1 mL of the sample, which was then vortexed and left at room temperature for 30 minutes. The transition in color from purple to yellow signifies the antioxidant capacity of the specimen. 1 milliliter of DPPH was mixed with 1 milliliter of methanol to create the blank. The absorbance of the reaction mixture was recorded at a wavelength of 517 nm. The scavenging activity was determined using the equation provided:

DPPH inhibition (%) = [(Absorbance $_{(Control)}$ − Absorbance $_{(sample)}$)] × 100

An IC_{50} value was also calculated by plotting % scavenging activity against the concentrations of sample extract. IC_{50} represents the 50 % inhibition at a particular concentration.

Bacteria Strains

The antibacterial properties of the biosynthesized ZnO nanoparticles utilizing *Achillea fragrantissima* were demonstrated against one Gram-positive bacterium, *Staphylococcus aureus*, and one Gram-negative bacterium, *Escherichia coli*. The bacterial strains were cultured on Luria–Bertani (LB) agar at a temperature of 30 °C for 24 hours, after which they were stored at 4 °C in a refrigerator**.**

Antibacterial activity of ZnO-NPs

The antibacterial efficacy of biosynthesized ZnO nanoparticles (ZnO-NPs) was evaluated using the agar disc diffusion method against the bacterial strains *Staphylococcus aureus* and *Escherichia coli*. A fresh overnight culture of each bacterial strain was evenly spread across separate agar plates. This investigation utilized various concentrations of the biosynthesized ZnO-NPs (15, 20 and 25 µg/mL^{-1}) and 20 µg/mL^{-1} of *A. fragrantissima* aqueous extract were dissolved in autoclaved distilled water to avoid contamination. Wells were created in plates that contained nutrient agar medium, which had been inoculated with 100 µL of each bacterial strain, and then incubated for a duration of 24 hours. Followed by the addition of 100 µL in separate wells of each solution that contains different concentration of ZnO-NPs and *A. fragrantissima* extract, as well as 15 µg/mL^{-1} of gentamycin as a positive control. The plates were stored in the refrigerator for a duration of 2 hours and subsequently incubated at 37 °C for 24 hours. The diameters of the inhibition zones were then measured and recorded in a table.

Statistical Analysis

Each analysis was conducted in triplicate, and the results are presented as mean ± standard deviation (n=3). Statistical evaluations were carried out using the Statistical Package for the Social Sciences software, version 25.0 (SPSS Inc., Chicago, Illinois, USA). To compare the means across different groups, one-way ANOVA was employed. A p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The Estimation of Total Phenolic and Total Flavonoids Contents

Total phenolic and flavonoid contents of the aqueous extract of *A. fragrantissima* was evaluated. Results showed that the plant is rich in flavonoids (24.18 ± 0.89^{b} mg RE/g extract) and phenolic compounds (29.39 ± 0.16^{a} mg RE/g extract). Phenolic acids and flavonoids are recognized as effective hydrogen donors [27], which contribute to a range of biological activities attributed to their functional groups, specifically carboxyl and hydroxyl groups [28]. Furthermore,

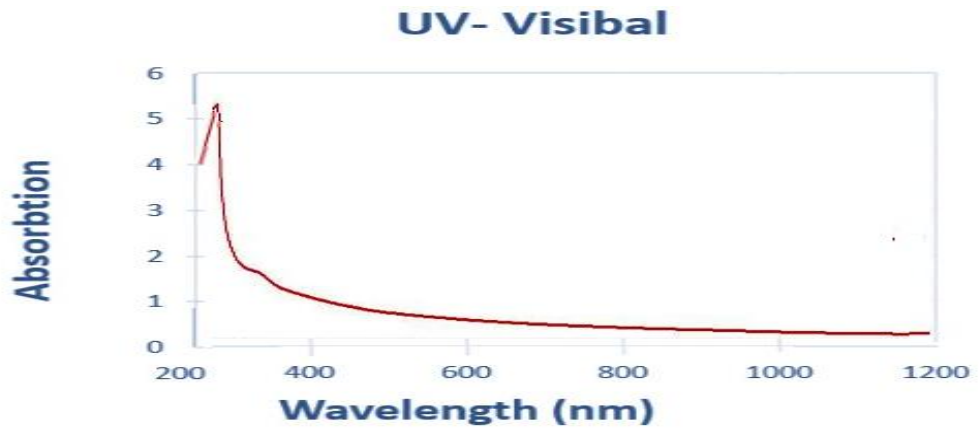

Fig. 1. Shows the UV-Vis Spectrum of ZnO Nanoparticles.

the presence of these biomolecules may facilitate the bio-reduction of metal salts into nanoparticles [29-31]. *A. fragrantissima* has previously been tested for chemical ingredients and biological activities by researchers [32,33].

UV-Vis Spectroscopy

The synthesis of ZnO nanoparticles was verified through the use of UV/Vis spectrophotometry. The maximum absorbance peak was observed at 378 nm in the UV-Vis spectrum, as illustrated in Fig. 1, which confirmed the synthesis of ZnO-NPs via *A. fragrantissima* aqueous extracts, which is close to the outcomes of an earlier study by Kaiyun Xu et al. [34], who examined the ability of *Selaginella convolute* aqueous extracts to bio-synthesize ZnO-NPs after preforming UV/Vis spectrophotometry at a unique peak 399 nm. Based on the current results, it is pertinent to suggest that high absorption peak at 378 nm might be correlated to ZnO's inherent band-gap absorption caused by electron transitions from the valence band (E_V) to the conduction band (E_C) (O_{2p}–Zn_{3d}). This is in agreement with previous reports by Suresh et al. [35] and Zak et al. [36]. It is widely recognized that metal oxide nanoparticles exhibit prominent absorption bands within the range of 200 to 800 nm at room temperature. In the current study, ZnO-NPs Demonstrated an absorbance maximum at 378 nm, which is a typical absorption band of ZnO-NPs as shown in (Fig. 1).

FTIR analysis

The Fourier Transform Infrared (FTIR) technique

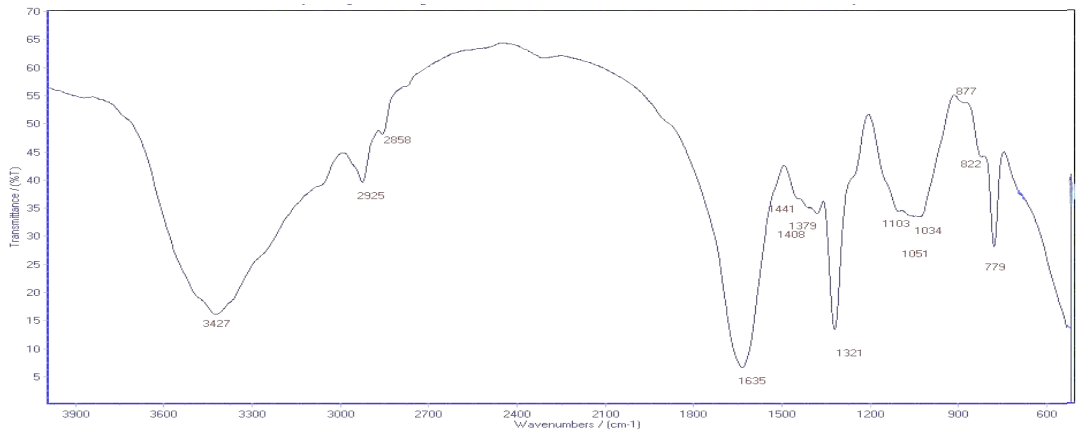

Fig. 2. FTIR Spectrum of *A. fragrantissima*.

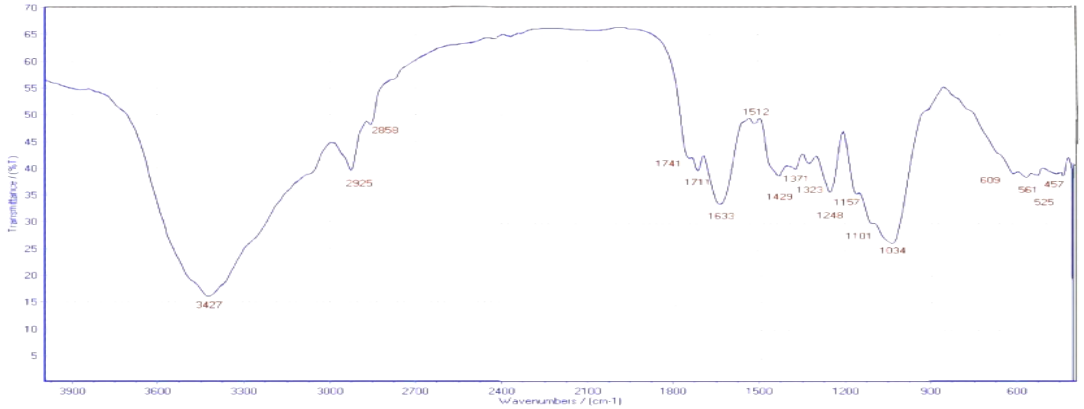

Fig. 3. FTIR Spectrum of Bio-synthesized Zinc Oxide Nanoparticles.

was employed to identify potential functional groups present in the aqueous extracts of *A. fragrantissima* that assist in the reduction and stabilization of ZnO nanoparticles. The peaks of *A. fragrantissima* aqueous extracts and biosynthesized ZnO-NPs are displayed in Figs. 2 and 3, respectively. The spectrum shows a very intense and broad band at 3427 cm^{-1} connected with the stretching vibration of hydroxyl group O-H. It can thus be suggested that the broad stretch peak between 3400 and 3430 cm^{-1} signifies the detection of an O-H stretch band in the extract and ZnO-NPs. This finding suggests the existence of O-H stretching associated with alcohol, phenolic, and flavonoid compounds, as has been documented in prior studies [37,38]. Moreover, the low intensity peak that arise at 2925 cm^{-1} corresponds to the stretching vibration associated with the –CH group in hydroxyl compounds or the N-H group in amines, as identified in previous studies [39,40]. The FTIR analysis of biosynthesized ZnO nanoparticles revealed absorption peaks at wavelengths of 475 cm^{-1} and 561 cm^{-1}, this is a clear evidence of the formation of bio-synthesized ZnO nanoparticles in the presence of the plant extract. The present findings seem to be consistent with other reports

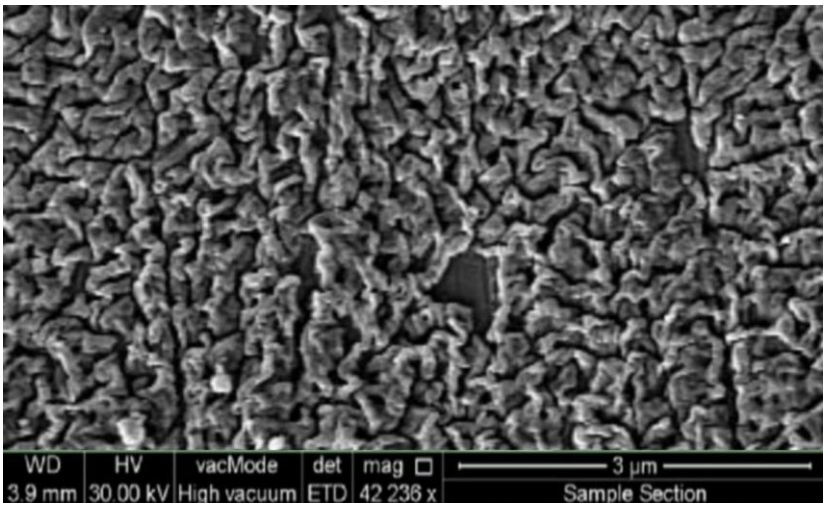

Fig. 4. SEM Image of Biosynthesized Zinc Oxide Nanoparticles.

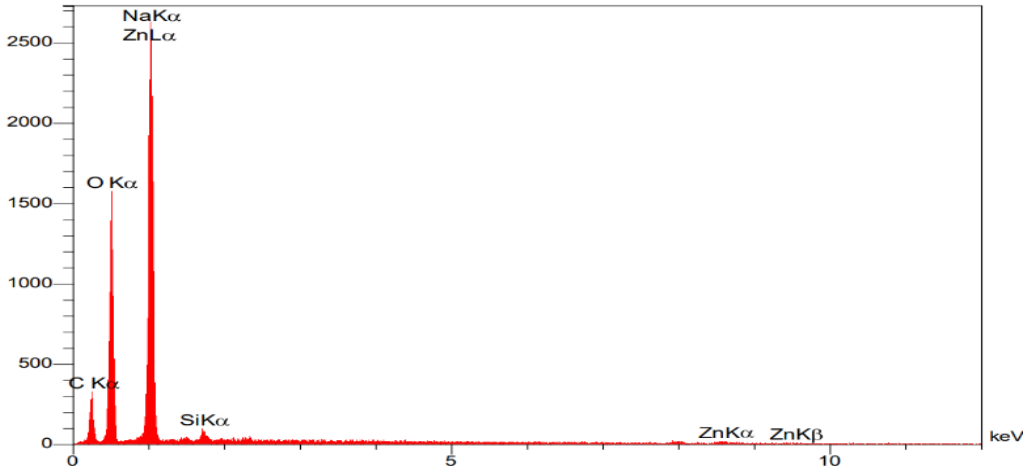

Fig. 5. X-ray Scattering Spectroscopy of Biosynthesized Zinc Nanoparticles.

which found that the typical absorption peaks of ZnO-NPs at wavelengths ranging from 400 to 500 cm^{-1} can be assigned to the existence of various biomolecules capable of functioning as surfactants, which adhere to the surface of ZnO nanoparticles (ZnO-NPs), contributes to the stabilization of these nanoparticles via electrostatic interactions [41, 42]. Consequently, it has been demonstrated that the aqueous extract of *A. fragrantissima* possesses the dual capacity to both reduce and stabilize ZnO-NPs [43,44].

Scanning Electron Microscope –SEM and EDX Analysis

The size and morphology of the green synthesized zinc oxide nanoparticles were examined using FE-SEM (Fig. 4), while the chemical composition of the biosynthesized ZnO nanoparticles was analyzed through EDX (Fig. 5). The SEM image clearly indicates that ZnO nanoparticles display an irregular spherical shape, with agglomeration likely resulting from magnetic interactions and the adherence of polymers among the nanoparticles [45]. The SEM image also confirmed the mean size of ZnO nanoparticles ranging between 50 and 87 nm. These results are in agreement with Islam et al. [46]. In addition, X-ray Energy Dispersive spectroscopy (EDX) techniques were employed to conduct a more in-depth investigation of the samples, aiming to enhance the understanding of the topographical features of the ZnO nanoparticles. The spectra demonstrated two distinct and clear peaks, each of which is due to the presence of the two elements zinc and oxygen, at the energies of 0.52 keV, 1.2 keV, respectively. As shown in Fig. 5. These results have a very strong correlation with already reported studies [47-49].

Antioxidant Activity of ZnO-NPs

The assessment of the free-radical scavenging potential of the aqueous extract of *A. fragrantissima* and the biosynthesized ZnO nanoparticles was conducted utilizing the DPPH radical scavenging assay (see Fig. 6). The findings indicated that the DPPH scavenging activity exhibited by both the extracts and the bio-fabricated ZnO nanoparticles was positively correlated with their concentration levels. The percentage of inhibition observed at the maximum concentration (2.5 mg/mL) of the *A. fragrantissima* aqueous extract was 40%, while this amount for the bio-synthesized ZnO-NPs was 71%. The ZnO nanoparticles exhibited the highest antioxidant properties when compared to the extract, possibly due to the binding of bioactive compounds to ZnO nanoparticles. This phenomenon may also be linked to the concurrent action of polyphenols, which function as antioxidant agents, alongside ZnO serving as a catalytic agent [50].

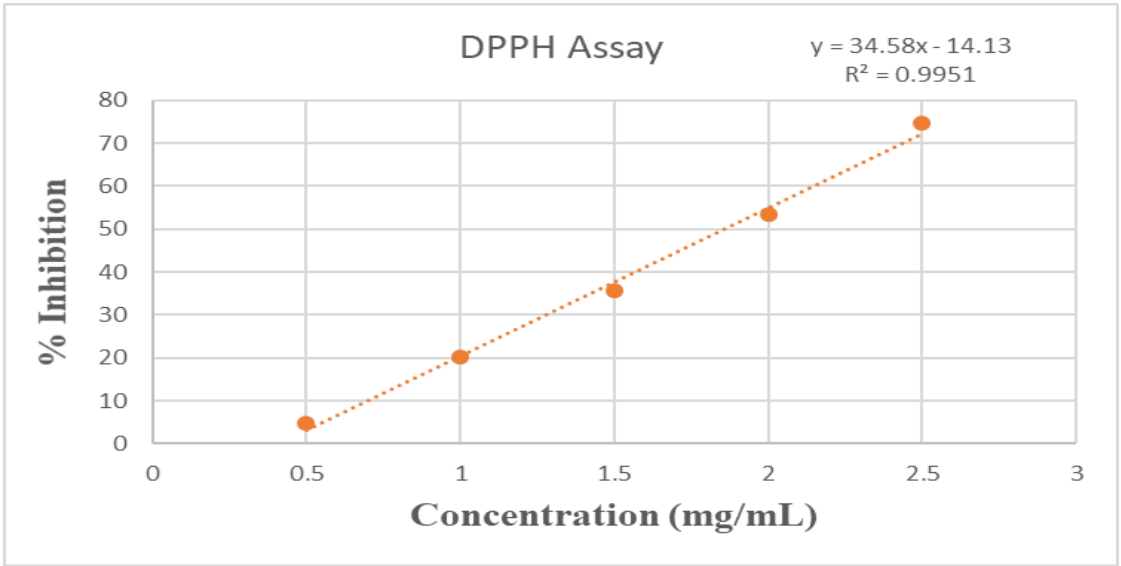

Fig. 6. Inhibition (%) of DPPH by Different Concentration of Biosynthesized ZnO-NPs.

Table 1. Evaluation of the Antibacterial Activity against Pathogenic Bacteria.

Bacterial strains	Diameter of Inhibition Zones (mm)			Gentamycin (15 µg mL^{-1})	(AFE) (20 µg mL^{-1})
	ZnO-NPs				
	15 µg mL^{-1}	20 µg mL^{-1}	25 µg mL^{-1}		
E. coli	10 ± 0.30^{d}	12 ± 0.72^{cd}	15 ± 0.50^{c}	13 ± 0.28^{c}	0 ± 0.00
S. aureus	14 ± 0.63^{b}	17 ± 1.12^{ab}	19 ± 0.84^{a}	15 ± 0.45^{cd}	0 ± 0.00

a, b, c means with different superscripts on the same column differ significant ($p < 0.05$).
*Data are expressed as mean ± standard deviation (n = 3).

Antibacterial Activity of ZnO-NPs

The antibacterial effect of the biosynthesized ZnO NPs was evaluated by disc diffusion assay against *E. coli* as GNB, and *S. aureus* as GPB. The findings are illustrated in Table 1. Overall, the data indicated that the biosynthesized ZnO nanoparticles derived from *A. fragrantissima* aqueous extracts exhibited a notable antibacterial activity against all bacterial strains evaluated. The most substantial inhibition was observed against *S. aureus* (25 ± 0.84) followed by *E. coli* (13 ± 0.50). However, aqueous extracts of *A. fragrantissima* did not detect any inhibitory sites in the tested bacterial strains. In addition, the antibacterial activity of the highest concentration of biosynthesized ZnO-NPs (25 µg mL^{-1}) was found to be similar to that of gentamycin, which was used as a positive control. The current investigation revealed that the biosynthesized ZnO nanoparticles exhibited considerable antibacterial efficacy against Gram-positive bacteria (*Staphylococcus aureus*) in contrast to Gram-negative bacteria (*Escherichia coli*). This observation aligns with a previous report conducted by Vijayakumar et al. [51] who reported that zinc oxide nanoparticles (ZnO-NPs) produced using *Laurus nobilis* leaf extract demonstrated a markedly greater antibacterial efficacy against Gram-positive bacteria (*Staphylococcus aureus*) compared to Gram-negative bacteria (*Pseudomonas aeruginosa*). Perhaps this is due to the composition and structure of GPB, such as the peptidoglycan layer, which could enhance the binding of ZnO-NPs to the cell wall. Conversely, the components of GNP may prevent such binding [52]. Furthermore, the findings showed that the inhibitory effect of biosynthesized ZnO nanoparticles, produced using aqueous extracts of *A. fragrantissima*, intensified as the concentration of ZnO nanoparticles increased. This observation aligns with the conclusions drawn by Gunalan et al. [53], according to the report, the growth inhibition showed a consistent increase with the rise in ZnO-NPs concentration in wells, which was attributed to the optimal diffusion of NPs in the agar medium.

CONCLUSION

Achillea fragrantissima extract was utilized in the bio-synthesis of ZnO-NPs through an environmentally friendly method. The ZnO nanocomposites produced were analyzed using UV-Vis, FTIR, and SEM-EDX techniques. The ZnO nanocomposites acquired are irregularly spherical and display a prominent absorbance peak at approximately 378 nm. Based on the findings from the FTIR analysis, it can be inferred that the components found in *Achillea fragrantissima* aqueous extract play a crucial role in reducing and stabilizing the nanocomposites. The outcomes also indicated that the ZnO nanocomposites possess remarkable antimicrobial properties against harmful strains (*E. coli and S. aureus*). Furthermore, these nanoparticles exhibit notable antioxidant activity, with a free radical scavenging capacity of around 70%. In conclusion, the study suggests that the ZnO-NPs synthesized through green synthesis using the aqueous extract of *Achillea fragrantissima* exhibit diverse biological importance, warranting further research for potential incorporation of the ZnO-NPs in pharmaceutical formulations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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