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Original Article

Synthesis of Nano-Hydroxyapatite from Sea Shell and Squid Bone Waste for Advanced Bone Applications via Precipitation Process

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Abstract *- Large quantities of seashells and squid bones are discarded globally as biowaste, leading to serious environmental concerns. This work explores the conversion of these wastes into nano-hydroxyapatite (nHAp) using a simple and sustainable precipitation technique. The aim of the study was to establish an economical synthesis route while assessing the material's suitability for biomedical applications. Hydroxyapatite is valued for its osteoconductivity - the ability to guide new bone formation and bioactivity- and its capacity to bond with living tissues, making it a promising candidate for bone regeneration. Comprehensive material characterization was carried out through FT-IR, XRD, SEM, TEM and EDAX. The analyses confirmed the development of phase-pure nHAp with uniform hexagonal and rod-like morphologies and high elemental purity. Antibacterial studies demonstrated significant inhibition zones against Listeria and Salmonella, with greater activity observed against Salmonella. The findings demonstrate that dual marine waste sources can be transformed into functional nHAp suitable for biomedical use. Beyond addressing waste management challenges, the study establishes a pathway toward scalable, low-cost biomaterials with potential applications in bone tissue engineering, implants, and regenerative medicine.*

Keywords *- Seashell, Squid Bone, Hydroxyapatite, Antibacterial.*

1. Introduction

The need for advanced biomaterials in the field of bone tissue engineering has been escalating due to the rising prevalence of bone-related diseases and the aging global population. Hydroxyapatite (HA), a naturally occurring mineral form of calcium phosphate, plays a crucial role in bone and dental structures due to its excellent biocompatibility, osteoconductivity, and bioactivity. As such, it has been extensively explored for use in bone grafts, implants, and coatings in orthopedics and dental applications. Despite its widespread use, traditional methods of synthesizing HA can be costly, energy-intensive, and environmentally damaging, necessitating the search for sustainable, cost-effective, and eco-friendly alternatives.

One promising approach for addressing these challenges is the use of natural sources, particularly marine waste, for HA synthesis. Sea shells and squid bones, often discarded as by-products in the seafood industry, are rich in calcium carbonate and phosphate—key precursors for hydroxyapatite. These organic wastes provide an ideal alternative source for HA production, offering a sustainable, cost-effective, and environmentally friendly option for the development of advanced biomaterials.

The synthesis of nano-hydroxyapatite (nHA) from these waste materials is particularly advantageous. Nanoscale HA particles possess enhanced surface area, improved mechanical strength, and superior bioactivity, all of which are critical properties for effective bone regeneration and healing. The precipitation method, which involves the controlled chemical precipitation of calcium and phosphate ions to form HA, is a widely used, scalable, and straightforward approach for synthesizing nHA. By optimizing this method, it is possible to produce nHA with tailored properties such as particle size, morphology, and crystallinity, which are crucial for its performance in biomedical applications.

This study focuses on the synthesis of nano-hydroxyapatite from marine waste, specifically sea shell and squid bone waste, using the precipitation process. The project aims to evaluate the feasibility of utilizing these natural waste materials as a source of calcium and phosphate, investigate the effect of synthesis parameters on the properties of the resulting nHA, and assess its potential for advanced bone applications. Furthermore, the project aligns with the principles of the circular economy by transforming waste materials into high-value products, thereby contributing to both environmental sustainability and the advancement of biomedical technologies.

Through this approach, the work aims to provide an eco-friendly and cost-effective pathway for producing high-performance biomaterials that can significantly impact the fields of bone tissue engineering and regenerative medicine.

Hydroxyapatite (HA) is a component that comprises many minerals like calcium, phosphate, and oxygen, along with the formula $Ca_{10}(PO_4)_6(OH)_2$. It resembles the natural substance in human bones quite a bit. Its biocompatibility, bioactivity, and osteoconductivity make this a suitable material for a variety of biomedical [1] purposes, particularly in bone tissue engineering. However, conventional methods for producing nano-hydroxyapatite (nHA) often involve expensive raw materials and complex processes, limiting their scalability and economic feasibility. In recent years, the utilization of biogenic waste materials as alternative calcium sources for nHA synthesis [7] has garnered significant interest. Sea shells and squid bones, primarily composed of calcium carbonate ($CaCO_3$), represent abundant and renewable waste resources [4, 13]. Recycling these wastes addresses environmental [2] concerns and provides a cost-effective route for synthesizing high-quality nHA.

The precipitation method is a popular way to make nHA because it is simple, inexpensive, and can create nanoparticles with a controlled shape and size. Marine shells are mainly made up of an inorganic substance called CaCO3 (calcium carbonate) in different forms like calcite, aragonite, and others, which makes up about 95% of their composition [3]. The remaining 5% is made up of organic materials like proteins, lipids, chitin, and more. These mixed materials make shells more treasured; hence, they are adopted for organic and inorganic applications. Nevertheless, every year, around 2.7 trillion tons of shell waste are produced, with only 450–1,000 billion tons being used for various purposes. The rest, about 50%, ends up as unwanted bio-waste. This waste can accumulate in marine environments, causing significant harm to the ecosystem. Shells also take a long time to break down, which can damage land quality, deplete natural resources, and harm aquatic life. Therefore, it is important to find ways to use waste shells to create valuable products like Hydroxyapatite without causing environmental or health problems.

Numerous reports have documented the conversion of seashells and squid bones into calcium carbonate. The present study focuses on synthesizing nano-hydroxyapatite from the waste material obtained from squid bone and seashells, investigating various procedures, which include antibacterial activity, XRD, FT-IR, TEM, and SEM with EDAX [5, 9]. The objective is to develop scalable and environmentally sustainable methods for producing high-quality hydroxyapatite from these marine resources [6], with the resulting nHAp composite being utilized in a range of biomedical applications [12]. By addressing existing research gaps, this work aspires to enhance sustainable resource utilization, broaden the range of biomaterials available for medical applications, and promote the integration of eco-friendly practices within the field of biomedical engineering [14].

2. Research Gap

The accumulation of seashell and squid bone waste extends beyond localized pollution, contributing to ecological imbalance in marine habitats, deterioration of land resources and added strain on waste management systems. While prior studies have mainly focused on single-source materials for hydroxyapatite synthesis, little attention has been given to combining multiple marine wastes to enhance efficiency and sustainability. In addition, many reported approaches rely on costly or chemical-intensive processes, with limited emphasis on scalable, eco-friendly methods that also validate antibacterial performance. To bridge these gaps, the present study investigates a dual-source precipitation route for producing nano-hydroxyapatite, offering both an environmental solution and a potential biomedical material.

2.1. Novelty

In comparison with existing research, this study introduces distinct advances in the synthesis of nano-hydroxyapatite. Earlier works have largely utilized single-source precursors such as eggshells, bovine bones or seashells or relied on chemical reagents that are costly and less sustainable. While these methods have produced hydroxyapatite with acceptable structural characteristics, they often face limitations in scalability and environmental impact. Recent studies (Lubna et al., 2022; Abere et al., 2022; Alam et al., 2024) highlight the importance of eco-friendly and renewable sources, but most still focus on individual waste streams. By contrast, the present work employs a dual-source marine biowaste system, seashells and squid bones, which ensures a more consistent calcium yield and promotes waste valorization. The adopted precipitation route is energy-efficient compared with sol–gel, pyrolysis or sonochemical techniques, yet it yields high-purity, nanostructured hydroxyapatite confirmed by FT-IR, XRD, SEM, TEM and EDAX. Importantly, the study goes beyond structural characterization by demonstrating antibacterial activity against two pathogenic strains, linking material performance with biomedical functionality. These aspects collectively establish the novelty of this work and position it as an advancement over existing approaches by integrating sustainability, cost-effectiveness and biomedical potential in a single framework.

This study advances existing research by demonstrating a dual-source approach that combines seashells and squid bones for the sustainable synthesis of nano-hydroxyapatite. While previous studies have predominantly relied on synthetic chemicals or single-source natural materials, this method ensures better resource utilization and consistency in calcium supply. The precipitation route adopted here is cost-effective and environmentally benign and yields high-purity nanostructures with confirmed antibacterial activity. By integrating environmental waste valorization with biomedical functionality, the present work provides a distinctive contribution that bridges sustainability and medical innovation.

3. Materials and Methods

Natural hydroxyapatite (NHAp) can be extracted from a wide range of calcium-rich biological waste materials, primarily in the form of calcium carbonate ($CaCO_3$) or calcium phosphate ($Ca_3(PO_4)_2$). These include livestock bones and marine industry by-products such as shells and internal skeletal structures. Utilizing such biowastes not only addresses waste disposal challenges but also supports sustainable material sourcing for biomedical applications. This study explores five key biogenic sources for NHAp synthesis: seashells and squid bone. Each of these offers distinct structural, compositional, and functional advantages, making them suitable candidates for conversion into hydroxyapatite for bone tissue engineering.

3.1. Seashells

Seashells are abundant marine biowastes composed predominantly of aragonite or calcite forms of $CaCO_3$. They are widely available in coastal regions and can be easily converted into HAp through sol-gel-assisted calcination followed by phosphatization. Their transformation into HAp is energy-efficient and cost-effective, aligning with eco-friendly manufacturing principles. Additionally, seashell-derived HAp often contains trace elements such as strontium (Sr^{2+}) and magnesium (Mg^{2+}), which are known to enhance osteogenesis and provide antimicrobial benefits. These properties make seashell-based HAp particularly attractive for use in bone graft substitutes, scaffolds, and composite matrices in regenerative medicine.

3.2. Squid Bones

Squid bone, or cuttlebone, is a lightweight internal shell structure characterized by a highly porous and lamellar architecture. It consists mainly of calcium carbonate and exhibits a honeycomb-like morphology that is advantageous for bone tissue engineering. When subjected to sol–gel processing and controlled sintering, squid bone can be converted into porous hydroxyapatite while retaining its native microstructure. This interconnected porosity facilitates cell adhesion, proliferation, nutrient exchange, and vascularization—features essential for successful scaffold integration and bone regeneration. Furthermore, the mechanical strength of squid bone-derived HAp can be optimized, making it suitable for load-bearing implant applications.

In this study, nano-hydroxyapatite (nHA) is synthesized from sea shell and squid bone waste using a precipitation method. The seashell waste, rich in calcium carbonate, is first calcined at 900°C to convert it into calcium oxide, while the squid bone waste, containing calcium and phosphate, is used directly as a calcium and phosphate precursor. The precipitation process involves mixing calcium nitrate (or calcium hydroxide) with phosphoric acid to form calcium phosphate, followed by pH adjustment to 9–10 with ammonium hydroxide, leading to the formation of a precipitate. The precipitate is then filtered, washed, dried at 60°C, and calcined at 800°C to obtain hydroxyapatite. To achieve nanoscale hydroxyapatite, the obtained powder is mechanically milled.

The synthesized nHA is characterized using X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Fourier-Transform Infrared Spectroscopy (FTIR), and particle size analysis. Bioactivity is evaluated through in vitro mineralization tests in Simulated Body Fluid (SBF) and cell culture studies using osteoblast-like cells to assess cytotoxicity and cell proliferation. This process offers an eco-friendly and cost-effective approach to producing high-quality nHA for advanced bone applications, while utilizing marine waste and contributing to sustainable biomaterials production. In this study, seashells and squid bones were first cleaned with boiling water to remove organic residues and then dried at 100 °C for two hours.

The dried materials were finely ground and subjected to calcination at 900 °C for three hours, resulting in the decomposition of $CaCO_3$ into CaO. The obtained CaO was dissolved in nitric acid to produce $Ca(NO_3)_2$ solution, which served as the calcium precursor. Diammonium hydrogen phosphate was gradually introduced to maintain a Ca/P ratio of 1.67, and the solution pH was carefully adjusted to 9-10 using NaOH to create conditions favorable for hydroxyapatite nucleation. The mixture was aged, filtered and oven-dried at 120 °C, followed by a secondary calcination at 600 °C to enhance crystallinity. This optimized precipitation pathway ensured the formation of high-purity nano-hydroxyapatite particles.

The FT-IR spectra confirmed the presence of phosphate, hydroxyl and carbonate groups, indicating the successful formation of hydroxyapatite with B-type carbonate substitution. XRD analysis revealed sharp diffraction peaks matching standard JCPDS patterns, verifying phase purity and high crystallinity, with no detectable secondary phases such as CaO.

SEM and TEM imaging demonstrated rod-like and hexagonal nanostructures with uniform distribution, which are known to support improved cell adhesion and biological response in biomedical applications. EDAX analysis confirmed the elemental composition, with calcium, phosphorus, and oxygen as the primary constituents, which is consistent with stoichiometric hydroxyapatite.

The antibacterial activity test revealed clear inhibition zones against both Listeria monocytogenes (Gram-positive) and Salmonella enterica (Gram-negative). Notably, greater inhibition was observed against Salmonella, which may be attributed to differences in bacterial cell wall structures. These results suggest that the synthesized nHAp possesses desirable physicochemical properties and exhibits antimicrobial potential, enhancing its applicability in bone grafts and implant coatings where infection control is critical.

3.3. Materials

Sea shell **Squid bone**

Fig. 1 Sea shell and squid bone

Figure 1, the gathered sea shells and squid bones underwent an initial washing with tap water multiple times, followed by a treatment with boiling water for a duration of 30 minutes. Next, deionized water is used to wash the leftovers in the shell, like algae and shell meat. The following materials are sourced from Merck: Diammonium hydrogen phosphate (NH_4) 2HP0$_4$, acetone, Nitric acid (HNO_3), deionized water, and Sodium hydroxide (NaOH). All chemicals utilized were of analytical reagent grade, boasting a purity of 99%.

3.4. Synthesis of Hydroxyapatite from Sea Shells and Squid-Bone

The flowchart Figures 2 and 3 show that the synthesis of nano-hydroxyapatite (nHA) powder from natural calcium sources such as sea shells or squid bones involves several key steps. First, the raw materials are dried at 100°C for 2 hours in a hot air oven to remove moisture, and then finely crushed using a pestle and mortar.

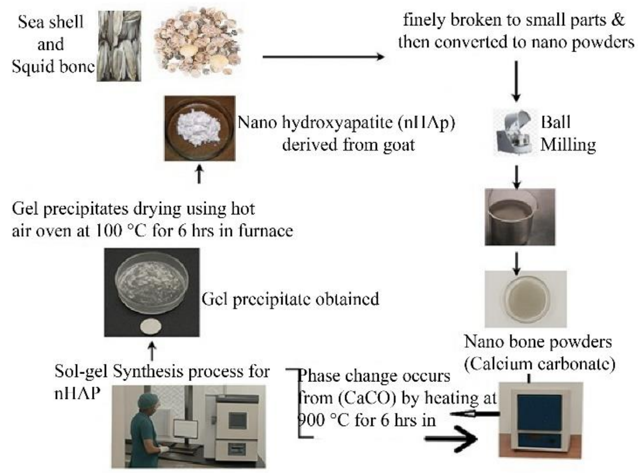


Fig. 2 Synthesis of Hydroxyapatite from Seashells and Squid-bone

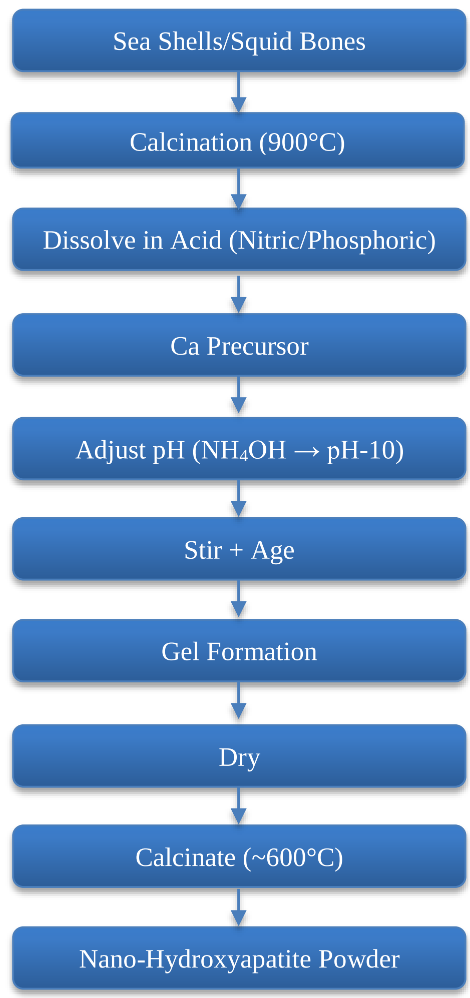


Fig. 3 Synthesis of Hydroxyapatite from Sea shells and Squid-bone

The powdered material is gradually heated in a muffle furnace, increasing the temperature by 10°C every 10 minutes over 3 hours, until reaching 900°C. This calcination step removes organic matter and converts calcium carbonate

($CaCO_3$) into calcium oxide (CaO). The resulting CaO is dissolved in nitric or phosphoric acid to produce a calcium ion (Ca^{2+}) solution, serving as the precursor. The pH of the solution is adjusted to 10 using ammonium hydroxide (NH_4OH), creating an alkaline environment suitable for hydroxyapatite formation.

This solution is stirred and aged, leading to gel formation. The gel is then dried and calcined again at approximately 600°C to enhance its crystallinity. The final product, nano-hydroxyapatite powder, is highly biocompatible and closely resembles natural bone, making it ideal for biomedical applications such as bone grafts and dental implants [18]. The precipitation process was optimized by maintaining the pH in the range of 9-10 through the dropwise addition of NaOH, creating favorable conditions for hydroxyapatite nucleation and growth.

The calcination of the raw materials was carried out at 900 °C for 3 hours to decompose $CaCO_3$ into CaO, followed by a secondary heat treatment at 600 °C to improve crystallinity of the final nHAp. For antibacterial testing, Listeria monocytogenes (Gram-positive) and Salmonella enterica (Gram-negative) were chosen because they represent two distinct bacterial groups of clinical and food safety importance. Their structural differences in cell wall composition allow a comparative evaluation of the antibacterial potential of synthesized nHAp.

$$CaCO_3 \longrightarrow CaO + CO_2 \uparrow$$

Hydroxyapatite nanoparticles were made using the precipitation method. First, 2.36 g (0.1 mole) of the calcined CaO from sea shell and squid bone waste was dissolved in 25% pure HNO3. After dissolving, the solution was mixed with 75% deionized water to create a 0.1 M $Ca(NO_3)_2$ solution. Next, 0.769 g of a 0.06 M solution of Diammonium hydrogen phosphate $(NH_4)_2$ HPO4 was incorporated into the $Ca(NO_3)_2$ solution to attain a Ca/P ratio of 1.67.

The 1M NaOH solution was then added incrementally, drop by drop, simultaneously stirring the solution with a magnetic rod to increase the pH to 9. A milky white precipitate was formed as NaOH was added. Deionized water was used to wash away the precipitate using Whatman 40 filter paper to remove any residue of ammonium (NH_4) ions and sodium (Na). The obtained precipitate was further dried at 120°C in a hot air oven. The composite scaffolds exhibited improved mechanical strength, reaching up to 0.99 ± 0.19 MPa [11].

The series of chemical reactions engaged in the process is described here.

$$CaO + 2HNO_3 \longrightarrow Ca(NO_3)_2 + H_2O\uparrow \quad (1)$$

$$10\,Ca(NO_3)_2 + 6\,(NH_4)_2\,HPO_4 \longrightarrow Ca_{10}(PO_4)_6(OH_2) + 6\,NH_4NO_3 \quad (2)$$

$$Ca_{10}(PO_4)_6(OH_2) + 6NH_4NO_3 \xrightarrow{900\,°C\,/\,3hr} Ca_{10}(PO_4)_6(OH_2) + 6NH_4NO_3 \text{ (Hydroxyapatite)}$$

4. Results and Discussion

4.1. FT-IR Analysis of HAp Derived from Sea Shell and Squid Bone Waste

Figure 4 presents the FTIR spectrum of the synthesized hydroxyapatite (HAp) at 900°C. The spectrum displays key peaks at 3447, 2924, 2310, 1750, 1642, 1334, 1043, 873, 601, and 569 cm-1.

The asymmetric stretching of the phosphate group in HAp correlates with the acute and intense peak at 1043 cm-1. The bending mode (υ4) of the PO43-group is associated with the peaks at 601 and 569 cm-1.

Like bone mineral, the peak at 873 cm-1 marks the existence of carbonated apatite with B-type substitution in the tetrahedral position. Whereas the peaks at 2400 cm-1 and 1750 cm-1 are ascribed to the amine (NH) and amide groups, respectively, the 2924 cm-1 peak is connected to the stretching mode of the C-H bond. Along with a minor band at 1642 cm-1, a broad band spanning 3000 to 3700 cm-1 points to the existence of absorbed water [16, 17].

Both hydroxyapatite samples have peaks with some variations and additional peaks. The strength of the peaks is higher in case two. Overall, the synthesized samples of the hydroxyapatite have been presented in all samples, asserting that the material is indeed hydroxyapatite, consistent with previous studies.

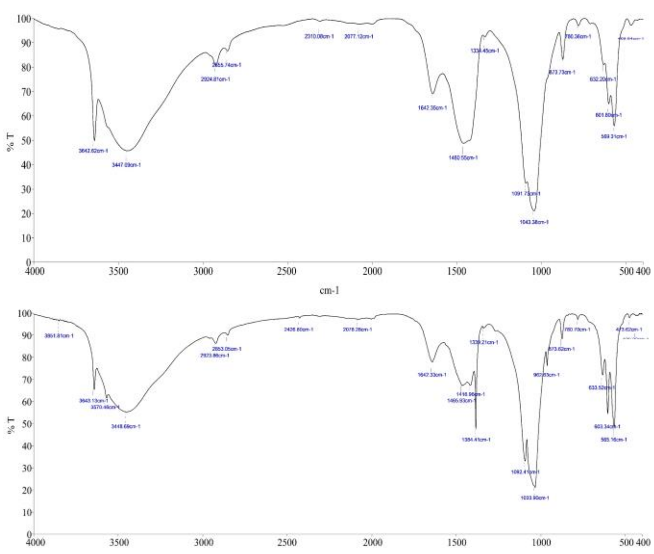


Fig. 4 FTIR spectrum of the HAp derived from sea shell (top) and squid bone waste (bottom)

4.2. XRD Analysis of HAp Derived from Sea Shell and Squid Bone Waste

Figure 5 illustrates the X-ray diffraction (XRD) patterns of hydroxyapatite (HAp) obtained from sea shell waste. Main diffraction peaks are seen at 2θ values of 25.87°, 31.80°, 32.19°, 32.54°, 32.94°, and 39.86°, which correspond to the (hkl) indices of (002), (211), (112), (300), (202), and (310), respectively. These peaks match well with the standard values for HAp from the JCPDS File no. 09-0432. No further impurity peaks were found, even if certain peaks were not completely resolved. This indicates that the synthesized material is primarily pure HAp.

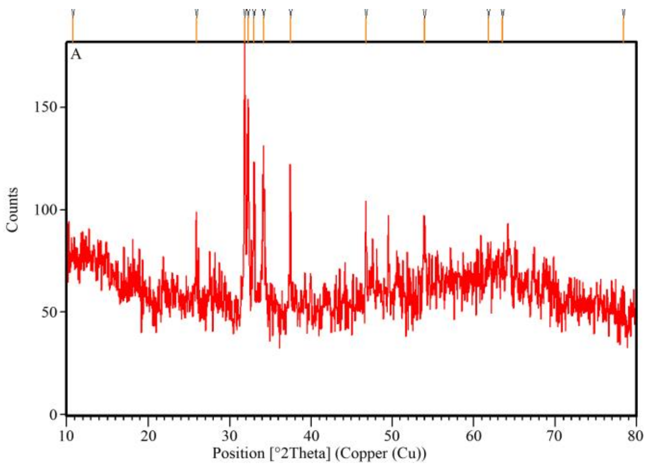


Fig. 5 XRD pattern of the HAp derived from sea shell waste

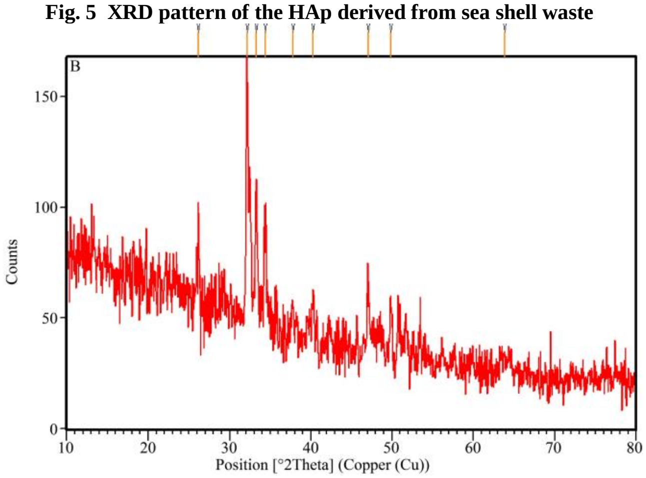


Fig. 6 XRD pattern of the HAp derived from squid bone waste

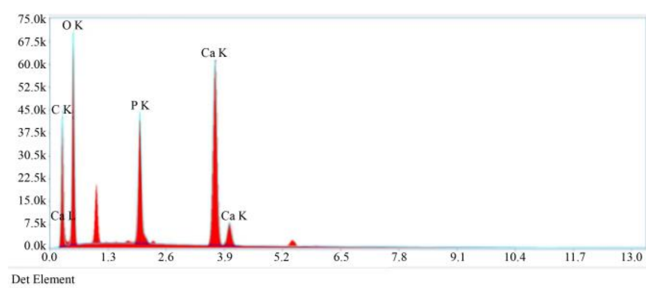
Figure 6 illustrates the X-ray Diffraction (XRD) patterns of hydroxyapatite (HAp) obtained from Squid bone waste. Figure 4 presents the X-ray Diffraction (XRD) pattern of hydroxyapatite (HAp) synthesized from squid bone waste. The XRD profile exhibits distinct diffraction peaks located at 2θ values approximately 25.9°, 31.8°, 32.2°, 32.5°, 32.9°, and 39.8°, which correspond to the crystallographic planes (002), (211), (112), (300), (202), and (310), respectively. These peaks are in good agreement with the standard reference data for hydroxyapatite (JCPDS File No. 09-0432), indicating successful phase formation. The intense peak at 31.8°, associated with the (211) plane, reflects a high degree of crystallinity. The overall absence of impurity peaks such as those corresponding to calcium oxide (CaO) or other calcium phosphate phases confirms the phase purity of the synthesized material.

Furthermore, the moderate broadening of diffraction peaks suggests the presence of nano-sized HAp crystals. These observations affirm that the thermal and chemical treatment of squid bone waste effectively formed pure, nanocrystalline hydroxyapatite, making it highly suitable for biomedical applications like bone tissue engineering and dental regeneration.

4.3. EDX Mapping Analysis and FE-SEM of HAp Derived from Sea Shell and Squid Bone Waste

The EDX spectrum has helped to establish the elemental composition of the produced HAp. Figures 7 and 8 show the EDX spectra of HAp derived from Sea shell waste (Figure 5) and squid bone waste (Figure 6).

The EDX spectrum shows peaks for oxygen, phosphorus, calcium, and carbon in both samples, indicating that these elements are present in the material. This indicates the formation of hydroxyapatite, which demonstrates its extreme purity.



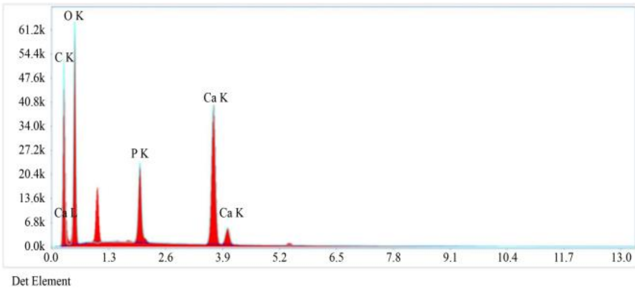
Quant Results

Element	Weight %	MDL	Atomic %	Net Int.	Error %	R	A	F
C K	41.05	0.11	52.51	1191.30	10.05	0.9196	0.1090	1.0000
O K	42.29	0.05	40.61	2208.76	10.30	0.9291	0.0767	1.0000
P K	4.34	0.01	2.15	1930.17	3.64	0.9515	0.7607	1.0158
Ca K	12.32	0.02	4.72	3434.03	1.86	0.9633	0.9443	1.0161

Fig. 7 EDX spectrum of HAp derived from sea shell waste

The FE-SEM images of HAp derived from sea shell waste (on the left side of Figure 9) and squid bone waste (on the right side) show that the HAp has a hexagonal and rod-like shape. These rods formed through a process of crystallization, nucleation, surface regulation, and growth, where nanoplates attach in an organized way.

This is possible because the nuclei tend to grow along the c-axis, with phosphate and calcium ions being attracted to it. The shape of these structures helps improve antibacterial activity and cell viability because of their high surface area and aspect ratio.



Quant Results

Element	Weight %	MDL	Atomic %	Net Int.	Error %	R	A	F
C K	47.28	0.06	57.66	1442.75	9.82	0.9252	0.1280	1.0000
O K	41.48	0.05	37.97	1983.09	10.30	0.9342	0.0785	1.0000
P K	2.52	0.01	1.19	1011.19	3.67	0.9553	0.7692	1.0145
Ca K	8.72	0.02	3.19	2193.60	1.86	0.9663	0.9517	1.0186

Fig. 8 EDX spectrum of HAp derived from squid bone waste

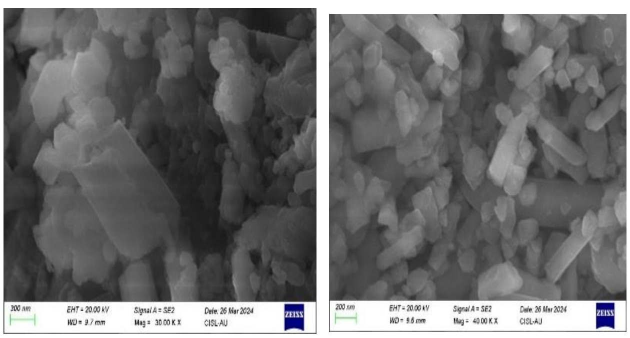
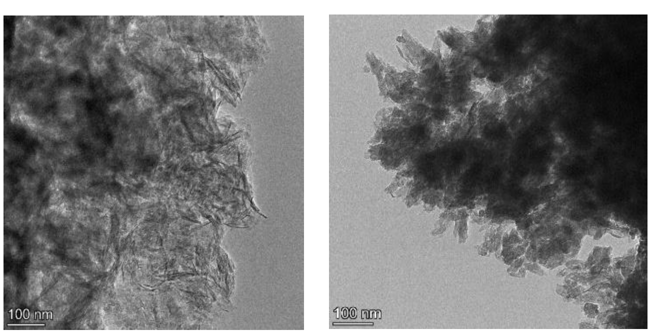


Fig. 9 FE-SEM image of HAp derived from sea shell waste (left side) and squid bone waste (right side)

4.4. HR-TEM Analysis of HAp

Figure 8 presents HR-TEM images (top left and top right) and the corresponding Selective Area Electron Diffraction (SAED) patterns (bottom left and bottom right) of HAp derived from sea shell and squid bone waste. Irregular needle-like structures and hexagonal rods are shown in the microstructure of HAp.

The results of HR-TEM are consistent with the FE-SEM outcomes. The SAED patterns (Figure 10) indicate that the HAp derived from sea shell waste is crystalline, whereas the HAp derived from squid bone waste shows some differences.



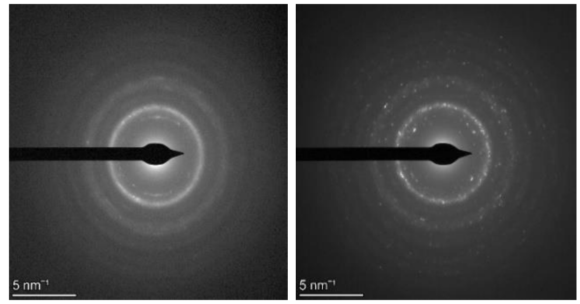


Fig. 10 TEM image of HAp derived from sea shell waste (top left side) and squid bone waste (top right side) and the corresponding SAED patterns

4.5. Antibacterial Activity Test

The synthesized hydroxyapatite with antibacterial [8] effectiveness was administered against the bacteria with two strains using the diffusion method with agar. The outcome of the results is shown in Figure 11. The results indicate that the samples exhibit effectiveness against both Listeria and Salmonella, while the negative control, deionized water, shows no antibacterial activity [10]. The zone of inhibition in Table 1 shows that HAp nanoparticles have antibacterial characteristics, suggesting possible microbial control. Several factors may elucidate these findings. Particularly, gram-negative bacteria have a thin peptidoglycan layer and a lipopolysaccharide component that define their cell wall structure, affecting the permeability of synthetic chemical molecules, which can disrupt respiratory functions and lead to cell death. The data suggest that HAp displays marginally greater antibacterial activity against Salmonella in comparison to Listeria. Consequently, this sample is deemed suitable for biomedical applications [19, 20].

Table 1. Antibacterial activity zone of inhibition against Listeria and Salmonella

Cultures	+Ve	-Ve	HAp from Sea shell	HAp from Squid bone
Listeria	16mm	_	6mm	6mm
Salmonella	19mm	_	8mm	11mm

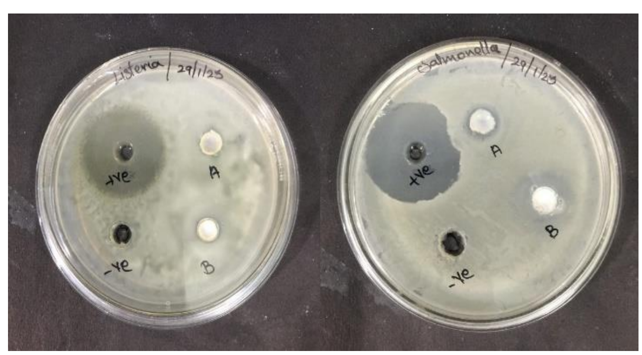


Fig. 11 Image of the HAp antimicrobial test results, and negative control against (a) Listeria, and (b) Salmonella,

5. Conclusion

Seashell and squid bone waste are promising sources for creating hydroxyapatite for biomedical use. This study promotes a simple, affordable, and eco-friendly precipitation method to produce nano-hydroxyapatite (nHAp) from these waste materials. The hexagonal and rod-like shapes of the hydroxyapatite are confirmed using Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM). Additionally, the phase purity XRD and Fourier-Transform Infrared Spectroscopy (FT-IR) are used to confirm the substance. The synthesized hydroxyapatite exhibits superior antibacterial activity against Salmonella compared to Listeria. Consequently, the findings indicate that hydroxyapatite may offer enhanced efficacy and biological safety for promising medical applications. By recycling seashell and squid bone waste through this method, society can significantly enhance waste management practices and promote ecological sustainability.

5.1. Future Scope and Limitations

The study presents an eco-friendly and cost-effective method to synthesize nano-hydroxyapatite from sea shells and squid bone waste with promising biomedical applications. However, further research is needed to test its antibacterial activity against a wider range of pathogens, including drug-resistant strains. Key material properties such as surface area, porosity, and drug-loading capacity were not examined. The mechanical strength remains insufficient for load-bearing applications, and essential biocompatibility studies, including cytotoxicity and in vivo testing, have yet to be conducted. Future work should focus on improving scalability, addressing raw material variability, and integrating advanced fabrication techniques like 3D printing. Additionally, optimization using pore size and porosity data can support detailed mechanical analysis and simulation to enhance structural performance for biomedical use.

Ethical Sourcing Statement

The squid bones used in this study were collected as waste by-products from seafood consumption and local markets. No additional animals were harvested for research purposes, and the study did not involve any live subjects. Since squid fishing is not restricted in the study region, the use of discarded squid bones complies with ethical research standards and promotes the valorization of biowaste for sustainable material development.

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