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RESEARCH ARTICLE

Antiproliferative Potential and Immunological Safety of *Filopaludina bengalensis* Extracts: Morphological and Biochemical Insights

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Abstract

Background: Mollusc-derived compounds are gaining growing attention as potential pharmaceutical agents and functional food supplements. This study investigated the nutraceutical potential of the freshwater mollusc *Filopaludina bengalensis*, which is widely distributed in West Bengal, India. The research encompassed species authentication and morphological characterization, along with an evaluation of its anti-inflammatory and anticancer activities, to determine its suitability as a natural source of nutraceutical compounds. **Materials and Methods**: *Filopaludina bengalensis* specimens were collected from freshwater habitats and identified using mitochondrial cytochrome c oxidase subunit 1 (COI) gene barcoding for molecular authentication. Morphometric measurements, including shell length and weight, were recorded. The flesh and extrapallial fluid were processed to obtain molluscan mass (FBM) and fluid (FBF) extracts. Proximate composition (moisture, protein, carbohydrate, lipid, and minerals) was analyzed. The cytotoxic activity of both extracts was evaluated in breast cancer cell lines (MCF-7 and MDA-MB-231) using the MTT assay. Anti-inflammatory activity was assessed by measuring nitric oxide inhibition in LPS-stimulated RAW264.7 macrophages, while oxidative stress and immune toxicity were evaluated through reactive oxygen species and nitrite production assays. **Results:** Molecular analysis confirmed the identity of *F. bengalensis*, validating the reliability of COI barcoding for species-level identification and phylogenetic analysis of freshwater molluscs. The mollusc exhibited an average length of 3.02 cm and weight of 5.57 g, yielding 0.5–1.0 ml of extrapallial fluid. Proximate composition analysis revealed high moisture (68.9%) and protein (50.4%) contents, moderate carbohydrates (33.8%), low lipids (4.3%), and notable mineral levels. Both FBM and FBF extracts showed dose-dependent cytotoxicity against MCF-7 and MDA-MB-231 cell lines, with FBF displaying superior potency (IC_{50} = 19 µg/ml and 49 µg/ml, respectively) compared to FBM (IC_{50} = 70.3 µg/ml and 142 µg/ml). In LPS-stimulated macrophages, both extracts significantly inhibited nitrite production, with FBF (IC_{50} = 49.56 µg/ml) showing stronger anti-inflammatory activity than FBM (IC_{50} = 77.52 µg/ml). Neither extracts induced nitric oxide or reactive oxygen species production in unstimulated macrophages, indicating low immune toxicity. **Conclusion:** The findings demonstrate that *Filopaludina bengalensis* possesses high nutritional value and exhibits selective anticancer and anti-inflammatory activities, particularly within its extrapallial fluid extract. The favourable safety profile and bioactivity of these extracts suggest their potential as natural nutraceutical candidates. Further studies are needed to isolate and characterize the active compounds and elucidate their mechanisms of action for therapeutic development.

Keywords: *Filopaludina bengalensis*, Nutraceuticals, Anticancer activity, Anti-inflammatory activity

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Introduction

Nutraceuticals, derived from plant or animal sources, including functional foods, fortified foods, and dietary supplements, offer health benefits beyond basic nutrition, including lipid-lowering, antioxidant, anticancer, anti-inflammatory, and immunomodulatory effects. Ongoing research focuses on their mechanisms, safety, and clinical efficacy, positioning nutraceuticals as valuable tools for future preventive and therapeutic strategies [1, 2]. While not replacements for pharmaceuticals, they provide cost-effective alternatives that closely mimic physiological biomolecules. Mollusc-derived compounds are increasingly recognized as promising pharmaceuticals and functional food supplements with ecologically vital marine and freshwater species such as oysters, clams, mussels, molluscs and squids providing food, pearls, shells, and bioactive compounds with significant therapeutic potential [3, 4]. Freshwater molluscs, particularly gastropods, are abundant across South and Southeast Asia, inhabiting rivers, lakes, ponds, and paddy fields. Characterized by robust, globular–conical shells and thick opercula, they are widely used in traditional remedies, including treatments for respiratory, cardiac, nervous, and hepatic disorders [5, 6]. Nutritionally, these species provide high-quality protein, essential amino acids, omega-3 fatty acids (EPA, DHA), bioavailable minerals (Ca, Fe, Zn, Se, Mg), and vitamins A, B12, and D. In India, states such as West Bengal, Jharkhand, Bihar, Maharashtra, and regions of the northeast utilize gastropods to support immunity, purify blood, prevent conjunctivitis, and as nutritional supplements [7]. Despite the recognized promise of mollusc-derived bioactive potentials, many widely consumed freshwater gastropods remain insufficiently characterized, underscoring the need to investigate species that combine high nutritional value with the potential to serve as sustainable sources of new therapeutic agents.

The abundance of molluscs in West Bengal's freshwater and marine ecosystems positions them as promising sources for drug discovery and large-scale production. Snails such as *Achatina, Bellamya, Lamellidens, and Pila* are increasingly valued as nutraceuticals, though their medico-food potential remains underexplored. *Lissachatina fulica* slime extracts exhibit anti-inflammatory activity, while *Bellamya bengalensis* demonstrates CNS-depressant, anti-inflammatory, and analgesic effects [8, 9]. *Filopaludina bengalensis* (currently the approved name of *Bellamya bengalensis*) provides protein-rich meat and calcium-rich shells, with ethnomedicinal and biomedical evidence highlighting its immunomodulatory and anti-inflammatory potentials. Lipid extracts inhibit NF-κB and ROS pathways, reducing edema, myeloperoxidase activity, nitric oxide as well as cytokine levels, while flesh extracts protect against oxidative stress, mitochondrial damage, and DNA fragmentation [10, 11]. Protein hydrolysates exhibit antioxidative and antihypertensive activities, whereas tissue extracts demonstrate hepatoprotective and anti-arthritic properties [12-14]. Furthermore, secretion extracts of *B. bengalensis* f. annandalei show potent antineoplastic activity, inducing cytotoxicity, apoptosis, and antiproliferative effects in human leukemia and hepatocellular carcinoma cell lines [15, 16].

Molluscs, due to their integrated nutritional and pharmacological potentials, are increasingly recognized as sustainable functional foods and reservoirs of novel therapeutic agents. Their species diversity and complex chemical nature confer notable anti-inflammatory and immunomodulatory activities, positioning them to address global nutritional needs while offering innovative therapeutic avenues. The association of chronic inflammation, immune dysregulation, and cancer motivated the researchers to explore the freshwater mollusc *Filopaludina bengalensis*, characterizing its physicochemical properties and evaluating its antiproliferative potential and immunological safety for prospective therapeutic applications.

Materials and Methods

Collection & Identification

Freshwater molluscs were collected from ponds in Paschim Midnapore, West Bengal, during the pre-monsoon period (February–May) and authenticated by the Zoological Survey of India, Kolkata, as *Filopaludina bengalensis* Lamarck (Viviparidae). Both the footpad (body mass) and extrapallial fluid were collected and extracted from the molluscs for subsequent experimental analyses.

Extraction

Molluscs were thoroughly washed with distilled water. Extrapallial fluid was carefully aspirated from live specimens using a sterile syringe beneath the shell margin and mantle, avoiding disruption of the mantle or shell. The fluid was centrifuged at 3500 rpm for 15 min at 4 °C to remove debris, and the clear supernatant was lyophilized (designated FBF). The remaining body mass was eviscerated, footpads collected, homogenized in phosphate buffer (1:10), and centrifuged under cold conditions to remove debris. The resulting supernatant was lyophilized (denoted FBM). Lyophilized powders of both fluid and mass extracts were stored at –20 °C until further use.

DNA Barcoding

Molluscan samples were authenticated using DNA barcoding, a precise method for species identification based on sequence variation in a standardized DNA region [17]. Genomic DNA was extracted from both tissue and fluid, and a 642 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene was amplified via PCR using primers 5′–GGTCAACAAATCATAAAGATATTGG–3′ (forward) and 5′–TAAACTTCAGGGTGACCAAAAAATCA–3′ (reverse). PCR products were subjected to bidirectional Sanger sequencing, and chromatograms were quality-checked to remove low-quality ends and ambiguous bases. Final sequences were formatted in

FASTA and compared against NCBI reference databases, with ≥98–99% similarity confirming species-level identification. The species origin of the fluid sample was confirmed, and its nucleotide sequence was uploaded in GenBank (Accession No. PX386700).

COI sequences of *Filopaludina bengalensis* and related taxa were aligned using MUSCLE in MEGA X, and phylogenetic reconstruction was performed by the Neighbor-Joining method with 1,000 bootstrap replicates. Evolutionary distances were estimated using the p-distance model, with positions containing gaps and missing data eliminated by complete deletion [18].

Morphometric characteristics and proximate composition

Collected molluscs were thoroughly cleaned, measured, and weighed. Whole-body proximate composition, including moisture, ash, protein, lipid and carbohydrate, was determined using standard protocols [19, 20]. Moisture content (1 g/sample) was measured by drying at 135°C for 2 hrs, and ash content (1 g/sample) by incineration at 550 °C for 4 hrs, expressed as a percentage of dry weight. Crude protein (0.1 g/sample) was quantified using the Kjeldahl method (N × 6.25) following acid digestion, distillation, and titration. Lipids were extracted using petroleum ether and chloroform/methanol (2:1) according to the Folch method, and measured gravimetrically. Carbohydrate content was calculated by difference:

1. Moisture (%) = [(wet weight – dry weight)/wet weight] × 100
2. Ash (%) = (weight of ash/weight of sample) × 100
3. Crude protein (%) = % N × 6.25
4. Crude lipid (%) = (weight of lipid/weight of sample) × 100
5. Carbohydrates (%) = 100 – (moisture + protein + lipid + ash)

Cell viability study in human breast carcinoma cell lines

The cytotoxicity of FBF and FBM extracts was evaluated using the MTT assay on human breast cancer cell lines MCF-7 (luminal) and MDA-MB-231 (triple-negative) [21]. Cells were seeded at 1×10^4 cells/well in 96-well plates containing complete DMEM with heat-inactivated FBS and penicillin–streptomycin, and allowed to adhere for 24 h. Cells were then treated with extract concentrations ranging from 3.125–200 µg/ml for 24 and 48 hrs. Subsequently, 50 µl of MTT solution (5 mg/ml) was added and incubated for 4 h. Formazan crystals were solubilized with 150 µl DMSO, and absorbance was measured at 590 nm (620 nm reference) using a microplate reader. Untreated cells served as controls. Assays were performed in triplicate, and cell viability was calculated as:

Cell viability = (A samples – A blank) / (A control –A blank) × 100%

A sample is an average absorbance in wells containing cells treated with a defined concentration of the sample; A blank is the absorbance of the blank (DMSO), and A control is the absorbance of the untreated cells used as a negative control. Cell viability was calculated relative to the untreated control and IC_{50} value was calculated using non-linear regression curve (log dose vs response).

Cell viability studies in RAW 264.7 murine macrophage cells

RAW264.7 murine macrophage-like cells, widely used in inflammation, immunology, and cancer research [22], were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified 5% CO_2 incubator. For cytotoxicity assessment, cell suspension (1×10^4 cells/well in 200 µl of complete medium) was seeded in 96-well plates and allowed to adhere for 24 hrs. Thereafter, cells were treated with lyophilized FBF and FBM extracts dissolved in PBS at various concentrations (12.5–400 µg/ml) and further incubated for 48 hrs under the same culture conditions. Subsequently, 20 µl of MTT solution (5 mg/ml in sterile PBS) was added and incubated for 3 h. The medium was then carefully removed, and the formazan crystals, formed by viable cells were solubilized in 100 µl DMSO per well, and absorbance was recorded at 595 nm using a microplate reader.

Nitrite Accumulation

Nitrite production in RAW264.7 culture supernatants was measured using the Griess reagent [23]. Cells (1×10^6 cells/ml) seeded overnight were treated with *Filopaludina bengalensis* extracts (12.5–400 µg/ml) and incubated for 48 hrs at 37 °C. Post-incubation, 100 µl of cell-free supernatant was mixed with 100 µl of Griess reagent and incubated in the dark for 15 min. Absorbance was recorded at 550 nm, with sodium nitrite (1–200 µM) as the standard. Nitrite levels were expressed in micromoles.

ROS Measurement

Reactive oxygen species (ROS) production in RAW264.7 cells was evaluated using the fluorescent probe H2DCFDA [23]. Cells (2×10^5/well) were seeded in 96-well black-wall plates overnight and treated with FBF and FBM at 25, 50 and 100 µg/ml for 24 hrs. Cells were washed with PBS, incubated with 1 mg/ml H2DCFDA for 30 min at 37 °C, and intracellular ROS-induced fluorescence was measured using a microplate reader (excitation 480 nm, emission 530 nm).

Nitric oxide assay with Lipopolisaccharide (LPS) induction in RAW264.7 cell line

Nitric oxide (NO) production in RAW264.7 cells is increased when cells are exposed to lipopolysaccharide (LPS), a commonly used marker of inflammation [24]. RAW264.7 cells (1×10^5/well) were seeded in 96-well plates and allowed to adhere for 24 hrs. Cells were then sensitized with 1 µg/ml LPS and treated with FBF and FBM extracts at 12.5–200 µg/ml for 24 hrs at 37 °C in a humidified 5% CO_2 atmosphere. Nitrite accumulation in the culture supernatant, reflecting nitric oxide production, was quantified using Griess reagent, with absorbance measured at 570 nm.

Percentage inhibition= (A-B) / (A-C) ×100

[A= absorbance of LPS+cell, B= absorbance of LPS+cell+sample, C= absorbance of only cell]. IC_{50} value was calculated using non-linear regression curve (log dose vs response).

Statistical analysis

GraphPad Prism 8.0.1 (GraphPad Software, La Jolla, CA, USA) was used for statistical analyses along with SPSS software. Data were presented as mean ± standard error of mean (SEM). Statistical significance was determined using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons.

Results

DNA Barcoding

DNA barcoding of the molluscan extrapallial fluid and mass sample, targeting the mitochondrial marker gene cytochrome c oxidase subunit 1 (COI), revealed the highest sequence similarity to *Filopaludina bengalensis* (family Viviparidae). BLAST analysis demonstrated 100% sequence identity with *F. bengalensis* (Accession No. PV174435), with all 642 base pairs of the sample sequence perfectly matching the reference. The species origin of the fluid sample was thus explicitly confirmed, and the corresponding nucleotide sequence has been deposited in the NCBI GenBank (Accession No. PX386700) for public access [25].

The Neighbour-Joining phylogeny based on COI sequences provides strong evolutionary support for the identification of the sample as *Filopaludina bengalensis*. In the tree (Figure 1), the present sequence clusters tightly with multiple authenticated *F. bengalensis* accessions, including isolates from different geographical regions, forming a high-bootstrap monophyletic clade with negligible intraspecific divergence. This clustering pattern indicates strong genetic coherence within the species. In contrast, the *F. bengalensis* clade is clearly and consistently separated from other viviparid genera represented in the analysis. Genera such as Mekongia, Notopala, Trochotaia, Angulyagra, and Larina appear as distinct, well-supported evolutionary lineages, each forming its own independent clade without any overlapping or intermediate branching with *F. bengalensis*. The long branch lengths and high bootstrap values supporting these genera highlight the deep evolutionary divergence between them and Filopaludina. This clear phylogenetic separation confirms that the sample belongs to *F. bengalensis*, emphasizing the discriminatory power of the COI marker in resolving species boundaries within Viviparidae and ruling out the possibility of cross-genus misidentification.

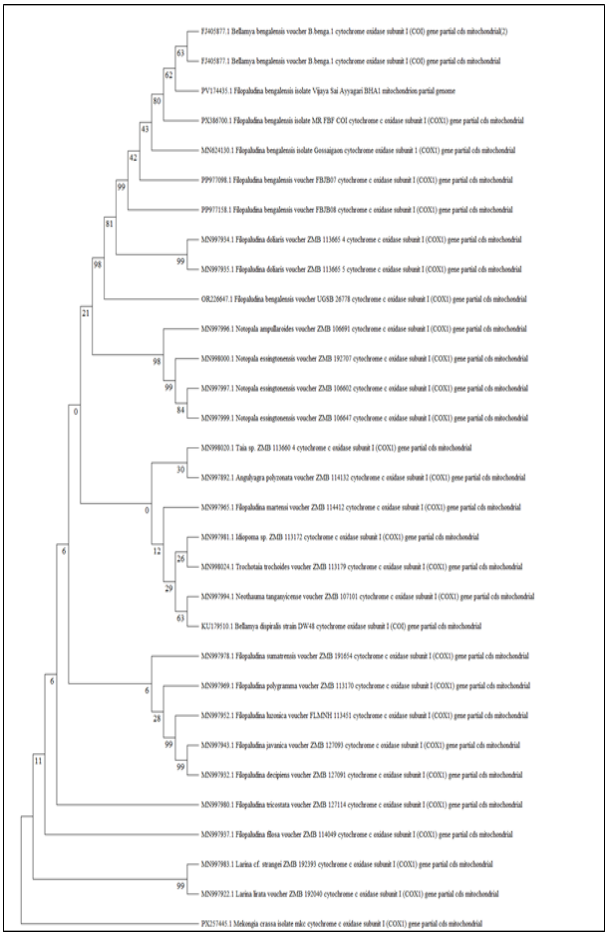

Figure 1. Neighbor-Joining Phylogenetic Tree Based on Mitochondrial COI Sequences of *Filopaludina bengalensis* and Related Viviparid Taxa. Evolutionary distances were calculated using the p-distance method and are expressed as the number of base differences per site. The scale bar represents the number of nucleotide substitutions per site.

Morphometric characteristics

The average length of the gastropod *Filopaludina bengalensis* Lamarck (n=50), including the outer shell, was estimated as 3.02 ± 0.15 cm ranging from 2 to 4 cm (Figure 2), with an average weight (with shell) of 5.57 ± 0.31 g (ranging from 4 to 7 g). The volume of extrapallial fluid aspirated from each mollusca varied with size, ranging from 0.5 to 1.0 ml.

Anatomical contributions to total molluscan mass were assessed from five randomly selected specimens (n = 5). The foot, representing the edible portion, averaged 1.03 ± 0.08 g, the remaining body 0.79 ± 0.18 g, and the shell 2.57 ± 0.12 g, with the remainder attributed to extrapallial fluid, located in the extrapallial space between the shell and the body part of the mollusca. Overall, the body accounted for the largest proportion of total mass, followed by the shell and foot.

Table 1. Proximate Composition of the Mass of Freshwater Mollusca *Filopaludina bengalensis*

Moisture content (%)	Ash (%)	Total protein content (%)	Total lipid content (%)	Total carbohydrate content (%)
68.87 ± 1.78	11.45 ± 0.54	50.44 ± 1.26	4.33 ± 0.41	33.78 ± 1.12

Moisture is reported as % wet weight (WW), however the other proximate composition of *F. bengalensis* mass expressed on dry weight (DW) basis. Data represent mean ± SEM values based on three independent measurements (n = 3) conducted on the molluscan species.

Figure 2. Morphological Features of *Filopaludina bengalensis* Lamarck

Proximate analysis of *F. bengalensis* flesh as demonstrated in Table 1 emphasizing the high moisture (68.87%), substantial protein (50.44%), moderate carbohydrates (33.78%), and low lipids (4.33%), reflecting a nutrient-dense profile in the molluscan flesh (mass) with minimal fat content. The ash value indicating the remarkable amount of mineral content.

Cell viability in MCF-7 (breast cancer) and MDA-MB-231 (triple negative breast cancer) cell line

The effect of molluscan mass (FBM) and fluid (FBF) extracted from *F. bengalensis* was evaluated on human breast cancer cell lines, as illustrated in Figure 3.

Both *F. bengalensis* extracts exhibited dose and time-dependent cytotoxicity against breast cancer cell lines, with the FBF consistently more potent than FBM. In MCF-7 cells, viability at 200 µg/ml decreased from 35.8% (FBM) and 24.1% (FBF) at 24 hrs ($p<0.0001$) to 31.3% and 15.5% at 48 hrs ($p<0.0001$), corresponding to IC_{50} values of 70.3 ± 2.7 µg/ml (FBM, 24 hrs) compared to 19 ± 1.5 µg/ml (FBF) and 37.3 ± 1.5 µg/ml (FBM, 48 hrs) contrasted with 14 ± 1.8 µg/ml (FBF). In MDA-MB-231 cells, viability at 200 µg/ml was 41.7% (FBM) and 28.4% (FBF) at 24 hrs ($p<0.0001$), declining to 22.1% and 15.5% at 48 h ($p<0.0001$), with IC_{50} values of 142 ± 3.5 µg/ml (FBM, 24 hrs) compared to 49 ± 1.6 µg/ml (FBF) and 11.5 ± 2.8 µg/ml (FBM, 48 hrs) contrasted with 9.2 ± 0.5 µg/ml (FBF). These results highlight the superior cytotoxic efficacy of the fluid extract as a potential anticancer agent.

Cell viability studies in RAW264.7 cells

Cell viability assays in RAW264.7 cells are crucial for assessing the safety and non-toxicity of a compound on immune cells. The cytotoxicity of the *F. bengalensis* fluid extract was evaluated using a cell viability assay, with untreated cells serving as the control.

The cytotoxic effects of both FBM and FBF on RAW 264.7 cells were evaluated using a viability assay (Figure 4), with untreated cells as the control group. At 12.5 µg/ml, treatment with the extracts caused a slight reduction in cell viability, whereas a mild increase was observed at 25 and 50 µg/ml, however, the changes were not statistically significant. Beyond 50 µg/ml, a concentration-dependent decline in viability was evident, with maximum cytotoxicity recorded at 400 µg/ml for both extracts ($p<0.01$). Overall, the minimal alterations in macrophage viability at lower concentrations highlight a favorable safety profile of the extracts, while higher doses (≥100 µg/ml) exhibited a clear dose-dependent cytotoxic response. Notably, the molluscan mass demonstrated comparatively lower toxicity than the fluid extract, aligning with the edible nature of the molluscan footpad.

Nitrite accumulation and reactive oxygen species (ROS) generation

Nitrite accumulation, serving as an indirect indicator of nitric oxide (NO) production, was measured in unstimulated RAW264.7 cells following treatment with different concentrations of the extract (Figure 5A).

The untreated control cells exhibited basal nitrite levels, while treatment with both FBM and FBF at varying concentrations resulted in minimal or no changes. Negligible NO production in unstimulated macrophages indicates that the extracts do not provoke macrophage activation or NO-mediated inflammatory signalling. However, at 400 µg/ml, the fluid extract induced a significant increase in nitrite production ($p < 0.0001$), consistent with its cytotoxic potential at this concentration as observed previously. Overall, these findings indicate that the extracts are safe up to 200 µg/ml, as they do not elicit adverse immunological effects. Similarly, the reactive oxygen species (ROS) assay (Figure 5B) revealed no significant changes in macrophage cells following treatment with either FBM or FBF, further confirming their non-toxic nature and lack of oxidative stress induction on the immune system.

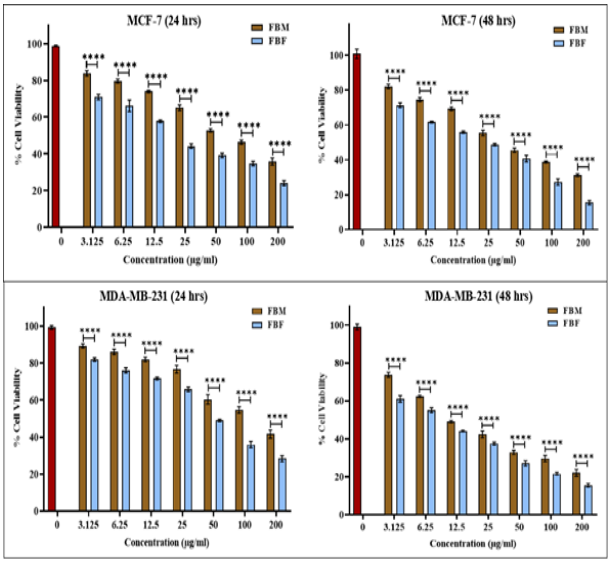

Figure 3. Cell Viability Study in Human Breast Cancer Cell Lines. A & B represent the effect in MCF-7 for 24 hrs and 48 hrs respectively, C & D denote the effect in MDA-MB-231 cell line post 24 and 48 hrs treatment with test samples respectively. Data represented as mean ± SEM (n=4), statistical analysis was done using one-way ANOVA followed by Tukey's post hoc analysis. * indicates significant difference in the cell viability in all the FBM and FBF treated groups compared to the untreated control (**** denotes $p<0.0001$)

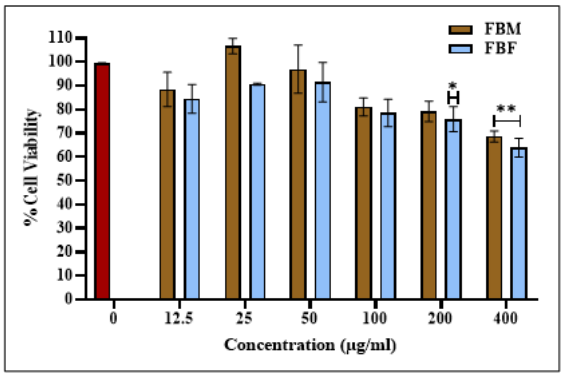

Figure 4. Cell Viability Study in RAW264.7 Cell Line after 24 hrs Treatment with Molluscan Extract in Different Concentrations. Data represented as mean ± SEM (n=4), statistical analysis was done using one-way ANOVA followed by Tukey's post hoc analysis. * indicates significant difference in the cell viability in respective FBM and FBF treated groups compared to the untreated control (* denotes $p<0.05$, ** denotes $p<0.01$)

Nitric oxide (NO) assay with Lipopolisaccharide (LPS) induced RAW264.7 cell line

In this study, the effects of molluscan mass and fluid extracts on the inhibition of NO production were assessed in LPS-stimulated RAW264.7 cells.

RAW264.7 macrophages were stimulated with LPS to establish an inflammatory model, characterized by enhanced nitrite accumulation as an indicator of inducible nitric oxide synthase (iNOS) activity. Treatment with graded concentrations (25–400 µg/ml) of FBM and FBF resulted in a marked, concentration-dependent reduction of nitrite levels ($p<0.0001$) in LPS-challenged cells (Figure 6). The inhibitory potency was reflected by IC_{50} values of 77.52 ± 0.53 µg/ml for FBM and 49.56 ± 1.16 µg/ml for FBF, demonstrating higher efficacy of FBF as significant anti-inflammatory potential.

Discussion

Marine and freshwater molluscs, particularly Cephalopoda, Bivalvia, and Gastropoda, are valued for their biodiversity, nutritional content, and bioactive properties, making them important as food and nutraceutical resource [3]. Snail meat, commonly consumed in Asia and parts of Europe, is rich in protein (up to 21% dry matter), essential amino acids, unsaturated fatty acids (>45% of total fat), and minerals such as calcium and magnesium. Both snail meat and mucus offer bioactive compounds, supporting their potential as health-promoting functional foods [26]. Marine organisms have gained attention for their immunomodulatory, anti-inflammatory, anticancer, antibacterial, and antiviral activities [27]. Notable examples include drugs derived from molluscs and symbiotic marine cyanobacteria, such as Adcetris®, Polivy™, and Blenrep™, and nutraceuticals like Lyprinol® and Biolane™ from the New Zealand green-lipped mussel (*Perna canaliculus*) [28]. Mucus from *Helix aspersa* contains glycosaminoglycans, mucopolysaccharides, antioxidants, and anti-inflammatory compounds, supporting its biomedical use in reducing colon inflammation [29, 30]. Various snail-derived extracts and hemocyanins have demonstrated anticancer activity against multiple human cancer cell lines, including breast, bladder, ovarian, prostate, leukemia, glioma, and colorectal cancers [31], attributed to bioactive proteins, peptides, minerals (Cu, Ca, Zn, Se), and fatty acids such as eicosapentaenoic acid (EPA), α-linolenic, linoleic, and γ-linolenic acids [32]. These findings highlight the potential of mollusc bioactives in reducing cancer cell viability, yet the biomedical applications of freshwater molluscs in inflammation, immunomodulation, and cancer remain underexplored.

Freshwater snails, including *Filopaludina bengalensis*, are nutrient-dense and traditionally consumed as functional food sources [27]. The proximate analysis revealed *F. bengalensis* flesh with high moisture (68.87%), protein (50.44%), moderate carbohydrates (33.78%), low lipids (4.33%), and notable mineral content. These findings are consistent with reports on Northeast Indian freshwater molluscs (*Cipangopaludina sp., Brotia costula, F. bengalensis*), which contain 22.8–26.8% crude protein, 2.7–3.8% fat, and polyunsaturated fatty acids up to 1.95% [33]. The present study emphasizes the significance of mitochondrial COI barcoding as a reliable tool for species authentication in freshwater molluscs. The unambiguous identification of *Filopaludina bengalensis* with 100% sequence identity ensures the

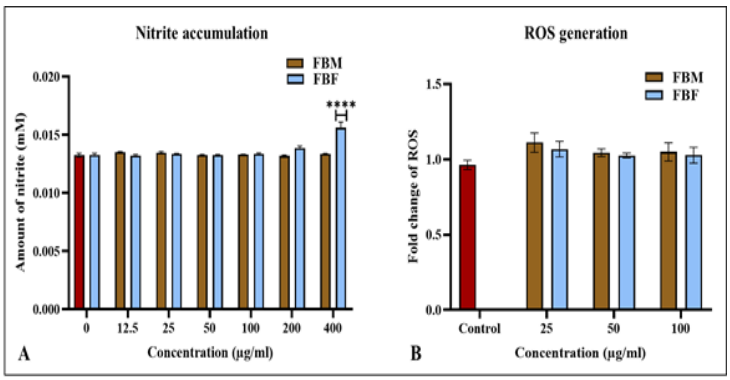

Figure 5. [A] Nitrite Accumulation and [B] Reactive Oxygen Species (ROS) Production in RAW264.7 Cell Line after 24 hrs Treatment with the Mass and Fluid of *Filopaludina bengalensis* in Different Concentrations. Data represented as mean ± SEM (n=4), statistical analysis was done using one-way ANOVA followed by Tukey's post hoc analysis. * indicates significant difference compared to the untreated control (****$p<0.0001$)

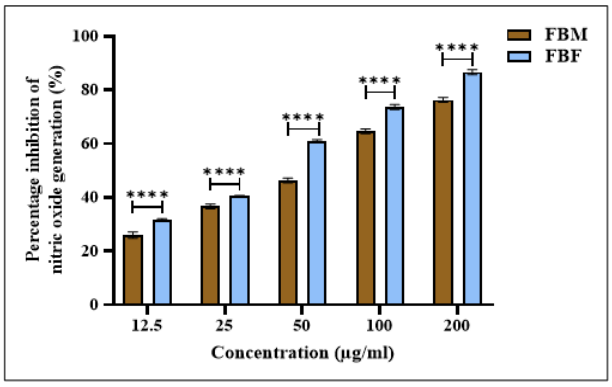

Figure 6. Effect on Nitrite Generation in LPS Induced RAW264.7 Cell Line Post 24 h Exposure with the Mass and Fluid of *F. bengalensis*. Data represented as mean ± SEM (n=3), statistical analysis was done using one-way ANOVA followed by Tukey's post hoc analysis. * indicates significant difference compared to the untreated control (****p<0.0001)

robustness of COI for resolving species boundaries, even among closely related viviparid taxa. Furthermore, the discriminatory power of this taxonomic marker is evident from the clear phylogenetic separation of *F. bengalensis* from related genera such as Mekongia, Notopala, Trochotaia, and Angulyagra. These findings thus provide a valuable reference for future ecological and evolutionary investigations in freshwater molluscs.

Secretion extracts of *Bellamya bengalensis* previously exhibited selective cytotoxicity against HepG2 and Huh-7 cells (IC_{50} = 12.88 µg/ml and 22.99 µg/ml respectively) [15] and myeloid leukemia cells U937, K562, HL-60 (IC_{50} = 22.21, 10.3, 12.79 µg/ml respectively), while sparing RAW264.7 macrophages and inducing NO production. Similarly, *F. bengalensis* fluid and mass extracts displayed dose- and time-dependent cytotoxicity against MCF-7 and MDA-MB-231 breast cancer cells, and minimal macrophage toxicity with stable NO and ROS levels, confirming safe immunomodulatory effects. In both the breast carcinoma cell lines, the *F. bengalensis* fluid extract exhibited substantially greater antiproliferative potency than the mass, underscoring a fundamental chemical and biological distinction between the two fractions. These findings align with previous reports showing that molluscan secretions can induce NO via the NF-κB pathway [16] particularly when combined with rIFN-γ, highlighting a potential mechanism for immunomodulatory activity. Both extracts revealed anti-inflammatory activity by inhibiting LPS-induced nitrite production in macrophages, reflecting iNOS inhibition with fluid extract (IC_{50} = 49.56 µg/ml) and mass extract (IC_{50} = 77.52 µg/ml), corroborating previous reports of *Bellamya bengalensis* extrapallial fluid suppressing prostaglandin and protease-mediated inflammation [14]. In the anti-inflammatory study also the better potency of the molluscan fluid suggests that it comprises of a higher concentration or bioavailability of the significant biomolecules. While the mass fraction aligned with previous descriptions of molluscan tissues as nutritionally protein-rich, also containing carbohydrates and lipids, earlier studies consistently show that molluscan fluids or mucus are particularly enriched in proteins, notably bioactive peptides [34]. Numerous anticancer and antimicrobial peptides have been isolated from marine molluscs [35, 36], notably the superior efficacy of *F. bengalensis* fluid extract likely reflects a peptide-rich profile. These observations strongly support the need for bioassay-guided fractionation, purification, and structural characterization to isolate the active peptide constituents, mediating the observed anticancer effects.

Although mollusc-derived bioactive compounds are known for their therapeutic and anti-inflammatory properties, research on freshwater mollusca *F. bengalensis* remains scarce. The present study exhibited the potential efficacy of the molluscan mass and fluid extract with highly selective cytotoxicity toward breast cancer cells while sparing immune macrophages. This selective toxicity is particularly significant in the context of current anticancer drugs, many of which are limited by severe off-target effects, nonselective cell damage, and immunosuppression. In contrast, the extract not only avoids activating immune cells but also suppresses LPS-induced nitric oxide production, suggesting a complementary immunomodulatory benefit relevant to emerging cancer–immune therapeutic strategies.

Overall, these findings position *Filopaludina bengalensis* as a promising source of biomolecules capable of aligning with modern targeted therapy paradigms by delivering tumor-specific cytotoxicity alongside supportive immune regulation. Future studies should focus on identifying and isolating the bioactive compounds and elucidating molecular mechanisms to facilitate drug development in oncotherapeutics.

In conclusion, marine and freshwater molluscs, represent a rich source of nutrients and bioactive molecules with considerable potential in functional foods and biomedical applications. The freshwater species *Filopaludina bengalensis*, the widespread freshwater mollusca are protein and mineral rich, and their identity can be reliably confirmed through DNA barcoding. The molluscan mass and fluid exhibit selective cytotoxicity against breast cancer cell lines (MCF-7 and MDA-MB-231) while preserving macrophage viability, coupled with pronounced anti-inflammatory activity through inhibition of LPS-induced nitrite production. These findings highlight the dual anticancer and immunomodulatory potential of *F. bengalensis* extracts, supporting their safe use in health-promoting interventions. Future work should focus on isolating specific bioactive compounds and explicating their molecular mechanisms, which could facilitate the development of targeted therapeutics and nutraceuticals derived from freshwater molluscs.

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Author Contributions Statement

S.M.- Conceptualization, Methodology, Experimental Investigation, Original Manuscript Preparation. S.J.- Sample Collection, Methodology, Experimental Investigation. S.M.- Experimental Investigation, Data Collection, Validation. B.S.- Experimental Investigation, Data Collection. R.C.- Experimental Investigation, Data Collection. S.M.- Experimental Investigation, Data Validation. J.J.J. - Supervision, Project Administration, Formal Data Interpretation. R.I.- Statistical Analysis, Software Analysis. M.R.- Hypothesis Development, Project Execution, Supervision, Review & Editing Manuscript. All authors reviewed the manuscript.

Conflict of Interest

The authors declare no conflict of interests.

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Clinical Trial Number

Not Applicable

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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