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Effect of turmeric nanoparticles on gene expression of biofilm gene in *Staphylococcus aureus*

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ABSTRACT

For centuries, turmeric has been used as a potent, bioactive, and nontoxic remedy for many ailments. Low bioavailability and aqueous solubility are its only drawbacks. This study developed a turmeric nanoparticle preparation method and analyzed its behavior, average particle size, composition, dispersion, crystal shape, structure, and phase purity. AFM, SEM, XRD, FTIR, and UV-visible spectrophotometry were used to test these. Before and after turmeric nanoparticle treatment, Ica A and Ica D gene expression and antimicrobial properties were examined. Turmeric nanoparticles were prepared using green biosynthesis because they are non-toxic, environmentally friendly, and sometimes more effective against microorganisms than more expensive, energy-intensive, and potentially ecologically hazardous physical and chemical methods. FTIR peaks indicated pure nanoturmeric, but absorption peaks in this study showed that the samples' main components were "curcuminoids." Additionally, turmeric nanoparticles were spherical and averaged 49.8 nm. Decomposition analysis and antioxidant testing showed that turmeric nanoparticles do not cause hemolysis or harm humans. Turmeric nanoparticles inhibited S. aureus growth more in aqueous nanoparticles. This inhibition occurred at 1000 g/ml turmeric nanoparticles. Perform real-time PCR to determine how turmeric nanoparticles affect *Staphylococcus aureus* before and after 1000 g/ml treatment. The results show that turmeric nanoparticles limit biofilm gene expression by half. Fold change decreases from 1.06 to 0.46, p-value = 0.015, but Ica D is not significantly affected, and gene expression decreases (1.06-0.77).

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Introduction

Turmeric (ginger) is a Zingiberaceae family of herbaceous evergreen plant. it is widely used as a food preservative and as a spice like curry spice and colorant; it is responsible for the bright yellow color of ballpark mustard.

Turmeric has long been used in traditional medicine around the world. The primary yellow bioactive ingredient in turmeric, Curcumin (diferuloylmethane), has been demonstrated to have various biological effects (Feng et al .2017; Verma et al .2018; Abdulrahman et al. 2024).

Since more than 30 years ago, numerous preclinical studies have shown Turmeric (*Curcuma longa*) and Curcumin's therapeutic potential against various human ailments (Ghasemzadeh & Hosseinzadeh. 2024).

Turmeric enhances the anticancer effect by inducing cell apoptosis. Its anti-inflammatory, anticancer, and

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antioxidant characteristics may find application in managing symptoms of arthritis, and pathology associated with chronic oxidative stress. Clinical settings have already employed Curcumin to lessen after-surgery inflammation (Nisha & Anbu.2017).

Particles that range in size from 1 to 100 nm are known as nanoparticles (NPs), and they have unique physical and chemical features that make them useful for drug delivery. Prepare materials as nanoparticles lead to higher solubility, stability, bioavailability, and pharmacological effects while reducing cytotoxicity to healthy cells, more significant dosage requirements, and physical and chemical degradation (Dhivya & Rajalakshmi .2018) , Many ways used for formulation nano dosage by delivery to liposomes, pro liposomes, polymeric nanoparticles (nanospheres and nanocapsules), nanoemulsions, solid lipid nanoparticles, in-organic nanoparticles, and biological nanomaterials such as red blood cells, DNA, and so forth as drug and vaccine carriers(Wang et al. 2011; Hasan .2015).

Turmeric, found in *Curcuma longa*, is highly effective and used as a standard home cure for various illnesses. Many studies indicate turmeric has excellent antibacterial activity, significantly increased with particle size reduced to the nano range. The action of turmeric nanoparticles against Gram-positive bacteria is good. Also, the water-based spread of turmeric nanoparticles was much more effective than turmeric itself against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Penicillium notatum* (Chaudhary & Sekhhon, 2012). Compared to Gram-negative bacteria, the antibacterial activity was more potent on Gram-positive bacteria and had a lower effect on fungi (Bhawana et al. 2011).

Since it can penetrate human and animal mucosa and skin, *S. aureus* is recognized as an opportunistic, pathogenic, and Gram-positive bacterium. It is mainly found in the anterior nares. *S. aureus* differs from other staphylococcal species due to the colonies' gold pigmentation and positive coagulase results. Also, it can mannitol-fermentation, and deoxyribonuclease tests.

Asymptomatic *S. aureus* typically isolates about 30% of the human population and can cause complications ranging from superficial to life-threatening contagions in nearly all tissues (Gnanamani et al. 2017).

Staphylococcus aureus is the bacteria that causes most skin and soft tissue infections (SSTIs)(Kwiecinski 2013). It causes a wide range of SSTIs, ranging from superficial illnesses like impetigo to deep, potentially lethal infections like cellulitis, pyomyositis, and fasciitis, as well as folliculitis, ecthyma, abscesses, boils, and carbuncles (Miller & Cho.2011).

After breaking through the barrier of the skin and the mucous membranes, the *S. aureus* complex is suited for the colonizing of many mammalian hosts, including humans, and for infectious activities. The pathogenesis of *S. aureus* infections depends critically on the capacity to attach on biotic and introduced abiotic surfaces, build complex biofilms, and get internalized (Becker. 2024).

This study aims to examine the influence of turmeric nanoparticles on the gene expression of biofilm-related genes in *S. aureus*, emphasizing the potential antimicrobial and anti-biofilm properties of these nanoparticles. This research aims to elucidate the molecular mechanisms by which turmeric nanoparticles may inhibit biofilm formation and disrupt established biofilms by analyzing changes in the expression levels of key biofilm-forming genes, such as icaA and icaD. The results may offer significant insights into the formulation of innovative therapeutic approaches to address *S. aureus* infections, especially those associated with biofilm-mediated antibiotic resistance, thus aiding in the progression of alternative antimicrobial therapies.

Materials and Methods

Sampling

The research was done between October 2023 and January 2024. A total of 100 patients (53 males and 47 females) with various skin infections were included in the study. The patients received medical care in AL-Sader City Hospital and AL-Najaf Education Hospital in the province of Najaf.

Preparation of turmeric silver nanoparticles

The preparation process of turmeric silver nanoparticles was modified after Al-Hayanni and El-Shora (2021). Twenty grams of turmeric dry powder were macerated with 200 ml of distilled water for three days to produce an aqueous extract of *C. longa* roots. The mixture was then vigorously stirred for four hours, at room temperature, the aqueous extraction was filtered through Whatman No. 1 filter paper (Alnuaimi et al. 2019). 1.6987 grams of silver nitrate were dissolved in 1000 milliliters of deionized water, stirring constantly for 30 minutes, to get ten millimolar silver nitrate. A 100 ml extract was poured into a 900 ml silver nitrate solution, and the mixture was agitated for 30 minutes at 25°C and 800 rpm in dark condition. Solution's color altered, indicating the formation of silver nanoparticles. After that, it is stored in dark bottles for a whole day. The final samples were stored in dark jars after the reaction mixture had been centrifuged for 10 minutes at 10,000 rpm to get

clear supernatant after 24 hours.

After five days, test tubes maintaining the solution containing the previously manufactured silver nanoparticles were placed in a cooled Ulra-Centrifuge and ran for ten minutes at a speed of 20,000 (rpm/min). Following the duration, the filtrate was disposed of, and the precipitate was collected and cleaned in ion-free distilled water. Next, distilled water was added, and the mixture was placed in a cooled Ultra-Centrifuge and run at 10,000 rpm/min for 10 minutes. This process was repeated three times until the filtrate was colorless and stored for later use.

Characterization of turmeric silver nanoparticles

Using the following techniques, the precise arrangement of the average particle size, produced particles, phase purity, crystal shape, structure, and distribution were all measured. We also examined standard industrial turmeric nanoparticles.

Atomic force microscopy (AFM Model, Aa3000, Advanced Angstrom, USA) has been carried out at the University of Tehran according to Jagadeesh and Siengchin (2024).

A **UV-visible spectrophotometer** (UV-1650 PC, Shimadzu) was used to measure UV absorbance in the 200–800 nm wavelength range (Singh & Avupati, 2017; Hettiarachchi et al., 2021).

For turmeric silver nanoparticles, **X-ray diffraction (XRD)** has been carried out on Shimadzu XRD-6000 (Japan) at the University of Tehran according to Zhang et al. (2016).

Fourier-transform infrared (FT-IR) spectroscopy has been carried out according to various investigators (Rohman et al. 2015; Pakkirisamy et al. 2017; Pawar et al. 2018; Wen et al. 2023). FT-IR (Tensor, Bruker, USA) with a resolution of 4 cm^{-1} and a scanning range of 400 to 4000 cm^{-1} was used as the highly effective method in identifying the different kinds of chemical bonds, or functional groups, present in a given substance.

Antibacterial activity of turmeric nanoparticles

We used the diffusion assay to determine the antibacterial activity of the synthetic turmeric nanoparticles. Turmeric nanoparticle dilutions (1000, 500, 250, and 150 µg/ml) were made starting at a stock concentration of 2 µg. We made a well on a Mueller-Hinton agar plate with a diameter of 7 mm using a gel hole. A volume of 100 µL of artificial particles was added to the well (Muntasir 2018).

RNA Extraction

After incubating three samples of *S. aureus* for 48 hours under ideal biofilm development conditions, RNA was extracted from all the samples with and without turmeric nanoparticles. After centrifuging each sample, the supernatant was thrown away. Normal saline was used to dilute each sample to a 3.0 McFarland concentration. Using the TransZol up reagent (Trans kit) and the manufacturer's instructions, the total RNA was separated for the S. aureus isolation. Using Nanodrop's OD measurement technology, the amount of RNA samples was examined (Boeco, Germany). For every sample, the concentration of RNA was adjusted to be the same.

Real-time PCR

The expression levels of biofilm gene (Ica A and Ica D) were assessed using real-time polymerase chain reaction (PCR) with the relevant primers, (Table 1). Real-time PCR was performed to analyze the gene expression before and after treatment with turmeric nanoparticles to analyze their effect on the growth of S. aureus. The bacteria were treated with a concentration of 1000 µg/mL, chosen based on the antibacterial test.

Table 1 Primer design used in this study.

Primer	DNA sequence
Ica A	F: TCTCTTGCAGGAGCAATCAA R: TCAGGCACTAACATCCAGC
Ica D	F: ATGGTCAAGCCCAGACAGAG R: CGTGTTTTCAACATTTAATG CAA

The ONE STEP master mix (Promage kit) used the amplified target gene, the reaction mixture, and the thermocycle condition (Table 2 and 3).

The housekeeping 16sRNA gene was used as an internal control to normalize the expression. Complementary DNA (cDNA) for mRNA was synthesized using the One-Step qRT-PCR Master Mix (Promega kit) according to the manufacturer's instructions. Forty-five polymerase chain reaction cycles were performed using a Real-Time thermocycler (Analytic Jena, Germany). The differences in gene expression were calculated through the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The results of each analysis were carried out in triplicate and are shown as means standard deviation (SD). The Statistical Package for the

Social Sciences (SPSS) software program, version 20, was used to conduct statistical analysis using Student's t-tests. The value of P value less than 0.05 was considered significantly different.

Table 2 GoTaq® 1-Step RT-qPCR reaction Mix (Promage kit).

No.	Component	Volume/ 20µl	Concentration
1	GoTaq® qPCR Master Mix, 2X	10 µl	1X
2	Forward Primer	2 µl	300 nM
3	Reverse Primer, 10X	2 µl	300 nM
4	GoScript™ RT Mix for 1-Step RT-qPCR, 50X or Nuclease-Free Water for Minus-RT Control	0.4µl	1X
5	RNA Template (500fg–100ng) or Nuclease-Free Water for No-Template Control	3.7 µl	100 ng
6	$MgCl_2$	1.6 µl	25 mM
7	CXR Reference Dye	0.4 µl	30 µM

Table 3 One-step RT-qPCR program.

Step	Temperature	Duration	C
Reverse transcription	≥37°C	15 minutes	1
RT inactivation/Hot-start activation	95°C	10 minutes	1
a. Denature	30°C	10 seconds	40
b.Anneal/Collect data	57°C	30 seconds	
c. Extend	44°C	30 seconds	
Dissociation	60–95°C		1

Results and Discussion

After 48 hours of incubation turmeric extraction with silver nitrate in the shaking incubator, the turmeric extract turned from dark yellow to dark blue, evidence of the formation of the turmeric nanoparticles (Fig. 1).

Figure (2) illustrates the characteristic feature of turmeric nanoparticles. In the UV-visible absorption spectrum, this synthesized form had an absorbance peak at 423 nm. We scanned the highest absorption spectra from 424 nm to 422 nm. The main confirmation of this outcome was the practical synthesis of turmeric nanoparticle extract. We took a picture of the turmeric nanoparticles' FTIR spectrum and saw peaks at 3695.4 cm^{-3}, 3422.42 cm^{-3}, 2920.02 cm^{-3}, 1462.95 cm^{-3}, 1351.24 cm^{-3}, 984.06 cm^{-3}, and 45.74 cm^{-3}. The peaks at 3695.4 cm^{-3} and 422.42 cm^{-3} correspond to the group, and 2920 cm^{+3} is the asymmetric stretching vibration of Csp2−H. Bands at 1462 cm^{-1} and 1351 cm^{-1} correspond to the aromatic stretching vibrations of the benzene ring. The other assignments are as follows: There were oxygen vacancies (VO) in the complex, as shown by the C–O–C peaks at 984 cm^{-3} and 545 cm^{-3} in Figure 2.

Fig 1. A- *Curcuma longa* after dried powder, B- Turmeric extraction, and C- Silver Nanoturmeric.

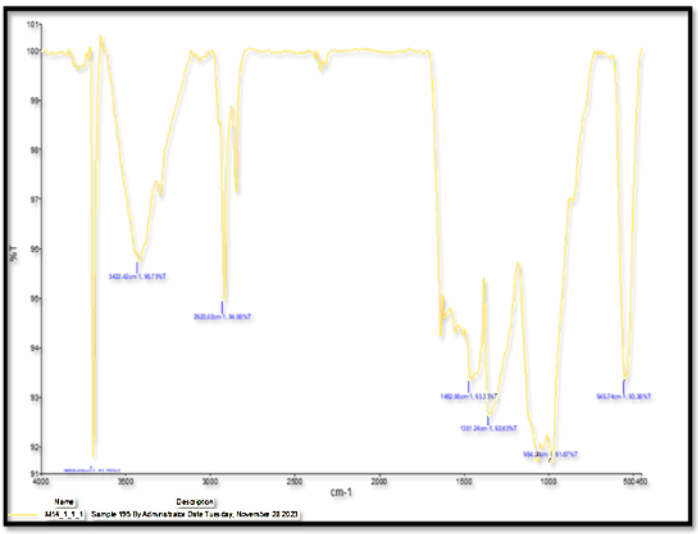

Fig 2. FTIR of turmeric nanoparticles (4000-400 cm−1).

Field emission scanning electron microscopy (FESEM) was used to look at the shape and structure of the turmeric silver nanoparticles in order to learn more about their properties. The image revealed an average size of 49.8 nm and a reasonably spherical shape of the produced, as shown in Figure (3).

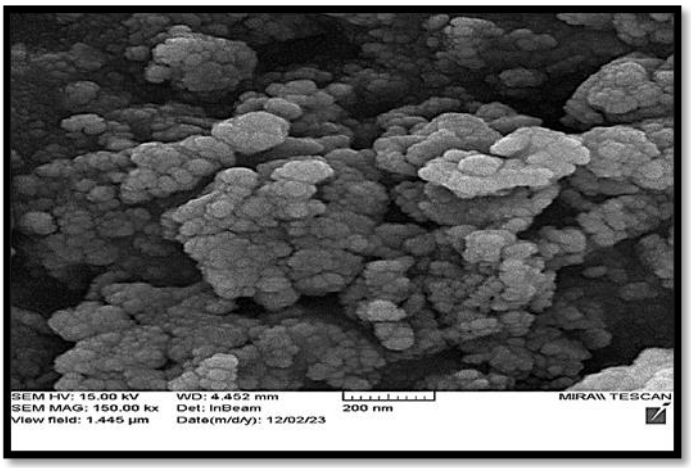

Fig 3. FESEM micrograph of turmeric nanoparticles spherical well scattered with a size average of 49.8 nm.

Atomic force microscopy (AFM) described the shape of the turmeric nanoparticles. The height measurements yielded a significant degree of accuracy in determining the elevation of the particles. The average turmeric nanoparticle diameter is in the usual range of size nanoparticles (1–100 nm), as seen in the granularity accumulation distribution charts of turmeric nanoparticles and the three-dimensional photographs in figure 4.

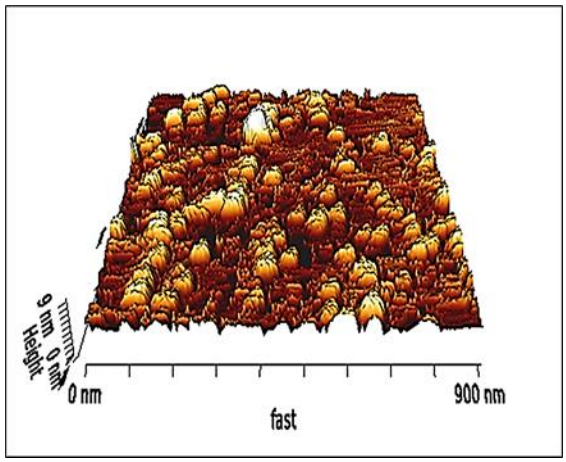

Fig 4. AFM analysis of turmeric nanoparticles.

The XRD (X-ray diffraction) verified the synthetic turmeric nanoparticle's spherical crystal structure. Additionally, the following was used to assess the crystalline size: The formula for Debye Scherrer $D = 0.94\lambda/\beta \cos\theta$ states that the nanoparticles' diameter was 15.74 nm (Fig. 5).

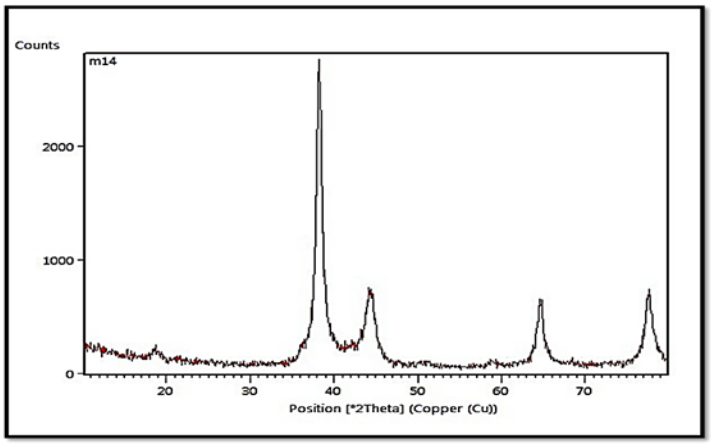

Fig 5. XRD analysis of turmeric nanoparticles.

We used Triton X-100 as a positive control indicator to detect hemolysis. A clean phosphate buffer saline solution was used as a negative control. Turmeric nanoparticles did not break down any of the blood samples that were tested at any concentration (1000, 500, 250, or 125 µg/ml) as shown in table (4).

Table 4 Hemolysis effect of turmeric nanoparticles

Sample	Hemolysis %
Triton X-100 (positive control)	100
PBS (negative control)	0
Nano-Curcuma 125 µg/ml	0
Nano-Curcuma 250 µg/ml	0
Nano-Curcuma 500 µg/ml	0
Nano-Curcuma 1000 µg/ml	0

After adding nanoparticles at 1000, 500, 250, and 125 g/ml doses to the DPPH (1-diphenyl-2-picrylhydrazyl) solution, we measured the absorbance at 517 nm 30 minutes later. The following made it possible to monitor color changes that showed how well the nanoparticles scavenged DPPH free radicals (Podder et al. 2018). As the concentration of turmeric nanoparticles increased, so did DPPH, which lessened their action. For turmeric nanoparticles, it was 75% in 1000 µg/ml, 71.9% in 500 µg/ml, 70.3% in 250 µg/ml, and 66% in 125 µg/ml (Fig. 6).

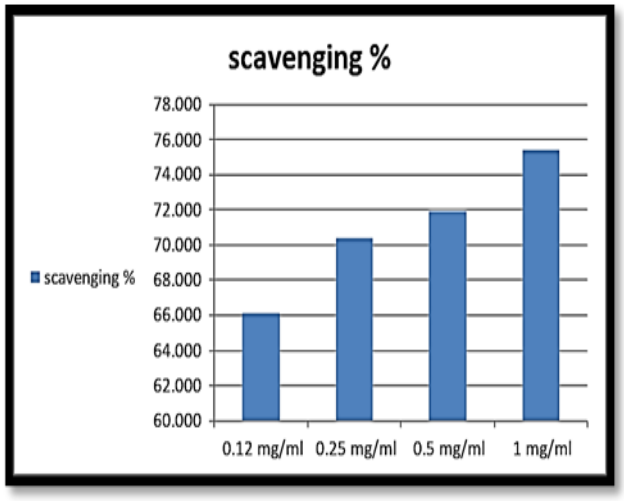

Fig 6. Antioxidant of turmeric nanoparticles.

The antibacterial activity of turmeric nanoparticles was shown at 1000 µg/ml, and the most significant inhibitory zone seen in most samples measured 23 mm.

Gene expression analysis:

The relative expression of genes Ica A and Ica D was determined with Ct values. The mean results in figure (7) showed that it affected the expression of regulator genes involved in biofilm formation. Real-time results (Figs 8 and 9) showed that the turmeric nanoparticle extract showed a significant effect on the expression of genes Ica A as the fold change decreased from 1.06 to 0.46, and *p* vula = 0.015 and the turmeric nanoparticle extract showed an effect on the expression of genes Ica D as the fold change decreased from 1.06 to 0.77 but it was no significant.

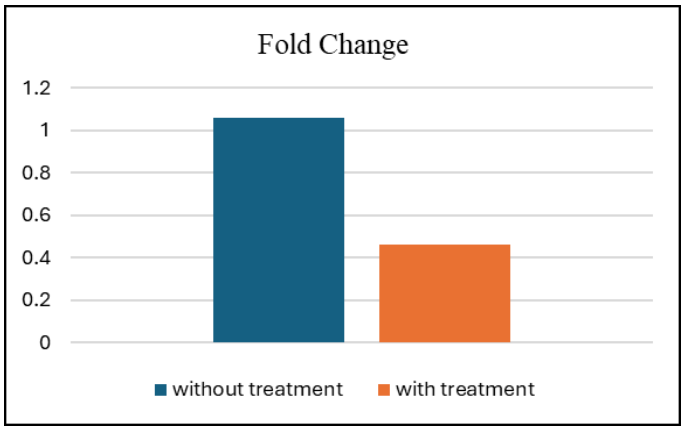

Fig 7. Effect of turmeric nanoparticles gene expression on biofilm gene.

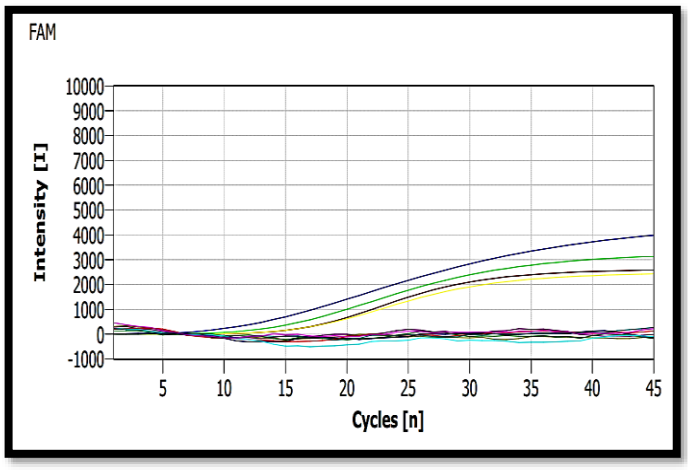

Fig 8. Ica A gene amplification plots by qPCR where samples included all study groups.

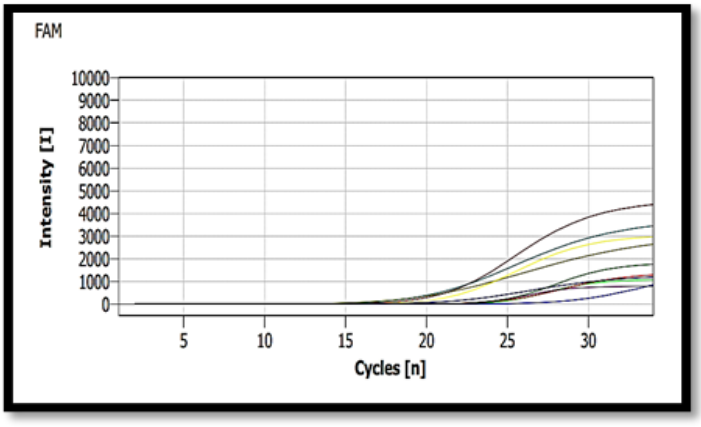

Fig 9. Ica D gene amplification plots by qPCR Samples included all study groups.

Discussion

Turmeric is not capable of producing potent therapeutic benefits due to the fact that it degrades rapidly and is not absorbed thoroughly by biological systems (Sharma et al.2007). Studies on nanomaterials have demonstrated the superior bioavailability of turmeric loaded with nanoparticles (Muqbil et al.2011; Fuloria.2022). However, scientists have conducted extensive research to develop more effective medication delivery systems. Particle size is a significant factor since it directly influences the drug release, cellular uptake, and physical stability of the nanoparticles (Elumalai & Shanmugam.2024). The outcomes of our study's UV–visible spectroscopy (Rahman et al. 2022), The study's acquired absorption peaks demonstrated that curcuminoids were the primary elements of the samples. Peaks in the FTIR spectroscopy spectra have been interpreted as representing pure turmeric nanoparticles. This is a strong and distinctive peak (Rohman et al.2015). AFM and FESEM were used to analyze the turmeric nanoparticles nano size, which was found to be typically between 1 and 100 nm. This result supports the formation of turmeric nanoparticles and fits the spherical crystal structure of the turmeric nanoparticle. Previous studies have also produced similar results whereby decreasing active component particle sizes to nanoparticle size increases effectiveness, solubility, and bioavailability (Dhivya & Rajalakshmi. 2018).

Turmeric nanoparticles shown enhanced efficacy in inhibiting the growth of S. aureus on Mueller-Hinton Agar media and suppressing the expression of biofilm genes, particularly Ica A, more effectively than Ica D.

Conclusions

In conclusion, turmeric nanoparticle was successfully developed by a green synthesized method. It is simple, natural, and easy method used for the synthesis of nano turmeric. Synthesized nanoparticles showed its efficacy against bacteria, *S. aureus*. The use of turmeric extract in the creation of nanoparticles yields improved efficacy, potent antimicrobial properties**,** and reduced manufacturing expenses.

The nano-turmeric extract has demonstrated efficacy in suppressing biofilm genes (Ica A, Ica D) expression, with a greater inhibitory effect observed on Ica A than Ica D.

Ethics approval and consent to participate

Ethical approval was sought from the Biotechnology College Ethics and Research Committee / University of Al-Qadisiya, and Patients were enrolled in the study only after written informed consent was obtained.

Conflict of interest

The authors declare that they have no conflict of interest.

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