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EVALUATION THE EFFECT OF SYNTHESIZED ZINC OXIDE NANOPARTICLES FROM *MYRTUS COMMUNIS* L. LEAVES EXTRACT ON *ESCHERICHIA COLI*

*Safa A. A. Ramina M. K.
Dept. Biotech. Coll. Sci. University of Baghdad

ABSTRACT

This study was aimed to estimate the effect of zinc oxide nanoparticles synthesized by *Myrtus communis* L. on the expression of the adhesion genes *fimH* and *fimA* in isolates of uropathogenic *Escherichia coli* (UPEC). The biosynthesized-ZnO NPs was characterized through several techniques including UV–vis spectral analysis, atomic force microscopy (AFM), Field Emission Scanning Electron Microscope (FE-SEM) and Fourier transform infrared (FTIR). For determination of MIC and sub-MIC of ZnO NPs, only 7 multi-drug resistant (MDR) and strong-biofilm producer *E. coli* isolates (harbored both *fimH* and *fimA*) were selected based on our previous work. Microtiter plate method was utilized and the results indicated that only three isolates (C1, C2 and C23) with MIC and sub-MIC (125 and 62.5 µg/ml), respectively, were selected for further experiments. Quantitative Real Time PCR was utilized for determining the gene expression of *fimH* and *fimA* in three isolates (C1, C2 and C23), with and without treatment with ZnO NPs. According to the results, the relative expressions ($2^{-\Delta\Delta Ct}$) of *fimA* gene in C1, C2 and C23 isolates of *E. coli* were downregulated in 0.51-fold after treated with 125 µg/ml of ZnO NPs, while the relative expressions ($2^{-\Delta\Delta Ct}$) of *fimH* gene in C1, C2 and C3 isolates of *E. coli* were upregulated in 4.42-fold after treated with 125 µg/ml of ZnO NPs.

Keywords: ZnO NPs, MIC, *fimH*, *fimA* ,Real-time PCR

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INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) bacteria is a rod-shaped, facultatively anaerobic, Gram-negative bacilli that do not produce spores and are usually observed in the digestive tract as a warm-blooded species' natural colonizer (mostly in mammals, but also in fish, birds, and reptiles). Although these bacteria are part of the normal flora, some strains can be harmful and cause gastrointestinal as well as UT infections (Sarowska *et al*, 2019). This bacterium is characterized by producing different virulence factors, which help the bacterial cells to invade and adhere the host. . One of these factors is type 1 fimbriae , which encoded by different genes. such as *fimH* and *fimA*, which contribute to formation of biofilm. The *FimH* gene is part of the Fim operon, which encodes a type 1 fimbriae surface organelle found in most *E. coli* strains (Zuberi et al,2017). It plays the most important role in its pathogenicity. Fimbriae type 1 adhesion factor (*FimH*) for colonization in extra-intestinal infections (Hojati *et al*,2025). It has also been reported that FimA, which makes about 95% of type I fimbriae, is the primary structural unit. In addition, the fimbriae assembly relies heavily on the surface expression of 4200 fimbrial filaments, which can be found only on fimbriated bacteria (Berne *et al*,2015). This property in UPEC strains make them good biofilm producers, which may contribute to their resistance to antimicrobials, which considered as the common therapeutic strategy. However, the emergence of antibiotic resistance has forced scientists to find alternative strategies. Hence, one of these strategies is nanotechnology. In recent years, nanoparticles have attracted a lot of attention as a result of their unique properties in terms of their optical characteristics, catalytic

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activity, and antibacterial properties. All these unique characteristics have enhanced their potential application in different fields such as biomedical, communications, and electronics. With these properties, nanoparticles have been able to exhibit unique applications that are being used across fields (Precious *et al*, 2019). Numerous nanoparticles, in particular ZnO, exhibit exceptional antibacterial activity against a variety of pathogens, including *Escherichia coli*. In addition, ZnO nanoparticles has proven several alluring uses in biomedicine and antimicrobial activities, catalysts, photodetectors, and solar cells (Alaa Alden &Yaaqoob,2022, Khalaf,2023). Researchers have turned to green synthesis for nanoparticle production because of its low cost, high efficiency, and wide availability (Ahmed *et al*,2017). Some plants, including *Myrtus communis*, regarded as one of the most important medicinal plants in Iraq due to the presence of active compounds like terpenes, essential oils, alkaloids, tannins, saponins, flavonoids, glycosides and phenols, have been reported as having been used in the biological method for creating ZnO NPs (Ali et al,2023). Sub-inhibitory levels of these compounds are being studied for their impact on biofilm development and signaling in bacterial cells, in addition to their inhibitory effects on pathogens. Pathogenic bacteria use quorum sensing (QS), also known as cell-to-cell signaling, to control processes including biofilm development and virulence factor production. Thus, the QS mechanism can be used to develop novel therapeutics. At sub-inhibitory concentrations, the nanomaterials are able to suppress QS and stop the growth of biofilms and pathogenicity in pathogens (Jamuna Bai & Ravishankar Rai,2018).The aforementioned advantages provide great potential in the utilization of nanoparticles as quorum quenching agent. This study was aimed to estimate the effect of zinc oxide nanoparticles synthesized by *Myrtus communis* L. on the expression of the adhesion genes *fimH* and *fimA* in isolates of uropathogenic *Escherichia coli* (UPEC).

MATERIALS AND METHODS

Collection, isolation and identification: A total of hundred fifty urine samples were obtained from patients with UTI, during the period from October 2022 to February 2023, with the approval of the College of Science Research Ethics Committee based on (CSEC/0123/0016). All these samples were supplied from Medical City hospitals in Baghdad. These samples were collected immediately from the patients and kept in sterile containers and transferred into laboratory for diagnosis. All these samples were subjected to several biochemical tests (indole, catalase, oxidase and citrate utilization) for isolating and identifying UPEC isolates (Abujnah *et al*,2015, Beneduce et al,2003).

Preparation of *Myrtus communis* extract

The *Myrtus communis* plant's leaves were collected, cleaned, and chopped into small pieces. An aqueous extract was produced by boiling 20 gram of fresh, small pieces in 200 mL of distilled water at 40 °C for 1 hour, followed by an overnight incubation. After the mixture had cooled to room temperature, it was centrifuged at 4000 rpm for 10 minutes. Leachate was removed (Jan *et al*,2021).

Biosynthesis of ZnO NPs using *Myrtus communis* extract: 20 g of zinc acetate ($ZnC_4H_6O_4$, 99%) (Himedia/India) was added to the leachate of *M. communis* and placed in a rotator for 24 hrs. Then, the mixture was centrifugated at 4000 rpm for 10 min. Deionized water was utilized for washing the leachate for 2 times. The leachate-containing tubes were centrifugated for 4000 rpm for 10 min. The leachate was filtrated using filter for 2 times. The leachate was placed in solid glass dish and putted in incubator until dry. The dried leachate was collected and kept in dark containers until use (Alaa Alden &Yaaqoob, 2022)

Characterization of ZnO nanoparticles

Different techniques were utilized in order to characterize ZnO including ultra-violate visible light (UV-Vis), atomic force microscopy (AFM), field emission scanning electron microcopy (FE-SEM) and fourier transforms infrared (FTIR) (Alaa Alden &Yaaqoob, 2022).

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Determination of the minimum inhibitory concentration (MIC) of ZnO nanoparticles

The ZnO nanoparticles' antibacterial activities were achieved against gram-negative bacteria (*E.coli*), 96 well plate was employed for this specific test which leads to minimum inhibition concentration (MIC) determination (Janaki *et al*,2015). Various concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9 and 1.95 µg/ml) of ZnO nanoparticles and negative control (synthetic ZnO nanoparticles) were added to the wells and then incubated for 24 hrs at 37 °C. After incubation, the MIC was determined.

Estimation of gene expression of *fimA* and *fimH:* Extraction of total RNA: Total RNA was extracted from three selected isolates of *E. coli* using the Qubit™ RNA HS Assay Kit according to the manufacturer's instructions (ThermoFisher®/ USA).

Synthesis of complementary DNA from Mrna: The synthesis of cDNA from RNA was performed using ProtoScript® First Strand cDNA Synthesis Kit (NEB/UK) according to manufactured instructions.

Real time quantitative PCR (RTq–PCR): The quantitative PCR technique was performed using the Luna Universal qPCR Master Mix. The goal of the quantitative PCR technique was to determine the cycling threshold (Ct) of genes (*fimA* and *fimH*) and housekeeping gene (*16S rRNA*). The primers of genes and their amplicon sizes were illustrated in Table (1). The components of qPCR that utilized in this study as follows:

Nuclease Free Water (3 µl), Reverse primer (10 µM/1 µl), Forward primer (10 µM/ 1 µl), Luna Universal qPCR Master Mix (10 µl) and Template DNA (5 µl) to reach the total volume (20 µl). The reaction mixture was amplified in a thermocycler (Eppendorf/ Germany) with the following PCR conditions: initial denaturation at 94.0 °C for 5 minutes, denaturation at 94.0°C for 30 second, annealing at 58.0 (*fimA*), 56.0 (*fimH*) and 60 (*16S rRNA*) °C for 40 second, extensions at 72.0°C for 45 second and final extensions at 72.0 °C for 5 minutes.

Real time quantitative PCR (RTq–PCR) for the detection of gene expression.

The cycling threshold (Ct) was calculated using the software used by a real-time cycler. Each sample was executed twice and the values of mean were estimated. The expression analysis of required genes was normalized against the housekeeping gene. Results were reported as a folding change in gene expression based on the ΔΔCt method's recommendations for data processing (Livak & Schmittgen. 2001). Differential CT values (Ct) were determined for each sample by comparing the CT values of target and housekeeping genes, as follow:

$\Delta Ct_{treated} = CT_{gene\ of\ interest} - CT_{Housekeeping\ gene}$

$\Delta Ct_{control} = CT_{gene\ of\ interest} - CT_{Housekeeping\ gene}$

The relative fold change in Ct values (ΔΔCt) for the genes of interest was determined. as follow: $\Delta\Delta Ct = \Delta Ct_{treated} - \Delta Ct_{control}$

The fold-change in gene expression was calculated as follow: Fold change=$2^{-\Delta\Delta Ct}$

Statistical analysis

Prism-Graph Pad was used to analyze the collected data. The ONE-WAY ANOVA was used to compare three sample groups with $p < 0.05$ considered significant.

Table 1. The primers and their amplicon sizes

Target gene		Primer's Sequence (5'-3')	Sizes of Amplicon (bp)	References
fimH	F	-GCTGTGATGTTTCTGCTCGT-	167	(Yazdi *et al*,2018)
	R	-AAAACGAGGCGGTATTGGTG-		
fimA	F	-TTGTTCTGTCGGCTCTGTCC-	261	This study
	R	-AGGCAACAGCGGCTTTAGAT-		
16s rRNA	F	-TTATCCCCCTCCATCAGGCAG-	134	This study
	R	-ATGGCTCAGATTGAACGCTGG		

RESULTS AND DISCUSSION

Collection, isolation and identification A total of a hundred fifty urine samples were collected. Only 52 isolates were identified by culture on selective and differential MacConkey and EMB media. On MacConkey agar, the isolated *E. coli* showed up as pinkish colonies, while on Eosin Methylene Blue, they had a green metallic sheen. Biochemical tests were indicated that isolates were indole-

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positive, catalase positive, oxidase –negative, citrate utilization-negative, whereas these isolates were identified as *E. coli*. The multidrug drug resistant *E. coli* was selected after performing the antibiotic susceptibility test that was established in our pervious study.Synthesized ZnO nanoparticles using *M. communis*After performing the procedure of synthesis of ZnO nanoparticles using *M. communis* for several times, 2 g of ZnO nanoparticles was obtained. The natural extract of *M. communis* was utilized for green biosynthesis of ZnO due to their effective chelating activities (Ahmed *et al*,2017,17). Characterization of ZnO nanoparticles UV–vis spectral analysis of ZnO nanoparticles The optical properties of the biosynthesized ZnO nanoparticles were investigated using a UV-Vis spectrometer. Figure (1) elucidates the absorbance spectrum of the ZnO sample within the nano-range at room temperature. The absorbance peak was found to be at around 287 nm which is similar to other findings reported by (Alaa Alden &Yaaqoob,2022, Salih *et al*, 2019) which reported that the attained UV-Vis peak indicates a direct electrons recombination between the valence and conduction bands at 287 nm. While (Jan *et al*,2021) reported that the absorbance peak was found to be at around 350 nm. Materials having a narrower band gap have higher photocatalytic activity because excited electrons can move quickly from the valence band to the conduction band, resulting in more rapid dye degradation (Jan *et al*,2021).

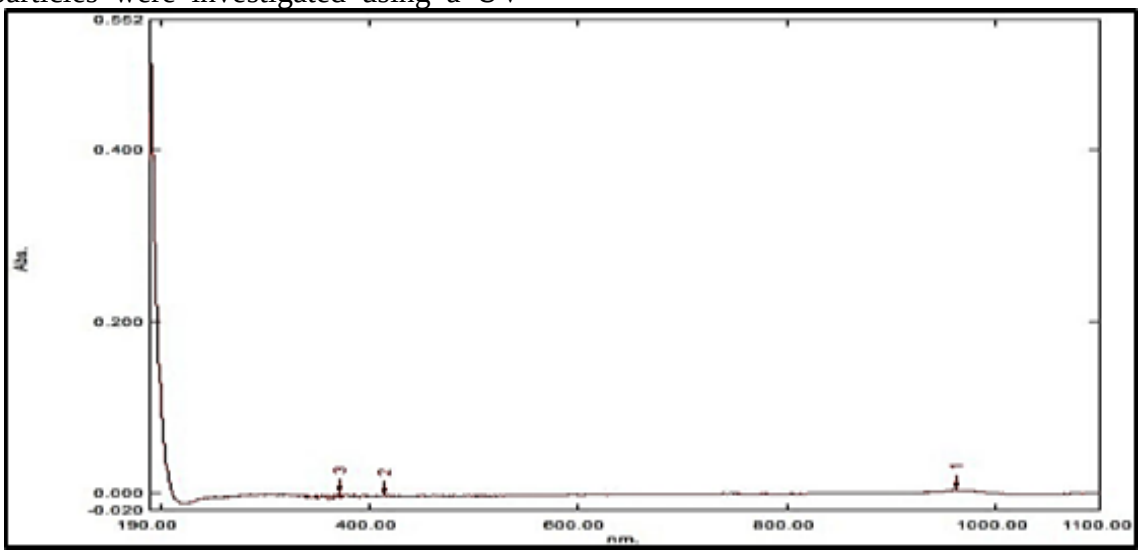

Figure 1. Characterization of ZnO nanoparticles using UV-vis light analysis Atomic force microscopy (AFM)

The surface roughness, topography, and morphology in this study were investigated using an Atomic Force Microscope (AFM) technique. This particular technique provides both two and three-dimensional images of the desired nanoparticles at an atomic level Figure (2). Table (2) shows that the average diameter of the scanned nanoparticles was estimated in the nano-scale; where the biosynthesized ZnO nanoparticles using *M. communis* were investigated via AFM technique. This particular surface analysis needs considerable attention as many factors could affect the outcomes of this analysis such as pollution. It is worth mentioning that the obtained nanoparticles exhibited spherical shapes with almost homogeneous alignment.

Table 2. Average diameter of ZnO nanoparticle using AFM analysis

Avg. Diameter: 61.83 nm	**<=10% Diameter: 25.00 nm**
<=50% Diameter:50.00 nm	**<=90% Diameter: 100.00 nm**

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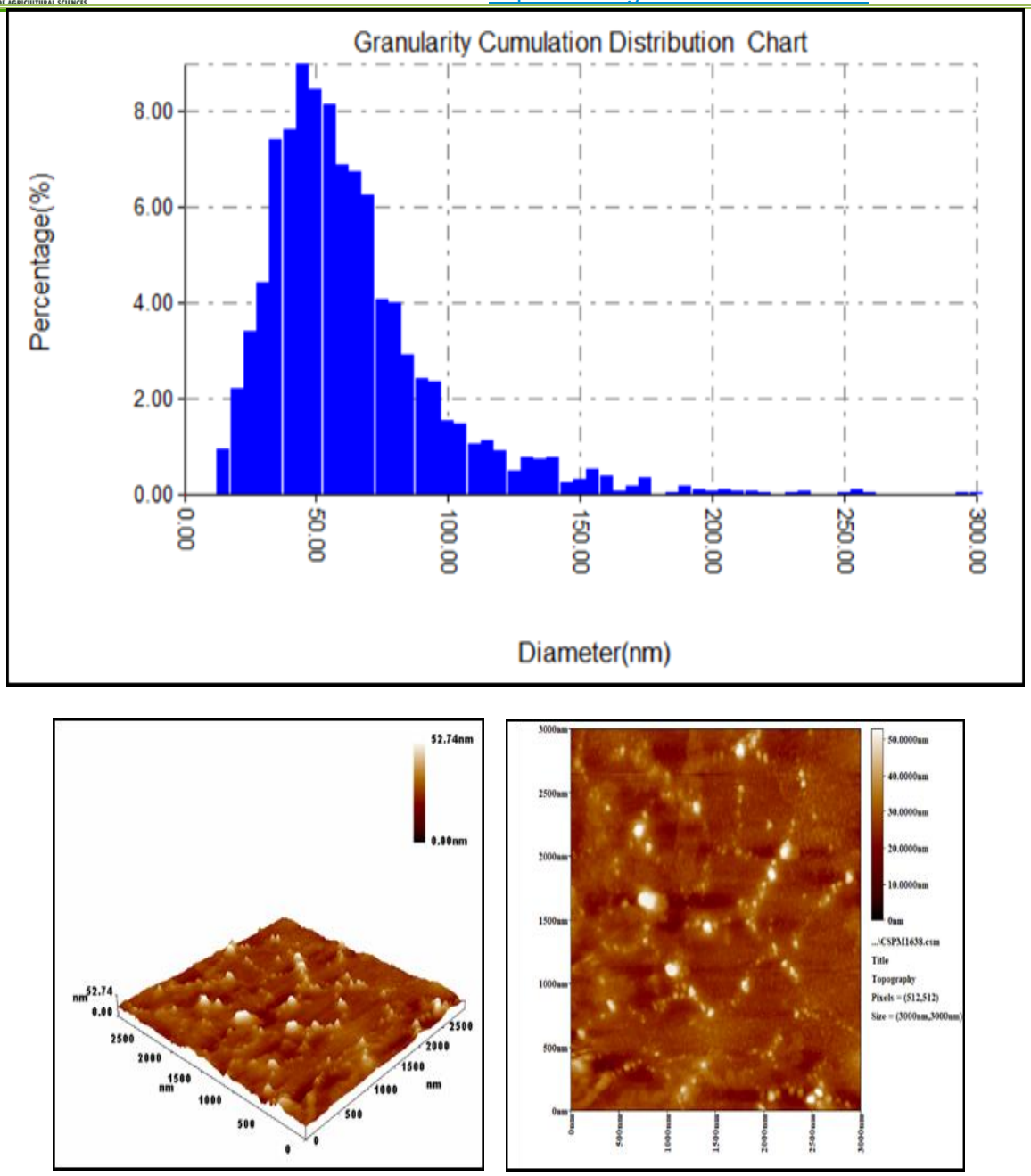

Figure 2. Characterization of ZnO using AFM analysis (A: Histogram of ZnO NPs, B: 3D of ZnO nanoparticles, C: 2D of ZnO nanoparticles)

Field Emission Scanning Electron Microscope (FE-SEM): The surface morphology obtained using the FE-SEM technique was investigated and the topographical analyses were presented based on the surface investigation. In Figure (3), the image illustrates a low glomeration degree.The *M. communis* addition to the mixture limits the degree of agglomeration. Moreover, the prepared ZnO nanoparticles sample exhibited spherical particles as well as plate-like structures. It is worth mentioning that the average nanoparticles' diameter was found to be around (30-35) nm using Image software. The obtained structure was found to be similar to other researchers' outcomes by which plate-like morphology was also obtained with almost similar particle diameter the size of the nanoparticle around (28.2-55) and (40-60) nm for (Alaa Alden &Yaaqoob,2022, Salih *et al*, 2019).

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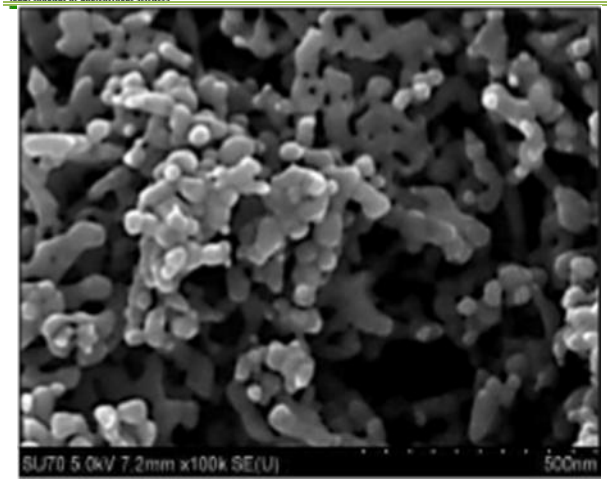

Figure 3. Characterization of ZnO using FE-SEM

Fourier transform infrared (FTIR)

The FT-IR investigation was introduced to locate the functional groups of the utilized reducing agent *M. communis* and also to study the possible effect of these functional groups in the ZnO nanoparticle production.The used range of the bio-synthesized ZnO nanoparticles via FTIR was ranging from (400-4000 cm-1), this in turn identifies the functional groups and the chemical bonds in the attained compound. Figure (4) presents the FT-IR spectrum of the utilized *M. communis*, zinc acetate, and *M. communis*-ZnO nanoparticles. Generally, the FTIR spectra of the ZnO nanoparticles revealed more than one main region. The first of which is 3446.56-3402.20 cm^{-1}, which attributed to hydroxyl group (O-H) stretching and indicated the presence of alcohol compounds, in agreement with (17) who found intense peaks at 3351.07 cm^{-1} due to the presence of same group (O-H). Also, the presence of nitro compounds was indicated at 1560.30 cm^{-1}, which attributed to N-O stretching. It reveals that the vibrational wavenumbers (1423.37, 1344.29 and 1068.58-1029.92 cm^{-1}) correspond to O-H, C-H and C-O groups, respectively, as shown in Figure (4), in agreement with (anaki *et al*,2015). Also, the figure (4) revealed that the presence of 3431.13, 1720.39, 1618.17 and 1086.56-1041.49 cm^{-1}, attributed to O-H, C=O, O-H and C-O, respectively, in agreement with (Nowrouzi *et al*,2021) who found similar peaks for the same functional groups. Figure (4) shows the presence of a wide range of peaks due to functional groups of both ZnO and *M. communis*, whereas there were different peaks including 3485.84-3377.12, 1718.46, 1560.30, 1340.43, 1419.51 and 1053.06-1026.06 cm^{-1}, which attributed to Alcohol (O-H stretching), Secondary Alcohol (C=O stretching), alkaline (C=C stretching), Secondary Alcohol (C-N stretching), Sulfate (S=O stretching) and Sulfoxide (S=O stretching), respectively, in agreement with (Jan *et al*,2021), who found similar peaks for the same functional groups.

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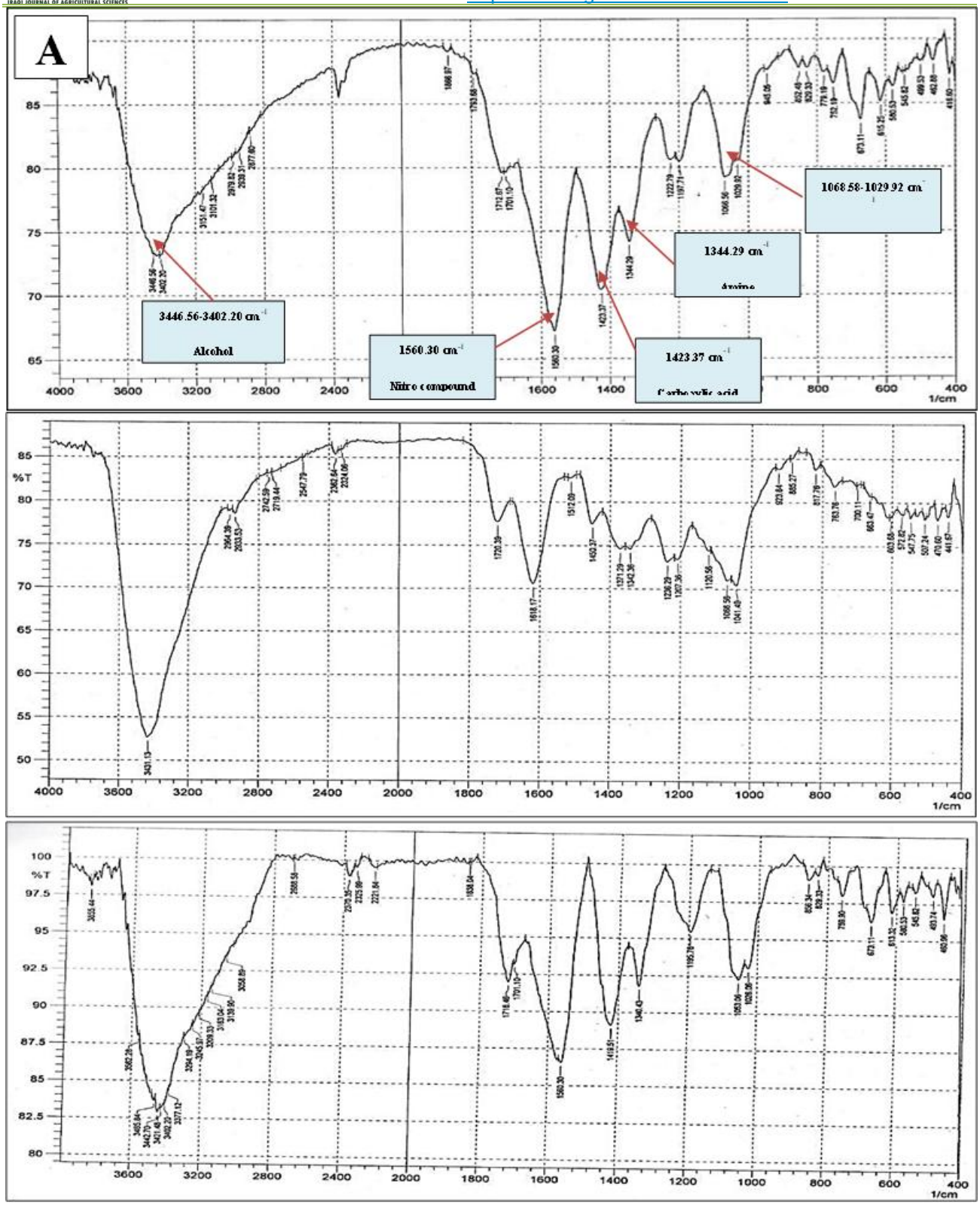

Figure 4. FTIR images for (A: ZnO nanoparticles. B: *M. communis*, C: ZnO NPs + *M. communis*).

Estimation of ZnO nanoparticles MIC

For determination of MIC and sub-MIC of ZnO, only 7 MDR and strong-biofilm producer *E. coli* isolates (harbored both *fimH* and *fimA*) were selected based on our previous work. Microtiter plate method was utilized and the results, as shown in Figure(5) , indicated that only three isolates (C1, C2 and C23) with MIC and sub-MIC (125 and 62.5 µg/ml), respectively, were selected for further experiments

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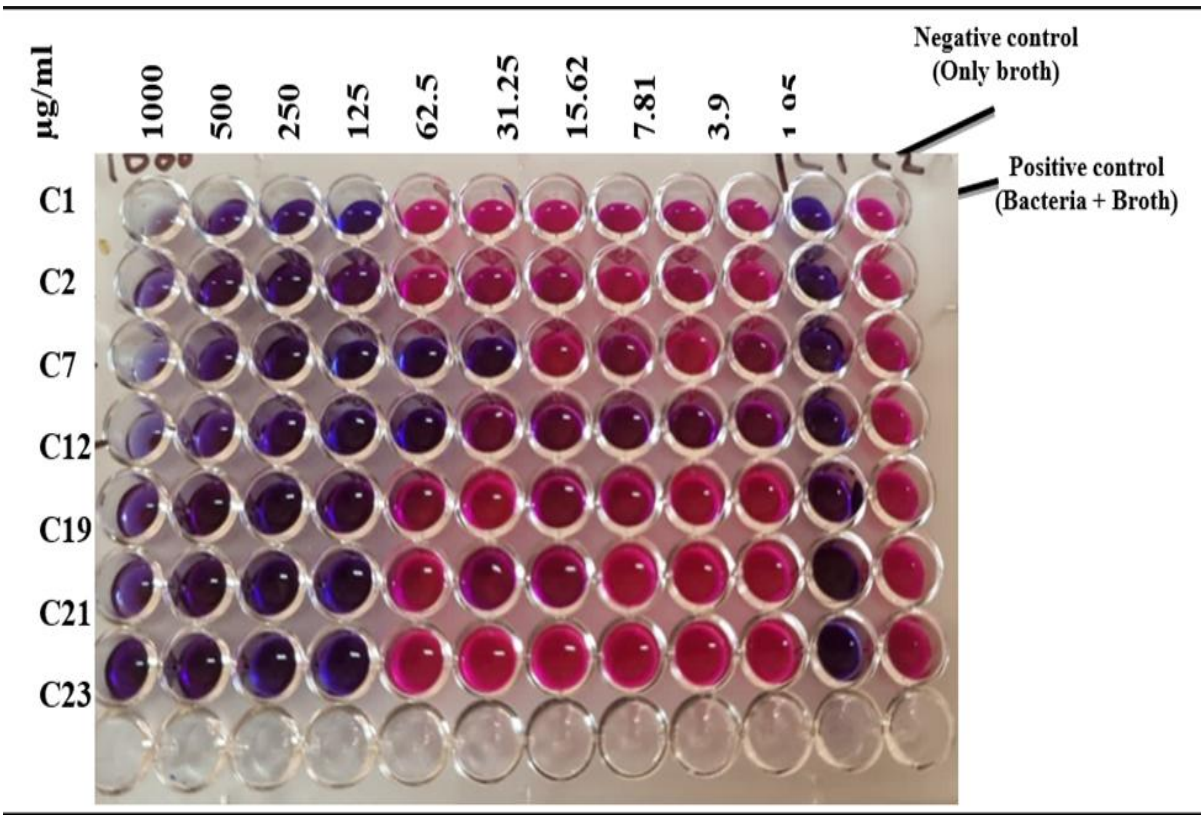

Figure 5. The results of MIC of *E. coli* using Microtiter plate method

Gene expression analysis

Quantitative Real Time PCR was utilized for determining the expression of the adhesion genes *fimA* and *fimH* in three isolates (C1, C2 and C23), with and without treatment with ZnO nanoparticles. The qPCR and the amplification results of each cycle was called CT (cycling threshold). The expression of genes was normalized to the scale of a housekeeping gene *16s rRNA* and quantified by the (ΔCt; dCt) value and folding ($2^{-\Delta\Delta Ct}$).In this experiment, three isolates were used, including C1, C2 and C23. Two tubes for each isolate, including control tube (free-ZnO nanoparticles tube). The tubes were treated with 62.5 µg/ml of ZnO nanoparticles. The analysis of results and determination the fold of expression for studied genes for each sample was performed according to the equations of (Livak & Schmittgen. 2001). According to the results in Table (3), the relative expressions ($2^{-\Delta\Delta Ct}$) of *fimA* gene in C1, C2 and C23 isolates of *E. coli* were downregulated in 0.51-fold after treated with 62.5 µg/ml of ZnO nanoparticles (p=0.112), while the relative expressions ($2^{-\Delta\Delta Ct}$) of *fimH* gene in C1, C2 and C23 isolates of *E. coli* were upregulated in 4.42-fold after treated with 62.5 µg/ml of ZnO nanoparticles (p=0.298).

Table 3. Gene expression of *fimA* and *fimH* in ZnO-treated isolates and non-treated isolates (control) of UPEC isolates (C1, C2 and C23).

Groups	Mean ± SE of *16srRNA*	Ct	Mean ± SE of *fimA* dCt	ddCt	Folding
Control	14.77 ± 0.36	22.37 ± 3.36	7.61 ± 3.20	0.0 ± 0.0	1.0 ± 0.0
Samples	20.30 ± 0.66	29.32 ± 2.75	9.01 ± 2.43	1.41 ± 0.85	0.51 ± 0.24
p-value	0.002	0.185	0.744	0.172	0.112
Groups	Mean ± SE of *16srRNA*	Ct	Mean ± SE of *fimH* dCt	ddCt	Folding
Control	14.77 ± 0.36	18.82 ± 0.55	4.05 ± 0.26	0.0 ± 0.0	1.0 ± 0.0
Samples	20.30 ± 0.66	22.85 ± 0.44	2.55 ± 0.71	-1.51 ± 0.95	4.42 ± 0.287
p-value	0.002	0.005	0.118	0.188	0.298

The results in the table above indicated that ZnO nanoparticles act as quorum quenching agent against gene expression of *fimA*; however, these nanoparticles induce the gene

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expression of *fimH.* In corresponding with this study, (Shivaee et al ,2021) reported that the MIC values for zinc oxide nanoparticles was 2500 µg/ml in all five isolates of *Klebsiella Pneumoniae* and the results of real-time PCR showed that the expression levels of *fimA* genes in isolates treated with zinc oxide decreased 9 fold, , compared with the control.In agreement with this study, (Hasan & Alsammak, 2023) reported that In *E. coli* show increasing in expression after exposed to sub-MIC of ZnO NPs at concentration (2500µg/ml), the most effected gene was *fim*H followed by *fim*B and *fim* I gene while same effect on *fim*C and *fim*E. In disagreement with this study, (Shakerimoghaddam *et al*,2017) reported that four UPEC isolates were exposed to sub-minimum inhibitory concentration of ZnO nanoparticles (1250 µg/ml) and the results were indicated that the presence of nanoparticles reduced the *fimH* expression level in all four isolates. Also, (Jamalan *et al*,2019) reported that the results showed that the IC50 of ZnO NPs for the T24-cells was 19.53 g. mL^{-1}, and that a concentration of 0.3 $g.mL^{-1}$ is harmless for this cell line. The minimum inhibitory concentration for the three UPEC strains employed in this investigation was 1250 $g.mL^{-1}$. These low ZnO solution concentrations decreased UPEC's ability to adhere to T24 cells by 28.77 to 44.71% and may have also reduced the expression of the *fimH* gene in UPEC.Hence, the increase in *fimH* expression may be attribute to the presence of *M. communis*; despite there is no clear evidence, but some studies reported role of plant extracts in increasing gene expression of *fimH*. For instance, *Vaccinium macrocarpon* extract increased the *fimH* expression in UPEC as part of a feedback mechanism after blocking *FimH* (Rafsanjany *et al*,2015) as well as acetone extract of *Apium graveolens* fruits caused a significant upregulation of *fimH* and *sfaG* in free floating, non-attached UPEC and significantly down-regulated these genes in adherent bacteria (Grube et al,2019).

Ethical Clearance

This study received approval from the Ethics Committee of Medical City Hospital and the University of Baghdad for Postgraduate Studies. Everyone presents signed a form to show that they agreed in writing.

CONCLUSION

This study demonstrated that ZnO nanoparticles at sub-MIC concentrations inhibit biofilm formation by decreasing expression of the *fimA* gene while having no effect on *fimH*. ZnO nanoparticles may be able to entirely block UPEC adhesion and biofilm development, however more research is needed on gene expression and bacterial adhesion to surfaces to confirm this.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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تقييم تأثير دقائق أوكسيد الزنك النانوية المصنعة من مستخلص أوراق الياس ضد بكتريا الاشريكية القولونية

صفا علاء الدين عبد الحميد رامينا ميخائيل خوشابه

قسم التقنيات الاحيائية – كلية العلوم – جامعة بغداد

المستخلص

تهدف هذه الدراسة إلى تقدير تأثير الجسيمات النانوية لأوكسيد الزنك المصنعة بواسطة *Myrtus communis L*. على تعبير جينات الالتصاق *fimH* و *fimA* في العزلات السريرية لبكتيريا الإشريكية القولونية المسببة للأمراض البولية (UPEC). تم توصيف جسيمات أوكسيد الزنك المصنعة حيويا من خلال العديد من التقنيات بما في ذلك مطيافية الامتصاص المرئي–فوق البنفسجي (UV–vis)، ومجهر القوة الذرية (AFM)، والمجهر الإلكتروني الماسح (FE–SEM)، وتحويل طيف الأشعة تحت الحمراء (FTIR). لتحديد MIC وSub–MIC للجسيمات النانوية لأوكسيد الزنك، تم اختيار 7 عزلات فقط من العزلات المقاومة للأدوية المتعددة (MDR) ومنتجة الأغشية الحيوية القوية *E. coli* (التي تحوي كلاً من *fimH* و*fimA*) بناءاً على دراستنا السابقة تم استخدام طريقة لوحة المعايرة الدقيقة وأظهرت النتائج أنه تم اختيار ثلاث عزلات فقط (C1، C2 وC23) ذات MIC و sub–MIC (125و62.5 ميكروغرام/مل)، على التوالي، لأجراء التجارب المتبقية. تم استخدام تفاعل البلمرة الكمي لتحديد التعبير الجيني لـ *fimH* و*fimA* في ثلاث عزلات (C1 وC2 وC23)، مع وبدون المعاملة بالجسيمات النانوية لأوكسيد الزنك. وفقًا للنتائج،انخفضت التعبيرات النسبية ($2^{-\Delta\Delta Ct}$) لجين *fimA* في عزلات C1 وC2 وC23 لبكتريا *E. coli* بمقدار 0.51 مرة بعد معاملتها بـ 125 ميكروغرام/مل من الجسيمات النانوية لأوكسيد الزنك ، في حين أن التعبيرات النسبية ($2^{-\Delta\Delta Ct}$) لجين *fimH* في عزلات C1 وC2 وC23 من الإشريكية القولونية ازدادت بمقدار 4.42 مرة بعد معاملتها بـ 125 ميكروغرام/مل من الجسيمات النانوية لأوكسيد الزنك.

الكلمات المفتاحية: تفاعل البلمرة الكمي، التركيز المثبط الادنى ،جسيمات أوكسيد الزنك النانوية، *fimH*،*fimA*.