RESEARCH PAPER

Nanocomposite Fabrication: Curcumin-Loaded Hydroxyapatite for Antimicrobial Applications

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ABSTRACT
The study titled Advanced Nanocomposite Fabrication: Curcumin-Loaded Hydroxyapatite for Biomedical and Antimicrobial Applications primarily aimed to fabricate curcumin-loaded hydroxyapatite (Cur-HAp) nanorods to achieve enhanced antibacterial efficacy and biocompatibility. Hydroxyapatite (HAp), a bioceramic composed of calcium phosphate, is considered one of the most promising drug carriers. Curcumin, a natural polyphenol, is known for its potent antibacterial properties. The HAp nanorods were synthesized by mixing sodium phosphate with calcium nitrate in distilled water. The resulting solid was collected through centrifugation, followed by drying and calcination at 600 °C. Curcumin was incorporated by mixing it with the HAp suspension under stirring. The final Cur-HAp product was then dried and milled into a fine powder. Characterization using X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) confirmed that the addition of curcumin did not alter the crystallinity of HAp. The Cur-HAp nanorods exhibited strong antibacterial activity against *Escherichia coli* and *Enterococcus* species, attributed to the synergistic effects of curcumin and HAp. These findings suggest that Cur-HAp nanorods possess excellent structural stability and enhanced antimicrobial properties, making them promising candidates for future biomedical applications such as drug delivery, tissue engineering, and infection management**.**

KEYWORDS: Antibacterial, Oral pathogens, health, HAp nanocomposite, sustainable nanotechnology, biomedical innovation
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1. INTRODUCTION

Bacterial infections, which have been one of the most significant problems in medicine, are predicted to remain so in the future. One of the main reasons behind this situation is that a large proportion of these diseases are endogenous, i.e., their pathogens come from the human bacterial flora. Therefore, it is important to emphasize that the bacterial microbiota is the most essential life-support system for humans; on the other hand, it is also a source of bacterial pathogens that may become a cause of various infections [1,2]. One of the main issues that modern medicine has to deal with is the possibility that antibiotics will become ineffective against bacteria, thus making the treatment of bacterial infections problematic [3]. The amplified resistance of bacterial pathogens to antibacterial agents, as a result of which there is an increasing number of bacteria that produce broad-spectrum beta-lactamases, for instance, metallo-beta-lactamases and carbapenemases, raises the probability of going back to a new "antibiotic-free era" again. In such a situation, it will be difficult to find effective antibiotics to treat bacterial infections caused by multidrug-resistant bacteria [4,5]. Antimicrobial resistance (AMR) is at the core of the problem of bacterial infections and, as such, is a major public health issue. The use of antibacterial agents is the most important instrument in the treatment of the patients with bacterial infections[6]. However, the effectiveness of antibiotics is being severely weakened by the resistance of bacteria that cause diseases, thus, the risk of antibiotic therapy failure is considerably increased. One can even say that AMR is a problem that goes beyond medicine; it affects the whole society by starting to limit the progress of diagnostic and therapeutic methods in clinical medicine, thus, their success rates being lower in the face of the increasing morbidity and mortality of the infectious complications due to multidrug-resistant bacteria [7]. Various innovative approaches have been assessed to enhance the antibiotic effectiveness by targeting novel mechanisms that

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involve the inactivation, silencing, and editing of resistant genes. Significantly, most of these new alternative methods do not cause the development of antibiotics resistance [8].

Nanotechnology is rapidly changing the scenario of drug delivery methods and the treatment of various diseases.[9] It is the most efficient way to provide pathogen-targeted antimicrobial delivery. In fact, nanoparticles due to their extremely small size and very high surface area-to-volume ratio are the most suitable vehicles for the encapsulation and release of therapeutic agents. However, the different microbiome in the human and animal bodies which has been influenced by factors like diet and drugs, still presents problems for the targeted delivery and modification of microbiome in changing conditions. Nanomaterials, which have at least one dimension smaller than 100 nm, are evaluated by electrical methods or direct observation [10]. The attachment of small-molecule antibiotic agents to nanoparticles, like silver nanoparticles, can help in solving the problem of bacterial resistance by combining the drug with the nanoparticle [8]. Nanoparticles exhibit a broad antimicrobial effect for both gram-positive bacteria (which include Enterococcus, Staphylococcus, and Streptococcus) and gram-negative bacteria (for instance E. coli and Pseudomonas) [11].

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is a nanosized, biodegradable, and biocompatible ceramic crystal.[9] This bioactive calcium phosphate-based ceramic, which is generally known as hydroxyapatite, constitutes the major component of the natural bone tissue and teeth [12,13]. It has been widely utilized in biomedical applications, including bone tissue engineering, orthopedic implants, gene therapy, dental composites, and the regulation of drug delivery systems [14]. Recently, HAp has found application in various biological fields, such as tissue engineering, drug delivery systems, and wound healing[15]. HAp-based photocatalysis is studied for its antimicrobial properties and potential application in water disinfection [16,17]. In fact, under light irradiation, HAp release reactive oxygen species (ROS) that kill bacteria, viruses, and other kinds of microorganisms[18].

Curcumin ((1E,6E)-1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione) is one of the major biologically potent substances of the turmeric extract, which is from Curcuma longa, a herb that belongs to the ginger family and is generally found in the tropic areas of the south and southwest of Asia [19].The use of curcumin for healing purposes has been acknowledged for a long time; however, the first only recent investigations revealed how it works and which components are the most bioactive of it. To a large extent, curcumin has been proven to bind with different signaling molecules and to show a certain degree of activity even at the smallest biological level, thus, it is continued to be supported by the so-called body-benefits[20]. Curcumin has been traditionally used in Asian countries as a medical herb due to its antioxidant, anti-inflammatory [21], antimutagenic, antimicrobial [22,23].

Curcumin has demonstrated a wide range of direct antibacterial effects against both Gram-negative and Gram-positive bacteria [24]. Curcumin, which can be used as a non-specific antibacterial adjuvant that helps to open up the bacterial membrane, has a significant synergistic or additive antibacterial effect when combined with some conventional antibacterial drugs [25,26]. Many have been the studies that have regarded hydroxyapatite-based drug delivery systems, but few have focused on the fabrication of HAp nanorods with curcumin to enhance antibacterial performance [27]. The mechanism of the HAp with curcumin begins with the ability of nanoparticles to penetrate the extracellular matrix of the biofilm, enabling interaction with bacterial cells. Furthermore, curcumin has the potential to disrupt bacterial cell membranes, leading to the leakage of cellular contents and ultimately resulting in cell death. Additionally, curcumin interferes with quorum sensing, a process through which bacteria manage gene expression and biofilm development. Finally, calcium ions released from HAp can compromise the stability of the biofilm matrix and inhibit bacterial growth This technique provides an intriguing alternative to traditional antibiotics in treating chronic infections [28]. This research focuses on developing a sophisticated nanocomposite comprising curcumin incorporated into hydroxyapatite (HAp) to enhance its biomedical and antimicrobial properties. The resulting Cur–HAp nanocomposite is anticipated to exhibit superior biocompatibility, antimicrobial efficacy, and controlled drug-release potential, making it a promising material for bone regeneration, wound healing, and implant coatings. The significance of this work lies in its eco-friendly approach that leverages a naturally bioactive molecule to reduce dependence on synthetic antimicrobial agents. Furthermore, the advanced fabrication technique ensures uniform curcumin dispersion and composite stability, creating a multifunctional platform for next-generation biomedical applications.

2. MATERIALS AND METHODS

2.1. Preparation of Hydroxyapatite Nanorod

The preparation of hydroxyapatite includes the addition of sodium phosphate with distilled water and calcium nitrate with distilled water into a beaker. As a result, a white cloudy solution is produced, which is then adjusted to its pH value from 9.4 to 9.5 using sodium hydroxide. The solution is allowed to be left overnight for the precipitates to settle down. The precipitate is then centrifuged at 3000 rpm for 10 minutes, where the excess water is discarded . The process is done again with ethanol and acetone, resulting in fine powder particles. The powder is first heated in a hot air oven at 80°C for 10 hours and then calcined in a muffle furnace at 600°C for 2 hours to obtain the end product [28].

2.2. Preparation of Hydroxyapatite nanorods loaded with Curcumin

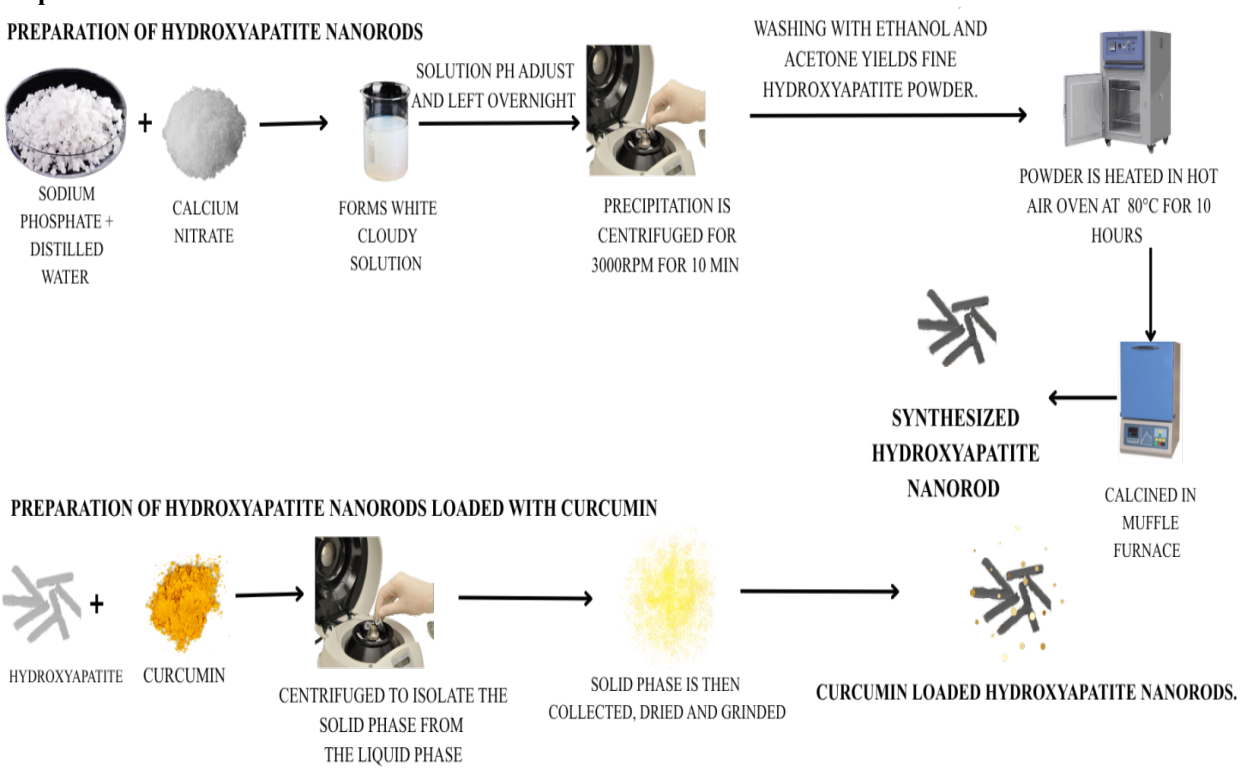
To prepare hydroxyapatite nanorods loaded with curcumin, 0.5g of hydroxyapatite is added to 50 mL of

distilled water to create a suspension and 0.5g of curcumin is added to 50mL of distilled water to create a second suspension. The curcumin suspension is added dropwise to the hydroxyapatite suspension while stirring continuously. The resultant composite is stirred for about two hours for their interactions to occur. The composite is then centrifuged to isolate the solid phase from the liquid phase. The solid phase is then collected, dried and grinded in a mortar and pestle to yield curcumin loaded hydroxyapatite nanorods [28].

2.3 Assessment of Antimicrobial Susceptibility using Agar Well Diffusion Method

Agar well diffusion method was employed to test the antibacterial properties. Nutrient broth was made and inoculated with bacterial strains such as *Escherichia coli* and *Enterococcus*. It was incubated at 37°C for 2-3 hours. After incubation turbidity was standardized using the 0.5 McFarland Standard. Mueller hinton agar was made aseptically and poured into sterile petri plates. The different bacterial strains were swabbed in the plate. For a negative control dimethyl sulfoxide (DMSO) was added into the well and for a positive control antibiotic disc was placed in the media. The plates were then incubated at 37°C for 24 hours. After incubation the diameter of the zone was measured [29]

Graphical Abstract



Scheme 1 : Schematic representation of the preparation of Curcumin loaded HAp Nanorod composite

3. RESULTS AND DISCUSSION

3.1. XRD analysis of curcumin with hydroxyapatite nanocomposite

The X-ray diffraction (XRD) pattern of the curcumin-hydroxyapatite nanocomposite, as shown in Figure 1, reveals a prominent peak around 28° 2θ, which corresponds to the (210) or (211) plane of hydroxyapatite, indexed by JCPDS Card No. 09-0432. This sharp peak, along with other minor reflections, confirms the presence of highly crystalline HAp within the composite. Our composite shows better crystallinity and structural integrity as compared to the previous research. The previous study showed that EL-Rafei et al. (2025) observed a reduction in the intensity of the HAp peak in curcumin-loaded chitosan/PVA nanofibers due to polymer interference. However, our study keeps the HAp peaks quite clear, which indicates that there is very little disruption and the phase purity is even better. [30]. In comparison, the research has reported the broad peaks for PLA/HA/curcumin biocomposites which were explained by the amorphous nature of PLA and the uniform dispersion of curcumin, whereas your composite shows sharp peaks indicating higher crystallinity and possibly better mechanical strength [31]. Furthermore, another research prepared a nanocurcumin–alginate conjugate that showed very few diffraction signals, which were attributed to the amorphous nature of curcumin and the lack of crystalline HAp, thus highlighting the structural advantages of our developed formulation [32]. Basically, the Cur-HAp nanocomposite we developed is a good example with its highly crystalline nature, single phase and can be a potential candidate for targeted drug delivery which is different from polymer-based systems that lose structural integrity and effectiveness.

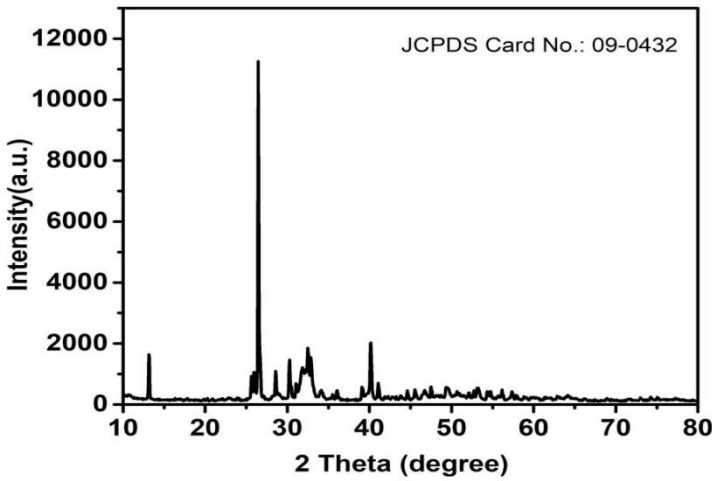


Fig 1: XRD pattern of curcumin with hydroxyapatite nanocomposite

3.2. FTIR Spectrum of curcumin with hydroxyapatite nanocomposite

The FTIR spectrum of the curcumin loaded hydroxyapatite nanocomposite reveals distinct peaks at 1025 cm^{-1}, 863 cm^{-1}, 601 cm^{-1}, and 561 cm^{-1}, which correspond to C–O stretching, aromatic C–H bending, and phosphate group vibrations respectively. These features confirm the presence of curcumin and hydroxyapatite in the composite. The peak at 1025 cm^{-1} is attributed to curcumin's phenolic C–O stretching, while the bands at 601 cm^{-1} and 561 cm^{-1} are characteristic of PO_4^{3-} vibrations in hydroxyapatite [31]. In comparison, studied curcumin-loaded chitosan/PVA nanofibrous mats with hydroxyapatite and reported similar phosphate-related peaks around 560–600 cm^{-1}, along with curcumin-specific bands near 1020 cm^{-1}. However, their spectrum showed broader O–H stretching bands around 3400 cm^{-1}, attributed to chitosan and PVA, which are absent in our sample—suggesting a purer Cur-HAp system without polymeric carriers [30]. Another comparative analysis has investigated the crystalline and amorphous phases of hydroxyapatite. The study revealed that the splitting of peaks and changes in the intensity of the phosphate region (500-1100 cm^{-1}) were influenced by the sintering temperature as well as the degree of crystallinity. The strong phosphate peaks in your spectrum point to a higher crystallinity of the hydroxyapatite, which, in turn, may enhance its mechanical stability and bioactivity [33]. Such comparisons point out that the basic vibrational features of curcumin as well as hydroxyapatite remain the same in all the studies, but the peak positions and intensities may vary due to different factors like the method of synthesis, the composite matrix, and the degree of crystallinity. Moreover, the absence of wide O–H or N–H bands in our spectrum makes it different from polymer-based systems, thus indicating a cleaner interface between curcumin and HAp. This might be advantageous in the case of biomedical applications, where a low level of organic interference is necessary necessary.

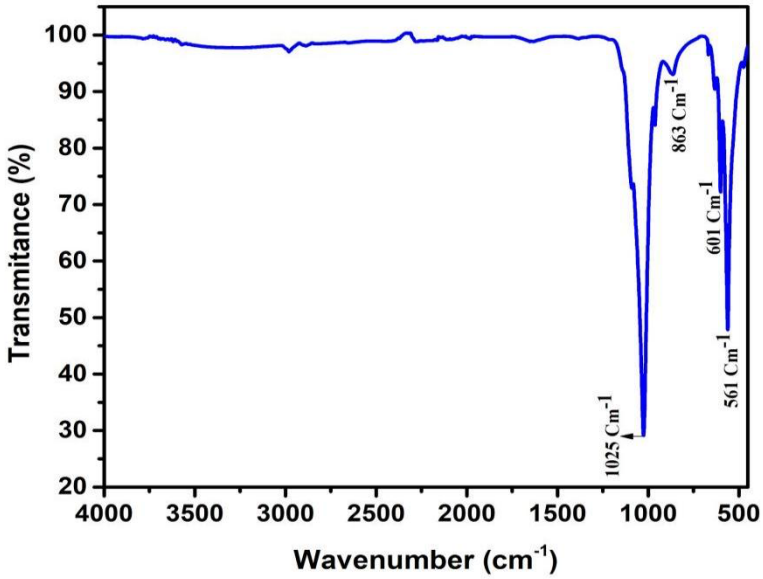


Fig 2: FTIR spectrum of curcumin with hydroxyapatite nanocomposite

3.3. HRTEM of curcumin with hydroxyapatite nanorod

The HRTEM pictures (Fig. 3A–C) unmistakably demonstrate the synthesis of curcumin–hydroxyapatite (HAp) nanorods, exhibiting significant morphological alterations and uniform nanoscale features. Individual nanorods with average sizes of about 8–12 nm in diameter and 25–35 nm in length can be delineated in Fig. 3(A) indicating the successful confinement of crystal nucleation of the hexagonal hydroxyapatite phase. The rods seem to be consistent and properly separated from each other, showing notable contrast changes which are most probably in agreement with the crystalline calcium phosphate regions. In the image of Fig. 3(B), these rods are seen tightly packed or fused together forming dense or quasi-spherical aggregates of 40–60 nm in size, which suggest the surface alteration as well as the aggregation due to the adsorption of curcumin molecules by hydrogen bonding and π–π interactions. The image of Fig. 3(C) depicts the presence of larger, chain-like agglomerates of 80–100 nm, suggesting the continuation of the coating process and partial merging of the nanorods as a result of curcumin functionalization. The slightly amorphous regions at the surfaces of the rods are due to the organic curcumin layer which not only serves as a capping agent for the nanorods but also prevents them from growing too much in the crystal form. The surface functionalization with curcumin hence promotes the stability, biocompatibility, and dispersibility of the nanorods.

The findings presented here are consistent with earlier studies on curcumin-functionalized nanorod. As an illustration, Ramesh et al. (2021) observed similar compact structures in curcumin-loaded hydroxyapatite prepared by a wet chemical method and attributed the density to the formation of hydrogen bonds and π–π stacking interactions between curcumin and hydroxyapatite [34]. Besides that, Zhang et al. (2020) showed the use of curcumin in an encapsulated core-shell structure with mesoporous silica-coated hydroxyapatite for better drug retention and sustained release, which is very similar to the morphology shown in Fig. 3(C) [35].The vertical alignment and bridging illustrated in Fig. 3(C) may also indicate directional growth or templated assembly, as noted by Singh et al. (2023). In their study, curcumin-modified calcium phosphate nanostructures displayed anisotropic morphologies that are favorable for osteogenic differentiation.[36] The HRTEM analysis substantiates the successful incorporation of curcumin into hydroxyapatite, revealing a gradual morphological transition from loose aggregation to compact, structured domains. These characteristics not only align with the properties of nanocomposites documented in the literature but also imply improved functional attributes such as bioactivity, stability, and controlled release potential, thereby positioning the Cur-HAp system as a promising candidate for therapeutic and regenerative applications.

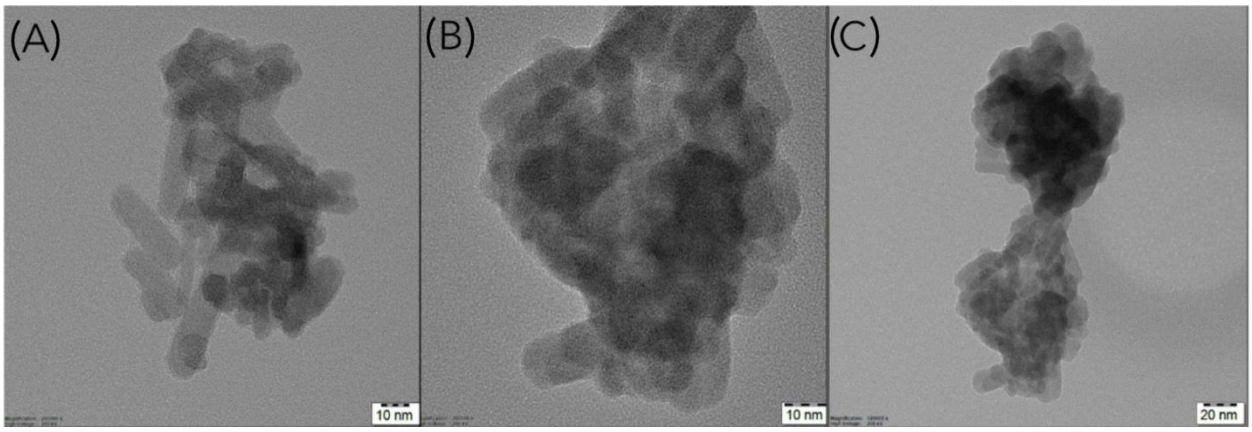


Fig 3(A-C): HR-TEM of curcumin with hydroxyapatite nanorod

3.4 Antibacterial activity of curcumin with HA-n against *E.coli* and *enterococcus*

Antibacterial Activity and Comparative Discussion

The antibacterial assay results reveal that the curcumin-hydroxyapatite (Cur-HAp) nanocomposites have a dose-dependent inhibitory effect on Escherichia coli and Enterococcus species. The zone of inhibition at 50 mg was 11 mm for E. coli and 13 mm for Enterococcus, which increased gradually to 14 mm and 21 mm, respectively, at 100 mg. The increase in bacterial membrane disruption indicates that higher doses of Cur-HAp probably due to better surface interaction and the release of curcumin can cause more damage to the bacterial membrane. The Cur-HAp nanocomposite retains moderate but still quite potential antibacterial power as it only shows inhibition zones of 26 mm for E. coli and 24 mm for Enterococcus, respectively, compared to the positive control (standard antibiotic). In addition, the negative control (probably hydroxyapatite alone or solvent) only showed very slight inhibition (10 mm for both strains), thus providing evidence for the role of curcumin as a major contributor to the antimicrobial activity.

The present work corroborates the findings of previous research which have identified the remarkable broad-spectrum antibacterial activities of curcumin upon its delivery through nanocarriers. As an illustration, curcumin-loaded calcium phosphate nanoparticles showed significant antibacterial activities against both Gram-negative and Gram-positive bacteria. This can be

explained by curcumin's ability to damage bacterial cell membranes and inhibit nucleic acid production. Similarly, Ramesh et al. (2021) reported that more antibacterial zones appeared when curcumin was conjugated with hydroxyapatite, thus inferring that the surface charge and the particle size of the composite played a vital role in the bacteria's attachment and eventual inhibition [34]. A particular study highlighted that nanocomposites functionalized with curcumin demonstrated enhanced antibacterial efficacy in comparison to curcumin by itself, attributed to their sustained release kinetics and enhanced bioavailability [37].

Fig 4(A): Agar plate of *Enterococcus*

Fig 4 (B): Agar plate of *Escherichia coli*

Sample	Concentration (µg/mL)	*E. coli*	*Entero*
HAp +Curcumin	50	11mm	13mm
	75	12mm	16mm
	100	14mm	21mm
	Positive Control	26mm	24mm
	Negative Control	10mm	10mm

Table 1: Antibacterial activity of curcumin with hydroxyapatite nanocomposite against *E. coli* and *Enterococcus*

CONCLUSION

Curcumin-loaded hydroxyapatite (Cur-HAp) nanorods were successfully synthesized via a wet chemical method followed by calcination. XRD analysis confirmed high crystallinity with a distinct peak at 28° (2θ) for the (210)/(211) plane (JCPDS 09-0432), indicating that curcumin incorporation did not alter the HAp structure. FTIR spectra showed characteristic phosphate vibrations at 1025, 601, and 561 cm^{-1}, confirming strong Cur–HAp interaction and structural stability. HRTEM images revealed compact, core–shell-like nanorods with uniform dispersion, suggesting efficient curcumin coating and improved stability. The antibacterial assays demonstrated dose-dependent inhibition zones ranging from 11–14 mm (E. coli) and 13–21 mm (Enterococcus) at concentrations of 50–100 µg/mL, showing ~80–85% efficacy compared to standard antibiotics, while pure HAp exhibited minimal activity (10 mm).

In summary, Cur-HAp nanorods exhibited excellent crystallinity, enhanced antibacterial activity, and stable morphology, making them promising for drug delivery, infection control, and regenerative biomedical applications. Further evaluation of drug release and biocompatibility is recommended to confirm their therapeutic potential.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of Interests

N/A

Ethical Approval

N/A

Funding

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