

Marine Algae-Derived Nanocomposites for Dual Therapeutic Action: A Green Synthesis Approach to Oral Cancer and Inflammation Control

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Abstract: Nanoparticles are increasingly being explored due to their distinctive physicochemical characteristics and wide range of biomedical applications. Among the various synthesis techniques, eco-friendly approaches such as green synthesis using marine algae have gained attention for their biocompatibility and sustainability. This research presents a green, one-pot synthesis method for fabricating Zirconium–Molybdenum Nanoparticles (Zr/Mo-NPs) utilizing the marine red alga *Gracilaria foliifera*. The synthesized nanoparticles were thoroughly characterized through multiple analytical techniques, including UV-Visible spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy-dispersive X-ray spectroscopy (EDX). UV-visible analysis identified distinct absorption bands at 243 nm and 367 nm, while FT-IR analysis highlighted the role of various functional groups in stabilizing the nanoparticle structure. XRD data revealed that the particles consisted of both crystalline and amorphous phases in equal proportions, indicating favorable structural integrity. SEM images illustrated predominantly spherical nanoparticles with minimal clustering, and EDX confirmed the elemental composition, detecting Zirconium, Molybdenum, Carbon, and Oxygen. In vitro biological evaluations showed notable cytotoxic effects against lung cancer cell lines, along with a concentration-dependent suppression of protein denaturation, highlighting their anticancer and anti-inflammatory potential. These outcomes indicate that Zr/Mo-NPs synthesized through *Gracilaria foliifera* offer a promising route for developing safe and multifunctional nanomaterials. Further investigations into their mechanisms of action and targeted therapeutic applications are recommended.

Keywords: Zirconium nanoparticles; Molybdenum nanoparticles; Green synthesis; *Gracilaria foliifera*; Oral cancer; Anti-inflammatory activity; Nanomedicine; Algae-based nanocomposites.

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1. Introduction

Oral cancer continues to pose a significant global health challenge, marked by persistently high mortality and morbidity rates. A major contributor to this trend is the tendency for diagnoses to occur only in the advanced stages of the disease, limiting treatment

effectiveness and survival outcomes [1]. Traditional interventions—surgical resection, chemotherapy, and radiation—are often invasive and bring a host of complications, including poor patient compliance and a notable decline in post-treatment quality of life [2].

Given these limitations, the emergence of nanotechnology in oncological research has been met with considerable enthusiasm. Its potential to revolutionize therapeutic strategies through targeted drug delivery, minimized off-target effects, and enhanced drug solubility is well recognized. Yet, the question remains: can these high-tech innovations be rendered sustainable and biologically safe without compromising efficacy?

In this regard, algae-based nanomaterials are receiving increasing attention. Their appeal lies not only in their compatibility with biological systems but also in their rich phytochemical makeup. Marine algae are known to produce a diverse array of bioactive compounds—polysaccharides, polyphenols, and carotenoids among them—that naturally possess anticancer and anti-inflammatory properties [3]. When these compounds are integrated into nanoparticle systems, the therapeutic effects can be significantly amplified, offering opportunities to both induce apoptosis in malignant cells and dampen chronic inflammation [4,5]. It seems likely that these mechanisms are synergistic, although this interplay warrants deeper investigation.

Interestingly, nanocomposites derived from algal sources are also gaining traction in dental and oral health applications. From improving the biofunctionality of restorative materials to enabling controlled drug release, their versatility is hard to ignore [6]. Nonetheless, a critical issue persists: conventional nanoparticle synthesis is often resource-intensive, involving harsh chemicals and generating hazardous by-products. This disconnect between innovation and environmental responsibility remains a thorn in the side of nanomedicine.

Green synthesis approaches offer a compelling solution. By using marine algae as both reducing and capping agents, researchers can sidestep toxic reagents and move toward scalable, environmentally friendly nanoparticle production. Among the various metal-based systems explored, Zirconium (Zr) and Molybdenum (Mo) nanoparticles stand out for their promising biological interactions. Both metals exhibit strong physicochemical stability and biocompatibility, and preliminary studies suggest they may play a dual role in mitigating inflammation and targeting tumor cells [3,7].

Gracilaria foliifera, a species of red marine algae, represents a particularly promising candidate for this purpose. It contains a suite of phytochemicals—including sulfated polysaccharides, flavonoids, and polyphenols—that are known to influence immune signaling pathways. These compounds are capable of modulating key inflammatory mediators like cytokines, interleukins, and cyclooxygenases, while also reducing oxidative stress through ROS scavenging [8,9]. Such properties may contribute not only to cancer inhibition but also to improved wound healing and immune resilience, factors that are critically important in oral pathology.

Moreover, the biosynthesis process itself seems to benefit from these phytochemicals. They facilitate the reduction of metal ions into nanoscale particles while simultaneously stabilizing them—possibly enhancing both shelf-life and biological activity. From a materials science perspective, this dual function is particularly valuable.

Zirconia nanoparticles are increasingly being used in dentistry due to their exceptional properties, such as high strength, biocompatibility, and aesthetic appearance [10]. Molybdenum-based nanoparticles, although less commonly featured in dental applications, bring their own advantages—chief among them, corrosion resistance and notable antimicrobial

effects [11]. Their underutilization may be due more to unfamiliarity than to any inherent limitations.

In the present work, we explored a one-step green synthesis of Zr/Mo-NPs mediated by *Gracilaria foliifera*. This was followed by thorough physicochemical characterization and in vitro evaluation of their biological activities. Early indications suggest a promising dual function—cytotoxicity against oral cancer cells and anti-inflammatory activity. Nonetheless, it would be premature to overstate these findings without further mechanistic insights. Future research must explore the molecular pathways involved, particularly the roles of apoptosis regulation and cytokine inhibition.

While still in its experimental stages, this approach appears to offer a sustainable, biologically potent avenue for oral cancer therapeutics and inflammatory control. It is a step toward reconciling ecological responsibility with cutting-edge biomedical innovation.

2. Materials and Methods

2.1. Materials.

Zirconyl nitrate and Molybdenum acetate salts were procured from SRL, India. Bovine serum albumin (BSA) and MTT reagent were obtained from Himedia, India. All other chemicals used in this study were of analytical grade and sourced from local suppliers.

2.2. Preparation of *Gracilaria foliifera* extract.

Gracilaria foliifera was washed with distilled water three times and shade-dried. The dried sample was ground into fine powder, and 10 grams of the powder was added to 200 mL of sterile double-distilled water. The mixture was filtered using Whatman No. 1 filter paper, and the obtained extract was kept at 4°C for later use.

2.3. Green synthesis of Zr/Mo nanoparticles

The one-pot synthesis process was used for the synthesis of *Gracilaria foliifera*-mediated Zr/Mo-NPs. Algal extract of *Gracilaria foliifera* was kept in a conical flask, whereas 25 mM Zirconyl Nitrate and 25 mM Molybdenum acetate solution were taken in two separate burettes. Through a titration process, both solutions were added dropwise to the algal extract. The mixture was incubated overnight on an orbital shaker, during which a yellowish-white precipitate was observed settling at the bottom of the flask. Following incubation, the mixture was centrifuged at 4500 rpm for 30 minutes to collect the precipitate. The resulting pellet was washed with distilled water and centrifuged again under the same conditions. The purified pellet (Zr/Mo-NPs) was then dried in a hot air oven at 60 °C for 24 hours to eliminate residual moisture. Finally, the dried nanoparticles were stored in an airtight container at room temperature for future use.

2.4. Characterization of nanoparticles.

The synthesized nanoparticles were characterized using various analytical techniques. UV-Visible spectroscopy (Thermo Scientific Evolution 600) was employed in the range of 200–800 nm to monitor optical properties. Functional group interactions were identified using FTIR spectroscopy (Bruker) in the 4000–400 cm⁻¹ range. XRD analysis was conducted to determine crystal structure and particle morphology. SEM and EDX (JSM-7001F, JEOL, Tokyo, Japan) were used to examine nanoparticle morphology and elemental composition, respectively.

2.5. Anti-Inflammatory assay.

The anti-inflammatory activity of the Zr/Mo-NPs was evaluated using the Albumin Denaturation Assay. A 1% BSA solution was added to each well of a microtiter plate, followed by various concentrations (20–100 $\mu\text{g/mL}$) of the nanoparticles. A negative control (1% BSA) and a positive standard (Diclofenac sodium) were employed to validate the anti-inflammatory activity. Plates were incubated at room temperature for 15 minutes, followed by heating at 55 °C for 20 minutes. Absorbance was measured at 660 nm to calculate the percentage inhibition.

2.6. Cytotoxicity assay in KB cells.

KB (Human oral epidermoid carcinoma cell line) cells (5000 cells/well) were seeded into 96-well plates and cultured until 70% confluence. Cells were treated with varying concentrations of Zr/Mo-NPs (10–100 μM) and incubated for 24 hours in a CO₂ incubator. Following treatment, 10 μL of MTT reagent (5 $\mu\text{g/mL}$) was added to each well, and plates were incubated in the dark for 3 hours. The medium was removed, and 100 μL of DMSO was added to solubilize formazan crystals. Absorbance was recorded at 490 nm using a microplate reader.

2.7. Statistical analysis.

All experiments were conducted in triplicate. Data are presented as mean \pm standard deviation (SD). The statistical significance was analyzed using Student's t-test, ‘*’ $p \leq 0.05$, ‘**’ $p \leq 0.01$, ‘****’ $p \leq 0.0001$.

3. Results and Discussion

3.1. Synthesis and characterization of nanoparticles.

3.1.1. Visual confirmation.

The successful green synthesis of Zr/Mo-NPs was visually confirmed by a color change from pale yellow to yellowish white precipitate, indicating the reduction of metal ions by bioactive compounds in *Gracilaria foliifera*.

3.1.2. UV-Vis spectroscopy.

The UV-Visible spectral analysis of the synthesized Zr/Mo-NPs exhibited distinct peaks at 243 nm and 367 nm, consistent with surface plasmon resonance typical of metallic nanoparticles, confirming their successful formation (Figure 1).

3.1.3. FT-IR spectroscopy.

FT-IR analysis identified key functional groups, such as N–H stretching (3359 cm^{-1}), which indicates an amine or amide group, C=C stretch (1633.93 cm^{-1}) indicates alkene or aromatic ring, sulfoxide (1036 cm^{-1}), and aliphatic iodide (525 cm^{-1}). These suggest the involvement of algal biomolecules in nanoparticle stabilization through electrostatic and hydrogen bonding interactions (Figure 2).

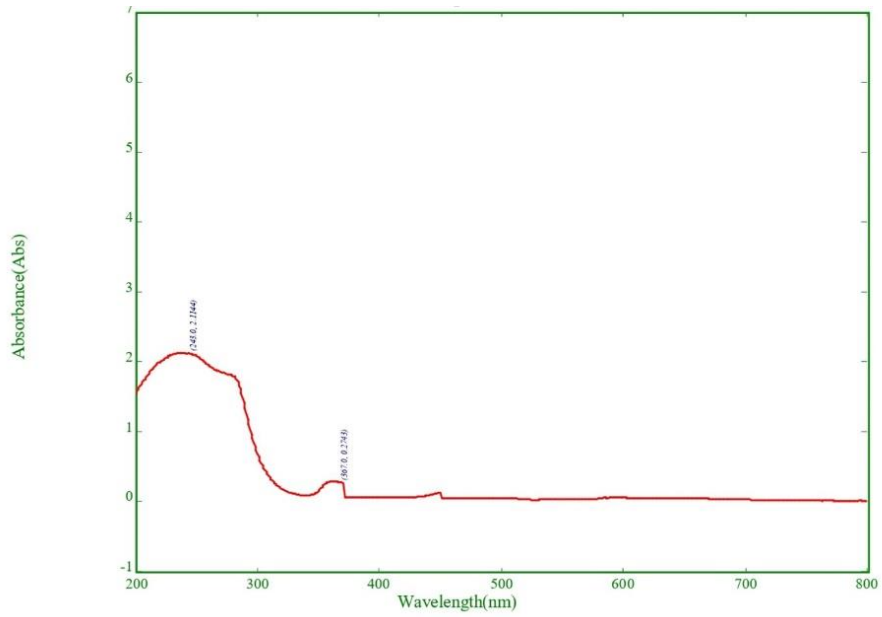
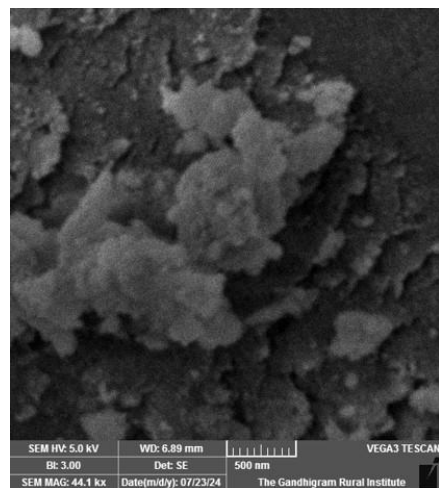
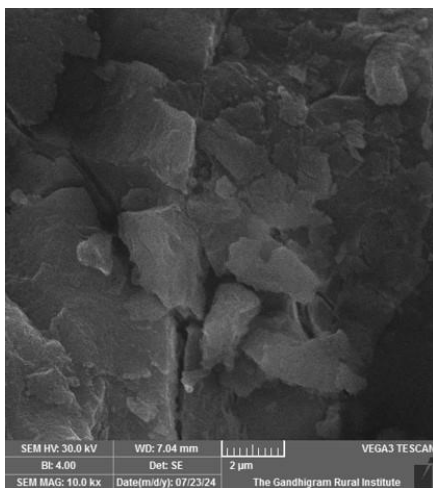
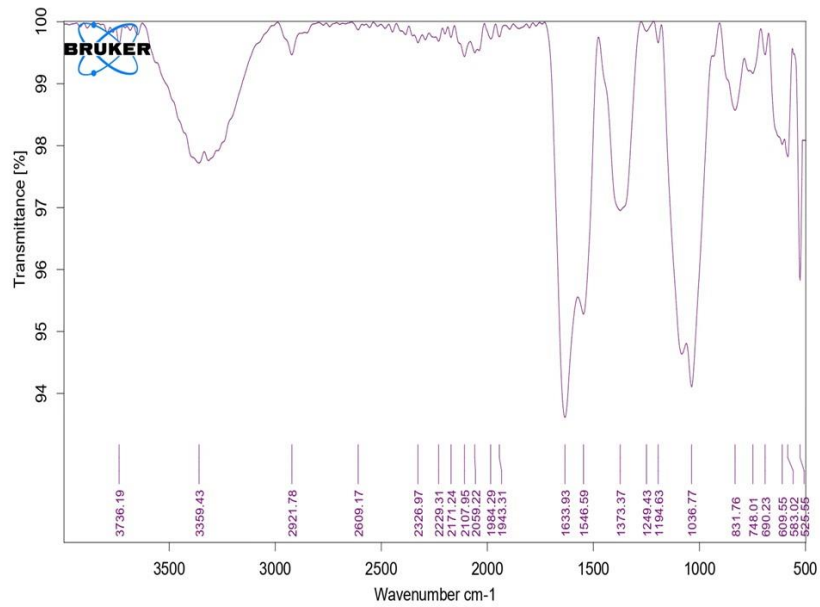


Figure 1. UV absorption spectrum of *Gracilaria*-mediated Zr/Mo nanoparticles.



(a) (b)
Figure 3. SEM micrographs of Zr/Mo-NPs: (a) 2 µm scale; (b) 500 nm scale.

Figure 2. FT-IR spectra showing different types of functional groups responsible for nanoparticle capping.

3.1.4. Morphology and elemental composition.

SEM imaging revealed that the nanoparticles exhibited a slightly irregular and agglomerated morphology with an average size ranging between 80 and 100 nm (Figure 3). EDX analysis confirmed the presence of Zirconium (43.77%), Molybdenum (7.36%), Oxygen (28.6%), and Carbon (20.21%) in the nanoparticle composition (Figure 4).

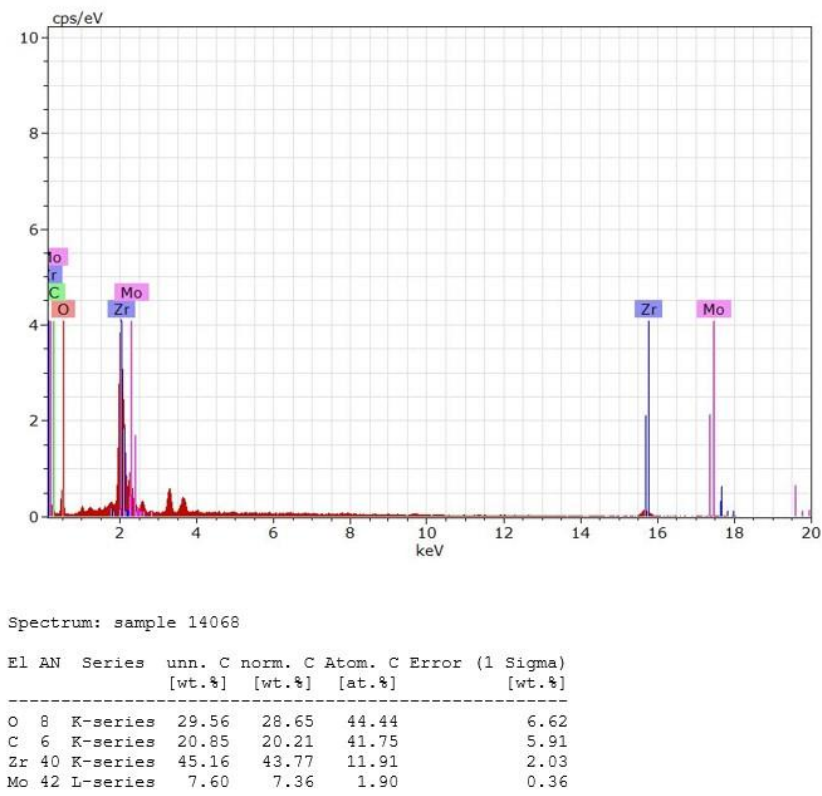


Figure 4. EDX spectrum indicating an elemental composition of synthesized Zr/Mo-NPs.

3.1.5. XRD analysis.

XRD results indicated that the nanoparticles exhibited a dual-phase structure comprising 50% crystalline and 50% amorphous components (Figure 5). The amorphous phase may be due to the stabilizing effect of organic compounds from *Gracilaria foliifera*.

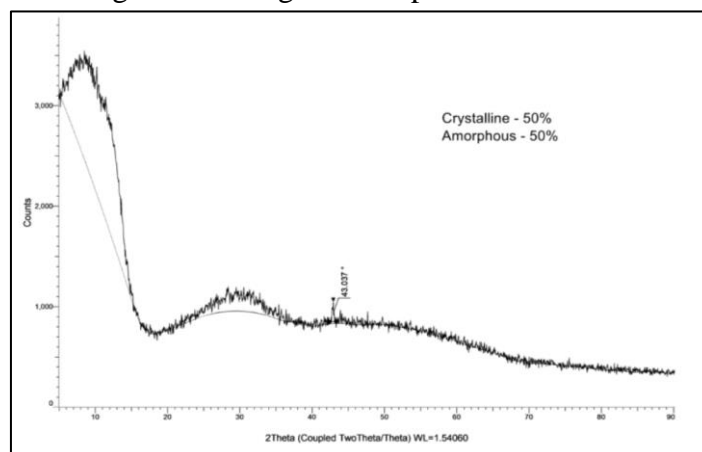


Figure 5. X-ray diffraction pattern of Zr/Mo-NPs showing mixed-phase characteristics.

3.2. Biological evaluation.

3.2.1. Cytotoxicity assay.

Cytotoxicity testing against the KB oral cancer cell line revealed a dose-dependent decrease in cell viability. At 10 $\mu\text{g/mL}$, 85% viability was retained, while only 15.2% remained at 100 $\mu\text{g/mL}$, suggesting strong anticancer potential (Figure 6).

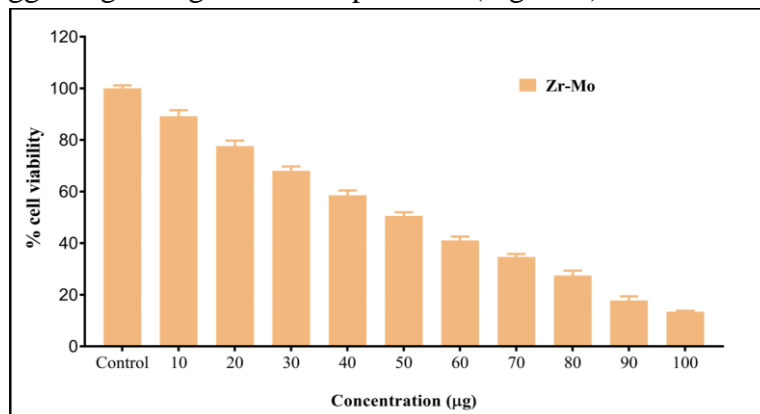


Figure 6. Cytotoxicity of Gracilaria-mediated Zr/Mo-NPs against KB cells. The statistical significance was analyzed using Student's t-test. $p \leq 0.0001$ between the control and treated group.

3.2.2. Anti-inflammatory activity.

The anti-inflammatory effect assessed via the albumin denaturation method demonstrated a dose-dependent increase in activity. Zr/Mo-NPs inhibited protein denaturation by 28% at 20 $\mu\text{g/mL}$ and 61% at 100 $\mu\text{g/mL}$, indicating significant anti-inflammatory potential (Figure 7).

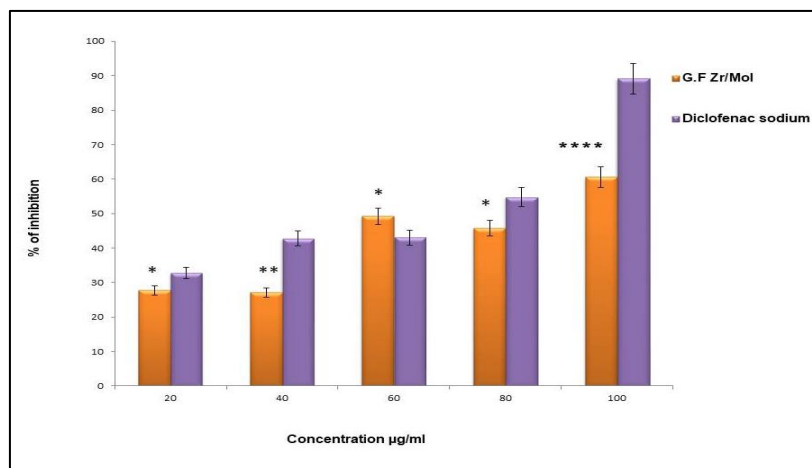


Figure 7. Anti-inflammatory activity of Zr/Mo-NPs at varying concentrations. The statistical significance was analyzed using Student's t-test, $*$ $p \leq 0.05$, $**$ $p \leq 0.01$, $****$ $p \leq 0.0001$.

3.3. Discussion.

The successful biosynthesis of Zr/Mo-NPs using *Gracilaria foliifera* was initially evident through a rapid and visible color shift—an indicative phenomenon often linked to surface plasmon resonance (SPR). This optical response arises from the oscillation of free electrons on the nanoparticle surface when excited by light, and it has long been considered a primary visual cue confirming nanoparticle formation. UV-Visible spectrophotometry further substantiated this, revealing strong absorption peaks at 243 nm and 367 nm. Notably, these spectral features correspond well with established data for zirconium dioxide and other metal

oxide nanoparticles synthesized via green routes, where peaks in the UV range are associated with electronic transitions influenced by phytochemicals acting as reducing agents [12].

Further investigation using FT-IR revealed a spectrum rich with peaks corresponding to various functional groups, supporting the presence of active biomolecules involved in nanoparticle stabilization. Signals indicative of sulfoxides, aliphatic amines, and alkenyl groups suggest strong coordination between metal ions and organic ligands. These interactions are likely responsible for not only stabilizing the nanoparticles but also enhancing their solubility and biocompatibility. Phenolic compounds, flavonoids, and polysaccharides from *G. foliifera* have been previously noted for their stabilizing and therapeutic effects, implying a dual role in both nanoparticle formation and bioactivity [13].

The XRD pattern of the synthesized Zr/Mo-NPs showed a combination of sharp and broad peaks, pointing to a composite structure consisting of both crystalline and amorphous domains. The crystalline nature suggests structural integrity, while the amorphous content, likely due to the organic coating, may promote interactions with cellular components. This balance is becoming increasingly appreciated in drug delivery systems, where rigidity ensures durability and amorphous zones facilitate biological interaction and reactivity [14].

Morphological analysis through SEM demonstrated that the nanoparticles were largely spherical, with diameters predominantly in the 80–100 nm range. Such size and shape are considered optimal for passive uptake via endocytosis, a crucial feature in therapeutic nanomedicine. While mild agglomeration was noted, this is not uncommon in biosynthesized nanoparticles, particularly when unmodified. This issue may be addressed in future work through surface functionalization or the incorporation of dispersants. EDX confirmed the elemental composition, with detectable levels of zirconium and molybdenum. Carbon and oxygen signals were also prominent, attributable to the phytochemical capping from *G. foliifera*, thus validating the algae-mediated synthesis approach.

In vitro cytotoxicity assessments using the KB human oral epidermoid carcinoma cell line revealed a clear dose-dependent inhibition of cell viability. The half-maximal inhibitory concentration (IC₅₀) was within a comparable range to other green-synthesized metal oxide nanoparticles investigated in similar cancer cell lines. Although this study did not directly probe molecular pathways, the literature suggests that nanoparticles of this type often induce apoptosis through reactive oxygen species (ROS) generation, disruption of mitochondrial function, and DNA fragmentation [15–17]. These findings imply a multi-faceted mechanism that could be further elucidated in future work.

Interestingly, the nanoparticles also showed a marked anti-inflammatory effect, as evidenced by their ability to inhibit heat-induced albumin denaturation. Since protein denaturation is a hallmark of inflammation, this result suggests the nanoparticles may modulate key inflammatory mediators. While this study did not directly assess molecular targets, the dose-responsive trend observed hints at interference with well-known inflammatory pathways, potentially involving cyclooxygenase inhibition or suppression of pro-inflammatory cytokines such as interleukins and prostaglandins [18,19]. This dual functionality—both cytotoxic and anti-inflammatory—makes these nanoparticles particularly attractive for oral cancer, where chronic inflammation often accelerates disease progression and worsens prognosis.

From a sustainability perspective, the use of *Gracilaria foliifera* presents clear advantages. This marine alga is abundant, renewable, and rich in biologically active metabolites, allowing nanoparticle synthesis without resorting to hazardous chemicals or high-energy processes. Such biosynthetic approaches align well with ongoing efforts to promote

clean, green nanotechnology practices that are both scalable and environmentally responsible [20-22].

In summary, this study demonstrates that *G. foliifera*-mediated synthesis of Zr/Mo-NPs is not only feasible but also yields nanoparticles with significant biological relevance. The observed anticancer and anti-inflammatory activities, coupled with the eco-friendly synthesis process, underscore their potential as multifunctional agents in oral healthcare. Still, further research is warranted to validate these effects in vivo and to uncover the molecular mechanisms at play. Optimizations such as surface modification, targeted delivery, and in vivo pharmacokinetics may help translate this promising platform into practical clinical applications.

4. Conclusion

The present study demonstrates the successful green synthesis of zirconium–molybdenum nanoparticles (Zr/Mo-NPs) using *Gracilaria foliifera*, yielding biocompatible nanomaterials with dual anticancer and anti-inflammatory properties. Characterization confirmed their stable nanoscale structure and phytochemical coating, while biological assays revealed dose-dependent cytotoxicity against oral cancer cells and inhibition of protein denaturation, suggesting therapeutic potential. The use of marine algae as a natural reducing and stabilizing agent supports an eco-friendly, sustainable synthesis approach aligned with green chemistry principles. However, it is important to note that the anti-inflammatory activity was assessed through a preliminary non-cellular albumin denaturation assay, which does not account for complex cellular mechanisms, bioavailability, or toxicity. Therefore, further validation using cell-based assays and comprehensive in vivo studies is essential to elucidate the underlying molecular mechanisms and confirm the clinical applicability of these nanoparticles. Overall, biosynthesized Zr/Mo-NPs represent a promising multifunctional platform for biomedical applications, particularly in oral cancer therapy and inflammation management, pending further mechanistic and translational research.

Author Contributions

Conceptualization, D.D. and S.S.; methodology, D.D. and S.R.; software, S.R. and S.S.; validation, S.R., S.S., and G.J.; formal analysis, S.S. and G.J.; investigation, D.D.; resources, D.D.; data curation, S.R. and S.S.; writing—original draft preparation, S.R. and S.S.; writing—review and editing, D.D.; visualization, S.S. and G.J.; supervision, S.S. and S.R.; project administration, D.D. and S.S.; funding acquisition, D.D. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not Applicable.

Informed Consent Statement

Not Applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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