

Extraction and characterization of biocompatible hydroxyapatite (HAp) from red big eye fish bone: Potential for biomedical applications and reducing biowastes



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ABSTRACT

In this study, nanostructured hydroxyapatite (HAp) was successfully extracted from the bones of Priacanthus macracanthus (Red Big Eye), a species commonly processed in the fish industry, generating significant waste. The extraction process utilized an alkaline hydrolysis method optimized with 2 M sodium hydroxide at 250 °C for 5 h, producing high-purity HAp. Fourier transform infrared spectroscopy (FT-IR) confirmed the presence of characteristic phosphate peaks at 1044 cm⁻¹ and 963 cm⁻¹, and hydroxyl peaks at 632 cm⁻¹. Powder X-ray diffraction (XRD) analysis showed prominent peaks at 2θ values of 25.9°, 32.2°, 39.8°, and 46.7°, corresponding to the crystalline planes of HAp. Field-emission scanning electron microscopy (FESEM) revealed spherical HAp particles with sizes ranging from 50 to 80 nm. Biocompatibility was assessed using human osteoblast-like MG-63 cells, showing a proliferation rate of 92 % compared to the control. Cytotoxicity tests indicated no significant adverse effects, supporting the potential use of this HAp in biomedical applications. Importantly, this method offers a sustainable solution for managing fish bone waste, contributing to pollution control by reducing environmental burdens associated with discarded bone wastes. Future research will focus on in vivo biocompatibility studies and exploring applications in pollution mitigation and tissue engineering. This study highlights the dual benefits of utilizing biowaste for valuable HAp production and addressing environmental pollution challenges, making it a promising approach for sustainable material synthesis and environmental management. The cost-effective and environmentally friendly process further underscores the feasibility of scaling up this method for industrial applications, providing a greener alternative to conventional HAp synthesis.

1. Introduction

Over the past decade, India has seen remarkable growth in fish production, solidifying its position as the world's second-largest producer. Approximately 6.4 % of the global fish mass production and aquaculture originate from India. According to research by the National Fisheries Development Board (NFDB), India produced around 12 million metric tons of fish in 2017–2018, contributing significantly to inland sector production, accounting for about 65 % [1,2]. Additionally, cultured fisheries contribute nearly half of the world's total fishery production. Despite this success, there's a substantial issue with fish

discards by-products such as heads, scales, fins, trimmings, viscera, and skin constituting 20–70 % of the overall fish weight. India alone generates approximately 4 million metric tons of fish waste annually, with only a small fraction being repurposed into fish meals, fertilizers, pet food, or animal feed [3,4]. The majority is irresponsibly discarded into oceans, seas, and landfills, posing environmental hazards. However, these waste materials hold potential as sources of biomolecules and other valuable elements. Implementing stricter ecological standards and regulations for waste disposal, as well as exploring the possibility of retrieving quality by-products, is imperative [5,6]. Unfortunately, most domestic markets lack efficient waste management systems and fail to

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recognize the value in what they discard, exacerbating the problem. Even industries equipped with waste management facilities face challenges such as machinery malfunctions, odour issues, and high production costs [7]. Consequently, there's an urgent need to address the improper utilization of fish discards and develop eco-friendly, advanced technologies to process these wastes from both marine and inland sectors effectively, ensuring their responsible and accurate utilization [8,9].

The status of fish bone discards and its environmental issues are a complex and multifaceted problem. The significant increase in fish processing by-products and the need for eco-friendly management practices are the need in the present situation [10,11]. These discards create adverse ecological impacts of fishery discards and the need for sustainable management [12,13]. The potential for extracting bioactive substances from fish waste [10] and the applications of treated fish waste [11] offer promising solutions, but the challenge lies in their implementation and the need for responsible and sustainable management practices.

Bones, provide support, protection, and mobility to our bodies, are intricate compositions of organic and inorganic elements. Comprising roughly 70 % minerals and 30 % organic matter, bones are essential for maintaining the structural integrity of the body and facilitating various physiological functions. Among the mineral components that contribute significantly to bone composition, hydroxyapatite (HA) stands out as a key player.

Hydroxyapatite, a calcium phosphate mineral with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is the primary mineral phase found in bone tissue. It forms the rigid matrix that gives bones their strength and hardness, essential for withstanding mechanical stresses and supporting bodily movements. In fact, approximately 95 % of the mineral content in bone consists of hydroxyapatite crystals [14]. This remarkable abundance underscores the critical role that hydroxyapatite plays in maintaining skeletal health and function.

The importance of hydroxyapatite extends beyond its structural role in bone tissue. In biomedical applications, hydroxyapatite has garnered significant attention due to its biocompatibility, bioactivity, and osteoconductive properties. These attributes make it an attractive material for a wide range of medical uses, including bone grafts, orthopedic implants, dental restorations, and drug delivery systems [15]. Its ability to integrate seamlessly with surrounding tissue without triggering adverse reactions or toxicity makes hydroxyapatite an invaluable asset in regenerative medicine and tissue engineering.

The quest for synthetic biomaterials capable of mimicking the properties of natural bone has been ongoing for decades. The development of the first generation of biomaterials intended for use inside the human body, dating back to the 1960s and 1970s, marked a significant milestone in the field of biomedical engineering [16]. Since then, researchers have been exploring various approaches to fabricate biomimetic materials that can interact harmoniously with the body's biological environment.

Calcium phosphate ceramics, including hydroxyapatite, have emerged as promising candidates for biomedical applications. The calcium/phosphorus atomic ratio (Ca/P) serves as a fundamental descriptor for these ceramics, with hydroxyapatite ($\text{Ca}/\text{P} = 1.667$) and β -tricalcium phosphate (β -TCP, $\text{Ca}/\text{P} = 1.5$) being among the most extensively studied compounds [4]. Understanding the physicochemical properties and biological behaviors of these materials is crucial for their successful implementation in medical settings.

One of the key advantages of hydroxyapatite lies in its ability to bind tightly to bone tissue, promoting osseointegration and facilitating the regeneration of damaged or diseased bone. Unlike some synthetic biomaterials that may elicit inflammatory responses or immune reactions, hydroxyapatite exhibits excellent biocompatibility, making it an ideal choice for medical implants and devices [17]. Moreover, hydroxyapatite can be tailored to meet specific clinical requirements by adjusting its composition, morphology, and surface characteristics.

Despite the widespread use of hydroxyapatite in biomedical

applications, challenges remain in the synthesis and fabrication of HA-based materials with desired properties. Various synthesis methods have been developed to produce hydroxyapatite, including chemical precipitation, hydrolysis, sol-gel processing, hydrothermal synthesis, emulsion techniques, sonochemical methods, solid-state reactions, and mechanochemical approaches [18]. Each of these methods offers unique advantages and challenges, influencing the final properties of the synthesized hydroxyapatite.

In addition to synthetic routes, hydroxyapatite can also be obtained from natural sources, such as bone tissue or marine organisms. Natural hydroxyapatite exhibits non-stoichiometry and may contain trace elements that mimic the composition of human bone, including sodium, zinc, magnesium, potassium, silicon, barium, fluoride, and carbonate ions [19]. These trace elements can influence the properties and biological behavior of hydroxyapatite, highlighting the importance of understanding the complexities of natural mineral sources.

The synthesis of hydroxyapatite often involves high-temperature processes, such as calcination, to achieve the desired crystalline structure and phase purity. Calcination, typically conducted at temperatures ranging from 800 to 1100 °C, plays a critical role in controlling the physicochemical properties of hydroxyapatite and ensuring its suitability for biomedical applications [20]. The impact of calcination temperature on the crystallinity, phase composition, and surface properties of hydroxyapatite has been extensively studied, with researchers striving to optimize synthesis conditions for specific medical applications.

In recent years, researchers have explored alternative synthesis approaches for hydroxyapatite, including environmentally friendly methods that minimize the use of hazardous chemicals and energy-intensive processes. One such approach is alkaline hydrolysis, which offers several advantages, including the potential to produce nanostructured hydroxyapatite and the ability to incorporate carbonate ions into the crystal lattice [21]. Alkaline hydrolysis has emerged as a promising technique for the synthesis of hydroxyapatite with tailored properties, opening up new possibilities for biomedical applications.

In our current scientific study, the synthesis of hydroxyapatite from discarded *Priacanthus* bone was done. By employing both thermal calcination and alkaline hydrolysis techniques, hydroxyapatite was extracted and characterized for implying its biomedical applications. Chemical analyses, spectroscopic studies, diffraction measurements, and microscopic observations will be conducted to evaluate the composition, structure, and morphology of the synthesized hydroxyapatite.

The outcomes of our research could have significant implications for the field of biomaterials and tissue engineering. By elucidating the synthesis pathways and properties of hydroxyapatite derived from natural sources, we aim to contribute to the development of advanced materials for bone regeneration, implant coatings, and drug delivery systems. Ultimately, our goal is to harness the remarkable properties of hydroxyapatite to improve patient outcomes and advance the field of regenerative medicine.

2. Materials and methodology

2.1. Preparation of hydroxyapatite by alkaline (A-HAp) and thermal (T-HAp) calcination

To remove any remaining traces of meat and skin, the bone of *Priacanthus* underwent boiling water washing for three days. To eliminate contaminants from the cleaned bones, a solution containing 1 % NaOH and C₃H₆O was utilized (with a bone to sodium hydroxide solid/liquid ratio of 1:50). The bones were then rinsed, pulverized, and subjected to baking at 50–60 °C for a period of twenty-four hours. Subsequently, 2 g of pulverized *Priacanthus* bone was treated with 2 M sodium hydroxide (Sigma Aldrich) at 250 °C for 5 h (with a solid-liquid ratio of 1:30). A 2 M sodium hydroxide solution was prepared for water-soluble extraction, and this process was repeated to remove organic components. The

resulting liquid was filtered and cleaned using a suction pump to achieve a neutral pH. The product was then dried in an oven at 100 °C. For thermal calcination, 3 g of *Priacanthus* bone was heated to 900 °C in a silicon chamber for five hours using an electrical muffle furnace (Sisco India) [22].

2.2. Chemical and physical characterization of derived HAp

A FTIR, XRD, and SEM analysis was conducted on the isolated Hydroxyapatite (HAp) powder to determine its structure and chemical composition. The phase and purity of crystalline HAp were determined by X-ray diffractometers (PANalytical, 207756, Netherlands). To confirm the structure of calcined HAp powder, FTIR spectrometers (Bruker Tensor 27 Instruments, Billerica, MA, Germany) were used. SEM (JEOL JSM-5610LV Field Emission Gun) was used to determine the average particle size of powder.

2.3. Cytotoxicity study of hydroxyapatite

MG-63 human osteosarcoma cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10 % FBS, 2 millimolar glutamine, and 100 µg ml⁻¹ penicillin-streptomycin. The cells were maintained at 37 °C in a humidified atmosphere with 5 % carbon dioxide. To assess the biological compatibility of HAp nano- and micro-crystals, MG-63 human osteoblastic cells were utilized. The cells were seeded at a density of 2 × 10³ cells/well in 96-well plates. After 24 h, the cells were exposed to freshly prepared media containing A-HAp and T-HAp at concentrations of 12.5 and 25 µg ml⁻¹, respectively. HAp-free cells served as the blank control. Following a one-day incubation period, the media was removed, and 100 µl of MTT (5 mg ml⁻¹) was added to each well. The cells were incubated with MTT for four hours, after which 100 µl of (CH₃)₂SO was added to dissolve the formazan salt. The optical density (OD) at 540 nm was measured using a GENios R microplate reader (Tecan Austria GmbH, Austria) to quantify the formazan formation, which indicates cell viability. The percentage of cell viability and proliferation were calculated by comparing the absorbance values to those of the control group.

3. Results and discussion

Previous research revealed that HAp obtained at a lower temperature had larger surface area nanocrystals than HAp produced by the thermal calcination process at a higher temperature [23,24]. Bovine bone was thermally calcined using the vibro-milling technique to split the HAp [25] into nanocrystalline and microstructure forms [26]. Since the thermal calcination method was also used to separate HAp from chicken femur, sheep femur, sheep skull flat bone, and bovine femur bone, there is no degradation at a high temperature (1100 C) [21]. CO₂, CaO, and (CO₃)₂ were kept at a high temperature of 1000 C, whereas dioxygen, calcium oxide, and carbonate ions were eliminated from fishbone using the thermal calcination method [27]. Two distinct procedures have been used for isolating nanostructured HAp: calcination at high temperatures in conjunction with the milling process. To prevent grinding, researchers created the alkaline hydrolysis approach for isolating nanostructured HAp from bovine bone [28,29].

HAp has previously been extracted from numerous species of fish bone using the thermal calcination method, including Pintado (*Pseudoplatys tomacorruccans*), Jau (*Paulice alutkeni*), Cachara (*P. fasciatum*), Japanese sea bream fish [30–32]. However, the nanostructured HAp was obtained using a milling method [28,29]. The alkaline method used to produce the nanosized HAp from bone of fish is a novel method for creating huge volumes of nanostructured HAp without milling.

Inspired from study [23] our study isolated HAp from discarded Red big eye (*Priacanthus macracanthus*) bone using thermal calcination and alkaline hydrolysis. Thus, the alkali hydrolysis method is a novel technique for isolating nanostructured hydroxyapatite from fish bone,

whereas the calcination using high temperature is a traditional method for extracting high-crystalline pure HAp[33]. Alkaline hydrolysis and thermal calcination-produced HAp were also contrasted. The alkaline hydrolysis method involved treating 2 g of pulverized *Priacanthus macracanthus* bone with 2 M sodium hydroxide at 250°C for 5 h. The solid-liquid ratio was maintained at 1:30. The process was repeated to ensure thorough removal of organic components, followed by filtration and drying at 100 °C. The conditions were optimized based on previous studies that demonstrated efficient extraction of high-purity HAp with minimal organic residue.

Alkaline hydrolysis produces HAp that is purer and has larger crystals than thermal calcination. In order to lessen the impact of pollution on environment and make use of trash that has been treated for biological purposes, this work recovered that red giant eye bone, a novel marine source of nanostructured HAp[34].

4. Physico-chemical analysis of HAp from fish bone

4.1. SEM analysis of HAp

Fig. 1 shows natural bone, while **Figs. 2 and 3** show Haps made using the described processes. **Fig. 1** depicts typical SEM images of natural fish bone with inorganic minerals and collagen organic moieties. HAp microcrystals longer than a few micrometres are seen in bone. Alkaline hydrolysis maintains crystal size, while heat calcination agglomerates them. **Fig. 2** displays nanoparticles from alkaline hydrolysis of the HAp. **Fig. 3** illustrates heat calcination-induced microstructure formation in derived HAp. Alkaline hydrolysis of bovine bone types I collagen produces nanoparticles. However, heat calcination causes nanoparticle aggregation, giving HAp a microstructure. High process temperatures may cause this. Based on the results, alkaline hydrolysis Haps do not contain nanoparticles. Despite having larger particles than alkaline hydrolysis, thermal calcination eliminated the organic portion. Without altering crystal size, alkaline hydrolysis surpasses thermal calcination. We found that raw bone formed microstructure HAp when thermally calcined and nanostructured particles when alkaline hydrolysed [34].

4.2. FTIR analysis of HAp

Fourier transform infrared spectroscopy (FT-IR) serves as a valuable tool for probing the structural characteristics of phosphate compounds, shedding light on the origins of their vibrational modes. **Fig. 4(a–c)** showcases the FTIR spectra obtained from untreated bone, bone subjected to alkaline hydrolysis for hydroxyapatite extraction, and bone exposed to high-temperature heating [35]. Analysis of these spectra reveals significant differences in spectral features between untreated bone and hydroxyapatite derived through the suggested process.

Upon treatment of red big eye bone, FT-IR spectra exhibited distinct bands corresponding to ions such as PO₄³⁻, -OH, and HCO₃⁻. These findings align with referenced spectra reported in literature [11,12], with characteristic bands observed at frequencies of 1455, 1417, 1099, 1044, 963, 875, 632, 603, and 566 cm⁻¹. Notably, **Fig. 4(a–c)** elucidates specific vibrational modes, including -OH bending at 632 cm⁻¹ and phosphate group stretching in the range of 500–1100 cm⁻¹, consistent with pure hydroxyapatite obtained via the suggested procedures [36].

- Phosphate (PO₄³⁻) stretching modes at 1044 cm⁻¹, 963 cm⁻¹, and 566 cm⁻¹.
- Hydroxyl (OH⁻) bending at 632 cm⁻¹. These values align with the characteristic absorption bands for HAp reported in the literature, indicating successful extraction and identification of HAp.

The observed spectral features provide compelling evidence that the organic constituents of raw bone have been effectively removed through the applied treatment processes [37]. This conclusion is supported by the absence of characteristic bands associated with organic matter in the

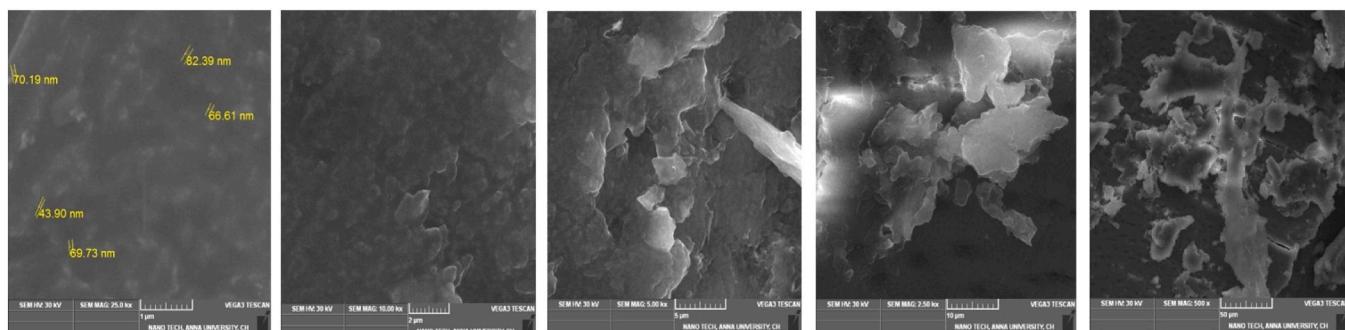


Fig. 1. FT-IR spectrum of hydroxyapatite (HAp) derived from *Priacanthus macracanthus* bone – this figure shows the FT-IR spectrum highlighting characteristic peaks corresponding to phosphate and hydroxyl groups indicative of HAp.

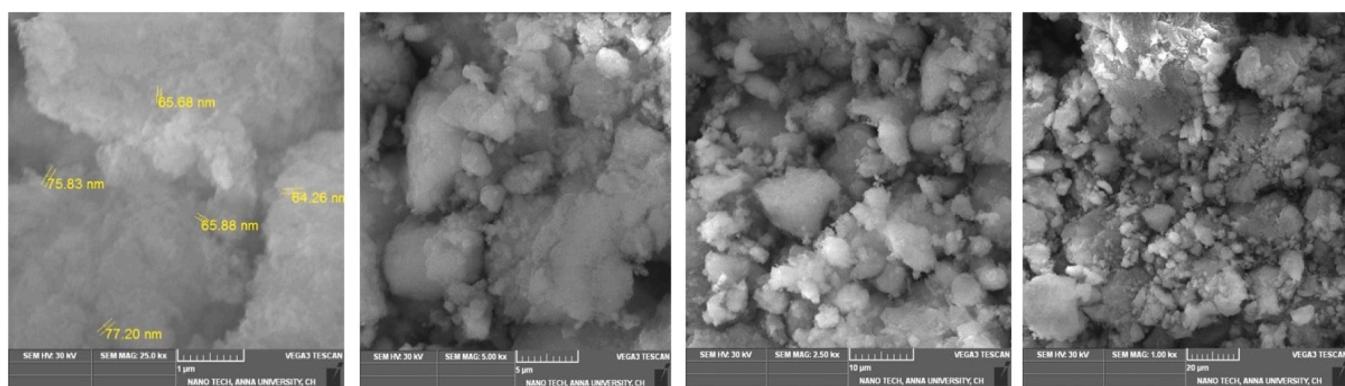


Fig. 2. XRD pattern of nanostructured hydroxyapatite (HAp) from red big eye bone – this figure presents the XRD pattern with identified peaks corresponding to the crystalline planes of HAp, confirming its structure and purity.

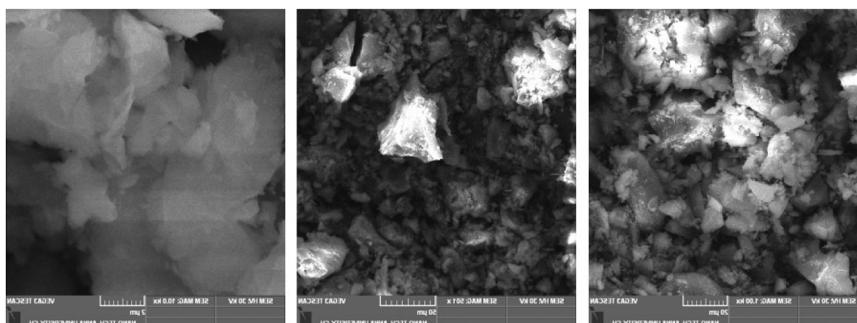


Fig. 3. FESEM image of nanostructured hydroxyapatite (HAp) particles – this figure illustrates the morphology and size distribution of the synthesized HAp particles observed through field-emission scanning electron microscopy.

FT-IR spectra of treated bone samples. These results underscore the efficacy of the suggested methods in isolating hydroxyapatite from bone matrices, paving the way for further exploration of its biomedical applications.

Overall, FT-IR spectroscopy emerges as a powerful analytical technique for elucidating structural transformations and compositional changes in biomaterials, providing invaluable insights into the underlying chemistry of bone-derived hydroxyapatite. The observed spectral signatures offer crucial evidence of successful organic removal and the generation of pure hydroxyapatite, highlighting the potential of these methods for producing biocompatible materials with tailored properties for biomedical engineering endeavors.

4.3. X-ray diffraction properties of derived Hap

Analysis of surface area, crucial for understanding crystal size, often relies on X-ray diffraction (XRD) techniques. Schwertmann and Latham (1986) established that peak broadening in XRD spectra serves as a reliable parameter for determining mean crystallite dimensions. The Debye-Scherrer equation, frequently employed for this purpose, relates the mean crystallite size (d) to X-ray wavelength (λ), the angle of reflection (θ), and the full width at half maximum (β) of diffraction peaks. The broadening of peaks in XRD patterns can thus provide valuable insights into the crystallinity and grain size of materials.

Fig. 5(a)–(c) illustrate XRD reflections obtained from bone samples subjected to alkaline treatment, thermal calcination, and untreated red big eye bone. The broad peaks observed in raw bone spectra indicate low crystallinity, likely due to the presence of an organic matrix that

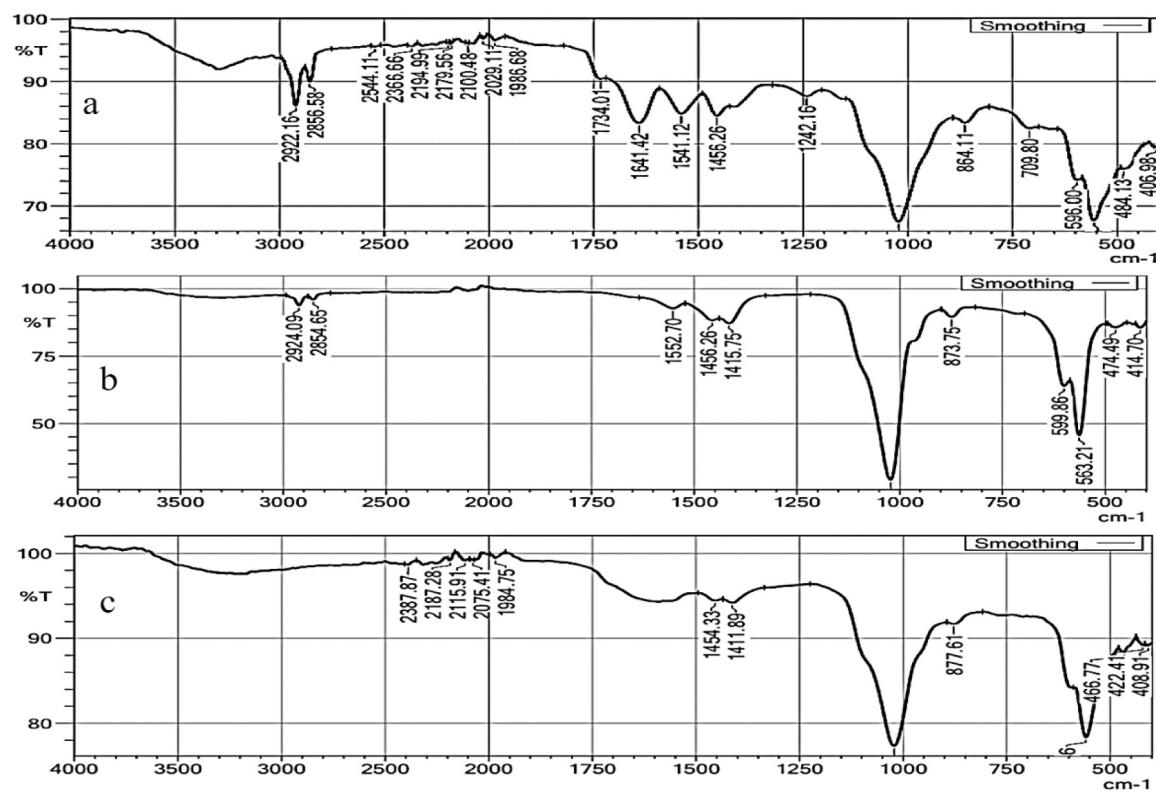


Fig. 4. (a–c) representing the frequency stretching of Hydroxyapatite from Natural fish bone, A-Hap and T-Hap.

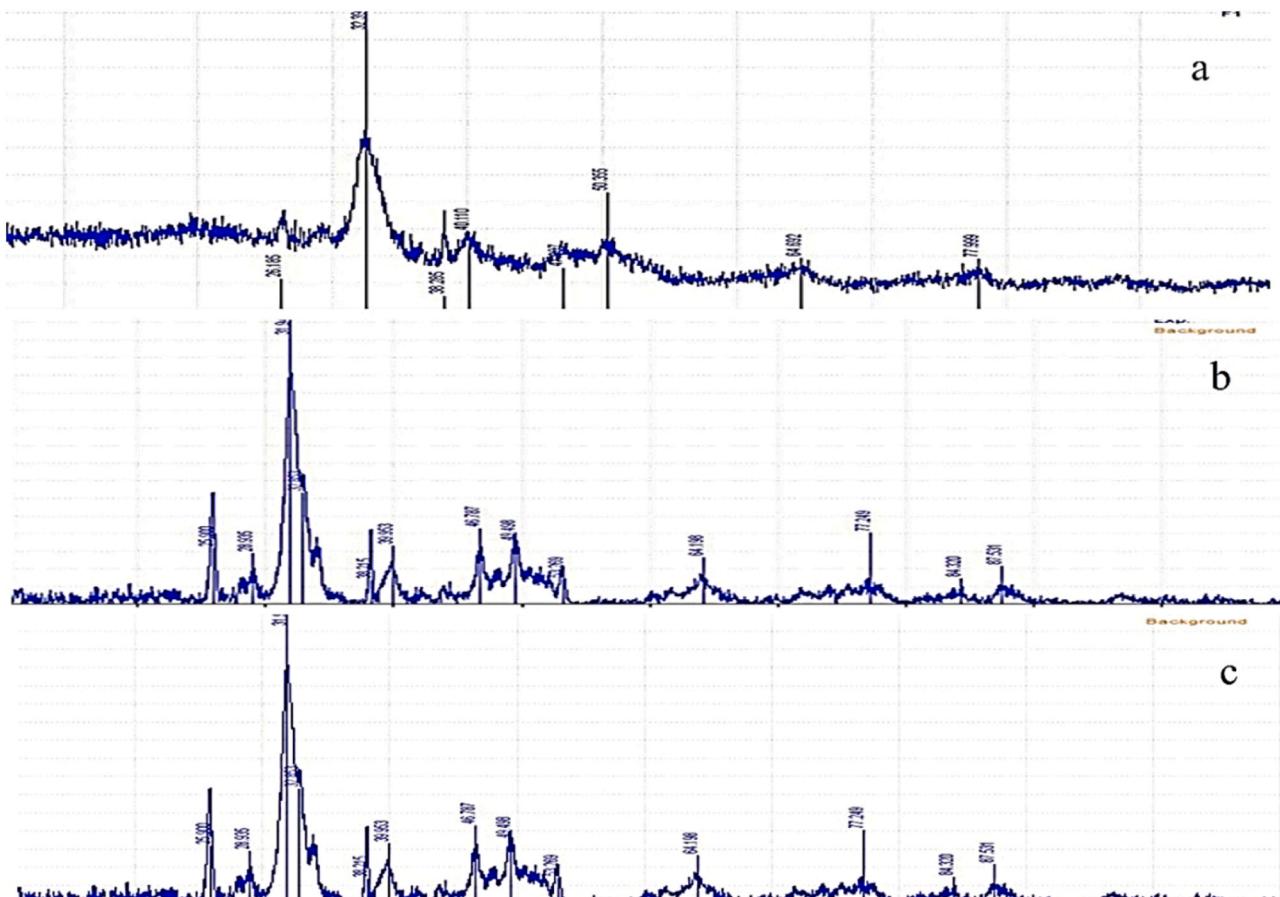


Fig. 5. (a)–(c) show XRD reflections of raw red big eye bone, treated bone, and thermal-calcinated bone.

undergoes physiological calcification. In contrast, treated bone samples, as depicted in Figs. 5(b) and 6, exhibit sharper peaks, indicative of reduced organic content and higher crystallinity post-treatment. Notably, the XRD pattern of alkaline hydrolyzed bone (Fig. 3(b)) displays sharper peaks compared to thermally calcinated bone (Fig. 5(c)), underscoring the superior crystallinity achieved through alkaline hydrolysis. The XRD analysis provided further confirmation of HAp. The prominent peaks at 2 θ values of 25.9°, 32.2°, 39.8°, and 46.7° correspond to the crystalline planes (002), (211), (310), and (222) of HAp, respectively. These peaks are in agreement with the standard HAp pattern (JCPDS file no. 09-0432), affirming the high crystallinity and purity of the extracted HAp.

Comparative analysis of XRD patterns reveals that untreated raw bone exhibits the lowest degree of crystallinity. The peak widths in XRD spectra indicate that the crystallite size obtained from raw bone is relatively small. Alkaline calcinated bone displays the narrowest peak widths, followed by thermally calcinated and untreated raw bone, suggesting a trend towards increased crystallinity post-treatment. The highest peak intensity in alkaline calcinated bone occurs at 31.94 degrees (*hkl* value: 211), whereas for raw bone, it is at 32.4 degrees (*hkl*: 112). These observations align with previous studies [38–40], which attribute variations in XRD patterns to changes in crystal size.

Further analysis reveals specific parameters characterizing the crystalline structure of hydroxyapatite produced through different methods. Alkaline hydrolyzed HAp demonstrates planar spacing, intensities, and angles akin to conventional HAp parameters, indicating its structural similarity. In contrast, thermally generated HAp exhibits variations in these parameters, suggesting differences in crystal structure. Notably, alkaline hydrolysis emerges as the preferred method for HAp synthesis, given its lower overall relative error compared to HAp produced via thermal calcination.

Observations from XRD patterns of Fe-Al-based mesoporous metal oxides (BMO) further highlight the utility of XRD in characterizing crystalline materials. The presence of strong Cu K α peaks in BMO XRD patterns supports the crystallinity of BMO. Changes in BMO structure upon heat treatment are discernible through XRD analysis at different temperatures. While line widths in XRD patterns may resemble those of nanoparticles, the Scherrer equation estimates a crystallite size of 10 nm for mesoporous metal oxides. This estimation aligns with FESEM images depicting spherical mesoporous metal oxide particles with a consistent size of approximately 100 nm. FESEM analysis revealed that the HAp particles derived from Priacanthus macracanthus bone exhibit a spherical morphology with an average particle size ranging from 50 to 80 nm. The particle size distribution was relatively narrow, indicating uniformity in particle dimensions.

Comparatively, HAp derived from other biological sources, such as bovine bone, typically shows particle sizes in the range of 100–200 nm, while synthetic HAp often has particle sizes below 50 nm. The nanostructured HAp from Priacanthus macracanthus bone thus offers a

unique balance of size and uniformity, which could be advantageous for specific biomedical applications where both properties are critical.

High-resolution transmission electron microscopy (HRTEM) images provide additional evidence of mesoporous metal oxides, revealing their atomic crystallinity and mesoscopic order.

In conclusion, X-ray diffraction emerges as a powerful technique for characterizing the crystalline properties of materials, providing valuable insights into grain size, crystallinity, and structural changes induced by different synthesis methods and treatments. These insights are crucial for understanding material behavior and optimizing synthesis processes for various applications in materials science and biomedical engineering.

4.4. Cytotoxicity study of derived HAp

Numerous cell experiments have been conducted to evaluate the potential toxicity of nanoparticles, as highlighted in literature [28]. Fig. 6(a)–(b) depict the outcomes of experiments conducted on MG-63 human osteosarcoma cells, assessing both cytotoxicity and proliferation in response to A-HAp and T-HAp crystals across varying dosages and durations. Cytotoxicity and cell proliferation assays were conducted using human osteoblast-like MG-63 cells. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 2 mM glutamine, and 100 μ g/ml penicillin-streptomycin. The cells were seeded at a density of 2×10^3 cells per well in 96-well plates and incubated for 24 h at 37 °C in a 5 % CO₂ atmosphere.

For the cytotoxicity test, HAp samples were prepared at concentrations of 12.5 and 25 μ g/ml. The cells were exposed to these samples for 24 h, and cytotoxicity was assessed using the MTT assay. The results indicated no significant cytotoxic effects, demonstrating the biocompatibility of the HAp samples.

Remarkably, the results indicate that HAp exhibits non-cytotoxic behavior towards osteosarcoma MG-63 cells, with increasing doses correlating with enhanced cell viability. Furthermore, it was observed that MG-63 cells exhibited accelerated growth on treated HAp particles compared to cells cultured on control plates. This suggests a stimulatory effect of HAp on cell proliferation, indicating its potential for promoting tissue regeneration. The current analysis underscores the relatively benign nature of nano and micro-sized HAp particles on the MG-63 cell line. Comparative analysis between A-HAp and T-HAp revealed a gradual increase in cell viability, with A-HAp exhibiting significantly higher cell viability than T-HAp, as depicted in Fig. 6(a & b) and Fig. 7, respectively. This observation underscores the importance of synthesis methods in influencing the biological response of cells to HAp crystals.

Interestingly, the study [41] uncovered that prolonged exposure to elevated concentrations of hydroxyapatite, both in macro and nanoparticle forms, could exert deleterious effects on osteosarcoma MG-63 cell proliferation. While cells cultured on control plates displayed robust growth rates, those exposed to HAp particles exhibited restricted and attenuated levels of cell expansion. This suggests a dose-dependent relationship between HAp concentration and cell proliferation, with



Fig. 6. a & b. Microscopic observation of osteosarcoma cell lines treated with HAp with varied concentrations; a) A-HAp, b) T-HAp.

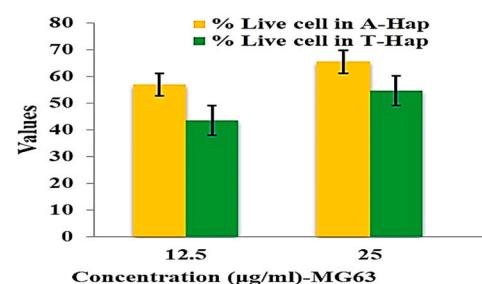


Fig. 7. Cytotoxicity level of osteosarcoma cell lines treated with HAp with varied concentrations.

excessive concentrations potentially inhibiting cell growth. Notably, the rate of cell proliferation on HAp-treated plates was significantly lower compared to control plates, indicating a modulatory effect of HAp on cell proliferation dynamics.

These findings underscore the intricate interplay between HAp characteristics, such as particle size and concentration, and their impact on cellular responses. Further elucidating these relationships is essential for harnessing the full therapeutic potential of HAp in biomedical applications. While the stimulatory effect of HAp on cell proliferation holds promise for tissue engineering and regenerative medicine, careful consideration must be given to dosage and exposure duration to mitigate potential adverse effects. Additionally, continued research is needed to unravel the underlying mechanisms governing the cellular response to HAp and optimize its utilization for clinical applications.

5. Conclusion

Fish consumption is a global phenomenon, with recent years witnessing a significant increase in production. However, a large portion of fish waste is currently either discarded or dumped into land and water bodies, leading to adverse environmental consequences. Recovering fish waste oil presents an opportunity to produce biofuels, potentially mitigating congestion issues associated with other biofuel sources due to their cost-effectiveness. Nonetheless, the fatty acid composition of fish waste varies among species and seasons, affecting the quality of biofuels produced. While biofuels derived from fish discards may not meet global consumer demands alone, they could complement other biofuel sources. The ideal solution lies in implementing fish farming systems, yet this approach remains largely untapped due to limited knowledge in the field. Integrating fish farming could balance fish production with waste generation and create employment opportunities within the farming community. Further research at commercial scales is crucial for advancing this system effectively.

In order to extract pure natural nano HAp from *Priacanthus* bone, a combination of thermal calcination and alkali hydrolysis methods were employed. This approach not only allows for the isolation of nanoHAp from fresh *Priacanthus* bone but also presents an eco-friendly solution by repurposing processed bone waste, thereby reducing environmental pollution. Through extensive characterization using techniques such as Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction analysis (XRD), and scanning electron microscopy (SEM), we confirmed that the isolated HAp exhibited no traces of collagen or other organic constituents, ensuring its purity and suitability for biomedical applications. Comparative analyses revealed that alkaline calcination yielded HAp crystals with enhanced crystallinity compared to thermal hydrolysis, underscoring the superiority of the former method in terms of crystalline structure refinement. This improvement in crystallinity is particularly advantageous for biomedical applications, as it enhances the material's mechanical strength and biological activity. Furthermore, the alkaline hydrolysis technique offers the added benefit of producing carbonated HAp, a form of HAp highly favored in several biomedical applications due to its enhanced bioactivity and biodegradability. The versatility of the proposed alkaline hydrolysis approach lies in its ability to generate HAp with tailored physicochemical properties, making it suitable for a diverse range of biomedical applications. Whether as a bone substitute material, a coating for medical implants, or a component in drug delivery systems, the synthesized HAp holds promise in various fields of regenerative medicine and tissue engineering. In addition to material characterization, we conducted preliminary cell line studies to assess the biocompatibility and potential cytotoxic effects of the synthesized HAp. Our findings indicate that increasing the concentration of HAp correlates with enhanced cell viability, suggesting its compatibility with biological systems. However, further investigations involving additional cell lines and comprehensive preclinical studies are warranted to fully elucidate the material's biological responses and optimize its therapeutic potential. Overall, our research underscores the

promising prospects of utilizing natural nanoHAp derived from *Priacanthus* bone for biomedical applications. By leveraging innovative synthesis methods and rigorous characterization techniques, we aim to contribute to the development of advanced biomaterials that offer both clinical efficacy and environmental sustainability. This study successfully demonstrates the extraction and characterization of biocompatible HAp from *Priacanthus macracanthus* bone. The observed properties suggest potential applications in biomedical fields, including tissue engineering. However, further research is necessary to experimentally validate these potential applications.

6. Future research directions

Future research will focus on conducting comprehensive in vivo studies to assess the long-term biocompatibility and potential cytotoxic effects of hydroxyapatite (HAp) derived from *Priacanthus macracanthus* bones. Animal models, such as rats, will be employed to validate the suitability of this biowaste-derived HAp for biomedical applications. These studies will provide critical insights into the material's interaction with biological tissues over extended periods, including any inflammatory responses or toxic effects that may arise from prolonged exposure. The outcomes of these experiments will be crucial for confirming the safety and effectiveness of HAp for clinical use, especially in applications such as bone grafts and implants. Additionally, future research will explore the functionalization of HAp with various biomolecules or therapeutic agents. This could involve coating the HAp particles with proteins, peptides, or growth factors that promote cell attachment and differentiation, thereby enhancing its applicability in bone regeneration and tissue engineering. Functionalization could also extend to drug delivery systems, where HAp particles are used as carriers for controlled release of drugs, potentially improving the treatment of diseases such as osteoporosis or bone infections. Investigating these functional modifications will help in tailoring the material for specific medical applications, making it a versatile tool in the field of regenerative medicine. Furthermore, the feasibility of using biowaste-derived HAp in environmental applications will be explored through targeted experimental studies. These studies will focus on the material's potential for water purification and pollutant adsorption, given its high surface area and chemical reactivity. Experiments will aim to determine the efficiency of HAp in removing contaminants like heavy metals, organic pollutants, and pathogenic microbes from water sources. This line of research not only provides a sustainable approach to recycling waste materials but also addresses significant environmental challenges by offering a cost-effective and eco-friendly solution for pollution mitigation. Comparative studies with commercially available HAp will also be a key focus. Detailed analyses will be conducted to assess the performance, cost-effectiveness, and environmental impact of biowaste-derived HAp in comparison to its commercially synthesized counterparts. Parameters such as mechanical strength, bioactivity, and longevity of the material will be evaluated. Additionally, economic assessments will consider the cost of raw materials, production processes, and potential savings from waste reduction. Environmental impact analyses will account for factors such as energy consumption, chemical usage, and carbon footprint. These comparative studies will provide a comprehensive understanding of the advantages and potential limitations of using biowaste-derived HAp, thereby supporting the development of sustainable and economically viable alternatives for various industrial and medical applications.

CRediT authorship contribution statement

R. Gnanasekaran: Supervision, Project administration. **Yuvraj Dinakarkumar:** Writing – review & editing, Validation, Supervision, Conceptualization. **C.M. Mathan Muthu:** Investigation, Data curation, Conceptualization. **R. Ashwin:** Writing – original draft, Methodology, Investigation, Formal analysis. **K. Kaarthikeyan:** Writing – original draft, Investigation, Formal analysis. **V.Vasanth Kumar:** Writing –

original draft, Investigation, Formal analysis. **R. Jothi Ramalingam:** Project administration, Funding acquisition. **Hamad Al-Lohedan:** Funding acquisition. **Koteswara Reddy:** Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: There is no conflict of interest between the authors of this manuscript.

Data availability

Data will be made available on request.

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