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Antitumor activity of edible fishes (*Channa striata* and *Anabas testudineus*) and gastropods (*Helix aspersa* and *Pila virens*) rudimentary mucus against HT-29 cell line and its biochemical properties

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Abstract

Background The mucus from fish and gastropods contains a wide range of bioactive molecules with biomedical properties. The fish and gastropods were collected from Oragadam lake, Kanchipuram district, Tamil Nadu, India. In this study, we wanted to examine the anticancer potential of fish and gastropods mucus. The biochemical components of the crude mucus were screened.

Results The biochemical analysis showed that the mucus of *Anabas testudineus* and *Pila virens* contained a high level of carbohydrates (2.8 and 1.5 mg/ml), the mucus of *Channa striata* contained a high level of lipids (0.9 mg/ml), and the mucus of *Helix aspersa* contained a high level of protein (1.3 mg/ml). The results showed morphological variations in the HT-29 cells upon treatment with crude mucus. Upon 24 h of gestation, the frozen cells began to shrink and seem round in shape. Using the MTT assay, the mucus crude extract was evaluated for its anticancer properties against the human colon cancer cell line (HT 29). The inhibitory concentration (IC50) was determined at 100 µg/ml after 24 h. Using specific staining techniques; fluorescent microscopy was utilized to examine the cell morphology and early and late apoptotic stages. Propidium iodide staining showed nuclear damage followed by DNA damage. This showed that the rudimentary mucus could prompt cell death and increased the number of fragments and mucus concentration, respectively.

Conclusion This study showed that the edible or commercially important fish and gastropod mucus have potential anticancer activity against HT-29 cancer cells.

Keywords Fish, Gastropods, Mucus, HT-29 anticancer

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Background

India has witnessed a noteworthy conversion in dietary food eating patterns with a substantial uptick in the population including fish in their diet, rising to 72.1% (Human Health Socio-Economic Asia India, 2024). In non-developed countries, terrestrial and marine and freshwater molluscs (gastropods and bivalves) are expansively picked and taken as a low-cost source of protein by economically and socially challenged groups (Anushree et al. 2020). Various fishes and molluscs have produced several bioactive substances which in chance are the energetic force towards bioprospecting and drug discovery to expose their potential to produce original bioactive compounds with pharmacological uses (Ngandjui et al., 2024).Colorectal cancer originates from the epithelial cells lining the colon or rectum within the gastrointestinal tract. A few components contribute to the high risk of colon cancer. This is due to the side effects of radioactivity treatment, operation, and annihilator medicines (Tabibzadeh et al., 2020). The search for natural products with anticancer properties for specific target and minimize the side effects treatment has been ongoing. However, clinics use a large range of anticancer medications; many are from natural or derived sources from various plants, animals, and microorganisms. Sixty percentages of the commercially available antitumour medicines are of natural source (Alves et al., 2018). Therefore, numerous natural compounds offer a wide range of applications in medicine and offer pharmacological significance. Given the wide variety of fishes and gastropod groups belonging to different habitats such as terrestrial, freshwater, and saltwater. Investigating the composition of mucus which protects the skin of fishes and gastropods from pathogens and other particles can be of greater significance. The effectiveness of mucus in its function relies on its ability to create a gel-like substance on the surface of epithelial cells (Bragadeeswaran et al., 2011). Goblet or mucus cells predominantly generate mucosal mucus, consisting of water and large molecules like mucins and glycoproteins, contributing to its gel-forming properties. (Lillehoj e t al., 2011). Now, bioactive metabolites are derived from fishes and gastropods are fine characterized in the antitumor properties during medical testing (Simmons et al., 2005). Cancer has become a growing public threat and despite the advancement in research and technology, there is an urgent need for the development of anticancer drugs. Marine organisms contain a reservoir of natural products with structural features (Alves et al., 2018). In addition, it comprises a variation of materials such as immunoglobulins, lectin, protease inhibitors, complements, and lytic enzymes which serve as defence substances against foreign pathogens (Kumari et al., 2011; Shephard, 1994).

Channa striata is commonly known as the snakehead fish. Snakeheads are yet to be explored as a source of medicine even though folk belief suggests *C. striata* is traditionally used to treat wounds, relieve pain, and boost energy, making it a commonly recommended remedy for promoting recovery (Rahman et al., 2018a). *Anabas testudineus* are carnivorous air-breathing species, which are displayed in waterways, lakes, pools, trench, and rice areas. They are widely used in traditional medicine (Singh et al., 2016). *P. virens* is a known snail under the family Ampullariidae. They are large freshwater gastropod molluscs having a thick calcareous operculum. Among the various invertebrates, mollusks are a novel source of pharmaceutically significant product and have an active innate system (Tincu & Taylor, 2004). They are considered to show anticancer, antibacterial, anti-inflammatory and antioxidant properties (Nagash et al., 2010). Molluscan metabolites have been tested for neuromuscular, antimicrobial, anti-neoplastic, and cytotoxic activity. The use of bioactive molecules has been technically verified to deal chemoprevention and defence against social ailments (Gayathri et al., 2017). *H. aspersa* is known as the brown garden snail and has been in human medicine for the treatment of wounds. Studies on the nutritional value of snails show a high protein but a low-fat content which can be used as an alternative food source (Cagiltay et al., 2011). In the human body, the oxidation process causes cellular destruction, tumour, and other worsening infections. Hence, the antioxidant compounds found in various mollusks could potentially shield cells from oxidative damage caused by oxidation processes (Nagash et al., 2010). The secondary metabolites derived from the fish and mollusks fall into an extensive variety of essential groups, with certain products predominating in definite fauna. It plays an imperative part as a defensive component in the life form by evacuating damaged cells owing to disgraceful mitotic boost (Lee et al., 2010). In recent years, triggering apoptosis has emerged as a focused strategy in the quest for antitumor drug development, with a specific emphasis on identifying agents that induce apoptosis in tumour cells, potentially representing an optimal approach for antitumor therapy (Chen et al., 2002). Hence, in the present investigation, we selected fishes and gastropods such as *C. striata, A. testudineus, P. virens*, and *H. aspersa* which are of commercial importance and tested their anticancer activity using its epidermal crude mucus.

Methods

Sample collection

We collected the ichthyofauna and gastropods *Channa striata*, *Anabas testudineus*, *Pila virens*, and *Helix aspersa* (Fig. 1a and b) from Oragadam Lake in the

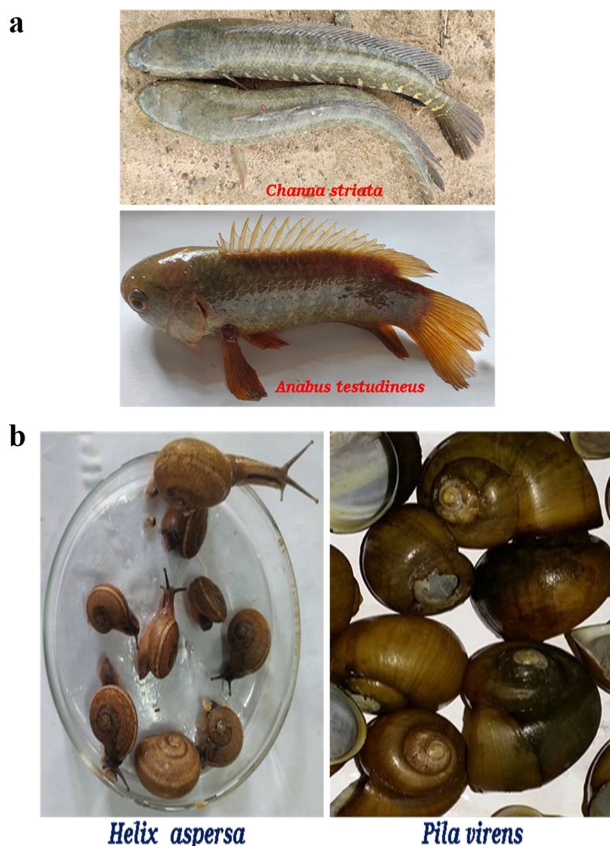


Fig. 1 **a** Fish samples—*Channa striata* and *Anabas testudineus* collected in the study area, **b** Gastropod samples *Pila virens* and *Helix aspersa* collected in the study area

Kanchipuram district of Tamil Nadu, India, in February 2023 at 10:00 a.m., following the monsoon season. The following medium-sized adult fish and mollusks were gathered from paddy fields, irrigation channels, ponds, and lakes (Lat. and Long. 12°38'28.9"N 80°03'26.1"E): *Channa striata*, *Anabas testudineus*, *Pila virens*, and *Helix aspersa*. The freshwater fishes and molluscs were transported to the laboratory and were maintained using an aerator. It was identified and authenticated by Dr. C. Arulvasu, Professor, Department of Zoology, University of Madras, Chennai, Tamil Nadu based on the morphological characteristics and the identification keys which were taken from palatine teeth patterns from fresh water fishes resources of India.

Extraction of crude mucus

Epidermal mucus was collected from healthy live fish *C. striata*, *A. testudineus,* and gastropods *P. virens*, *H. aspersa* upon placing them on a surgical procedure. Epidermal bodily fluid from the dorsal side of the fish was collected by a cell scrubber and exchanged into a tube with a buffer solution containing 0.013 M Tris, 0.12 M NaCl, and 0.003 M KCl (pH 7.4). The epidermal mucus was taken out from the gastropods by eradicating the peel from the shell with an antiseptic sharp-end metal rod into a glass beaker, and the mucus excretions were squeezed out aseptically from the soft body. The isolated mucus (3-5 ml) was stored in the refrigerator at 4˚C for further analysis. To prevent contamination by blood, intestinal fluids, and sperm, mucus collection avoided the ventral side. Subsequently, samples were stored at -80ºC until needed. Thawing of stored mucus samples was performed at room temperature, followed by homogenization using a pestle and mortar. The insoluble particles were eliminated by centrifugation at 4ºC for 10 min at 10,000 rpm. The supernatant was collected, and mucus was put into falcon tubes (15 mL), lyophilized, and stored at -20ºC for further examination.

Biochemical analysis of different mucus

Estimation of protein

The total protein present in the crude mucus was quantitatively estimated by the standard procedure method of Bradford (1976). Twenty microliters of *C. striata*, *A. testudineus, P. virens,* and *H. aspersa* crude mucus sample was diluted with five ml of Bradford chemical. Bovine Serum Solution (BSA) (for protein estimation) was prepared by taking 10 to 100 ug of protein in different test tubes. This was made up to 0.1 ml with NaCl solution. To this 5 ml of protein mixture was added. A blank solution was also prepared with 0.1 ml NaCl solution and 5 ml Bradford reagent. The colour variation in the sample was measured at range of 595 nm by UV–Vis double beam spectrophotometer (Shimadzu 160 Make). The UV peak range was noted; it represents the amount of protein presented in the sample and calculated using the standard value of protein.

Estimation of lipids

The lipid content in crude mucus samples from *C. striata, A. testudineus, P. virens,* and *H. aspersa* was determined following the method outlined by Barnes and Blackstock (1973). Specifically, 20 µl of each crude mucus sample was mixed with a chloroform/methanol (2:1) solution (5 ml), to which 0.5 ml of 0.9% NaCl solution was added and thoroughly shaken. After allowing the mixture to settle in a separation funnel for 12 to 14 h, the lower phase containing lipids was collected in a test tube and adjusted to a volume of 5 ml with chloroform. Subsequently, 0.5 ml of the lipid sample was digested with 0.5 ml of concentrated H_2SO_4 in a boiling water bath for 10–15 min. Following digestion, 0.2 ml of the acid digest was mixed with 5 ml of phosphovanillin reagent and allowed to stand for 30 min, after which the colour developed was measured at 520 nm using a Shimadzu

160 UV–visible double beam spectrophotometer. Lipid standards and blanks were prepared simultaneously, and the total lipid content was determined based on the lipid standard value.

Estimation of carbohydrates

The estimation of total carbohydrates in the crude mucus of *C. striata*, *A. testudineus*, *P. virens,* and *H. aspersa* was quantified using the methodology described by Roe (1955). To 20 µl of *C. striata*, *A. testudineus*, *P. virens,* and *H. aspersa* crude mucus sample, 1 ml (80 per cent) of ethanol was added and centrifuged at 4000×g for 10 min. Following centrifugation, 5 ml of the anthrone reagent was added to the supernatant, and the mixture was incubated in a gentle water bath for 15 min. Subsequently, it was cooled in a dark place at room temperature for 30 min. The resulting blue colour complex was then measured using a Shimadzu 160 UV–visible double beam spectrophotometer at 620 nm. The carbohydrate content of the coelomic fluid was calculated using the optical density of known concentration of standard and expressed as mg/ml.

Cancer cell lines and reagents

The human colon adenocarcinoma cell line (HT-29) was sourced from the National Centre for Cell Science in Pune, India. Essential laboratory materials including Dulbecco's modified Eagle medium (DMEM), trypan blue (TB), trypsin–EDTA, fetal bovine serum (FBS), 3-(4,5-dimethyl thiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT), sodium bicarbonate, dimethyl sulphoxide (DMSO), and antibiotic solution were procured from Hi-Media Laboratories in Mumbai, India. Additionally, laboratory equipment such as 96-well plates, 6-well plates, tissue culture flasks (25 and 75 mm^2), and centrifuge tubes of 15 and 50 ml capacity was obtained from Tarsons Items Pvt. Ltd. in Kolkata, India.

Cytotoxicity on Viro Cell Line

Vero cells maintained in cells (1×10^5 / well) were seeded in the 96-well plates with DMEM medium, supplemented with 10% of FBS, at 5% CO_2, and 37 °C. For experimental purposes, cells were detached, centrifuged, and then suspended and seeded into 96-well plates. The concentration of epidermal mucus ranged from 200 to 1000 µg/ml after 24 h of incubation. Following this, cells were exposed to 10 µl of 5 mg/ml MTT solution, and after the designated incubation period, formazan crystals formed, which were subsequently dissolved using an organic solvent. Absorbance was measured at 570 nm, with viable cells displaying a characteristic dark purple colour.

MTT Assay

The cytotoxicity and cell viability of crude skin mucus extract were assessed by the MTT method on vero and colon cancer cell line cells, followed by Mosmann, 1983. Human cancer cells were seeded at a density of 1×10^6 cells per well in 96-well plates containing Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS. Following a 24-h incubation period at 37 °C in a humidified atmosphere of 5% CO_2 and 95% O_2, allowing cell attachment, the culture medium was replaced with varying concentrations of crude mucus in each well for another 24-hour incubation. Control cells were maintained without the addition of crude mucus samples. After the incubation period, 20 µl of 5 mg/ml MTT dye was added to each well, and the plates were further incubated for 3 h at 37 °C. Subsequently, 100 µl of dimethyl sulphoxide (DMSO) was added to dissolve the formed crystals by gentle pipetting 2 to 3 times, and absorbance was measured at 570 nm. The IC50 value (half-maximal inhibitory concentration) is the concentration of a drug or compound that inhibits a biological or biochemical function by 50%. In the context of cancer cell line assays for animal studies, the IC50 value is calculated to determine the potency of a drug in inhibiting cancer cell proliferation or viability (Zbinden et al., 1981).

Morphological observation of HT-29 cells

After trypsinization, human cancer cells were seeded into 6-well plates and allowed to incubate for 24 h to facilitate attachment in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS. Following attachment, varying concentrations of crude mucus samples were introduced to the cell lines. n cells were cultured in a serum-free medium. After a 24-h incubation period, the cells were examined under an inverted microscope as described by Arulvasu et al. (2012).

Propidium iodide staining for nuclear damage

Fluorescent staining of apoptotic nuclei was conducted following the methodology outlined by Keum et al. (2002). Cancer cells seeded at a density of 5×10^4 cells/ml were seeded in 6-well plates containing a medium supplemented with 10% FBS. The cells were treated with crude mucus protein, while for control cells were maintained in serum-free media. Subsequently, upon completion of the incubation period, the cell culture media were aspirated, and the cells were washed with PBS before being fixed in methanol/acetic acid (3:1v/v) for 10 min. Following fixation, the cells were stained with 50 µg/ml of propidium iodide for 20 min. The stained cells were then

visualized under a confocal microscope (LSM 710, Carl Zeiss) at 20×magnification.

DNA fragmentation analysis

DNA extraction and agarose gel electrophoresis were conducted using a horizontal gel apparatus following the protocol described by Bortner et al., (1995). Cancer cells, plated at a density of 3×106 cells/ml, were cultured under controlled conditions. Various concentrations of the sample were added, and after 24 h of incubation, the cells were scraped and collected in Eppendorf tubes for DNA extraction. Agarose gel (0.8%) electrophoresis was performed with ethidium bromide-containing TAE buffer in a mini gel tank at 90 V for 1 h. Further DNA gel was examined for quantification under UV trans illuminator (Bio-Rad) and photographed.

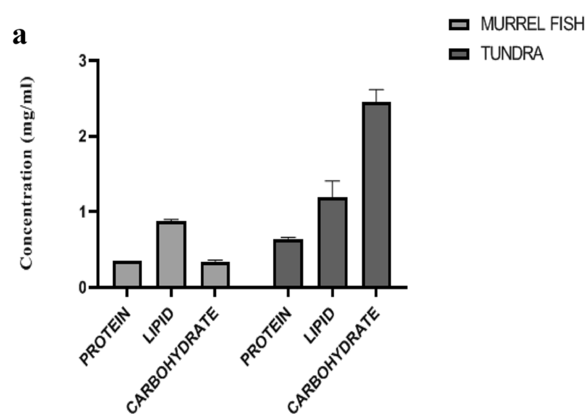
Statistical analysis

Statistical analysis was performed on the data from triplicate of the MTT assay, and the mean value along with its corresponding standard error was determined. The per cent change between the control and experimental data was calculated. Statistical analysis was conducted using one-way ANOVA (SPSS), and p value less than 0.01 is considered to be significant.

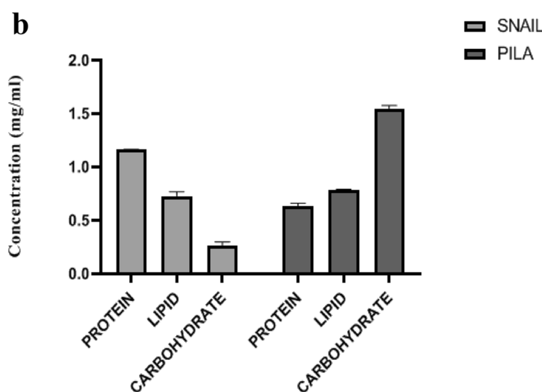
Results

Biochemical composition of crude mucus *C. striata, A. testudineus, P. virens,* and *H. aspersa*

The fish and gastropod's crude mucus of *C. striata*, *A. testudineus, P. virens,* and *H. aspersa* displayed a pale yellow colour. The estimated biochemical constituents, namely protein, carbohydrates and lipids from the mucus of specimens. Upon biochemical analysis, the estimates of mucus showed that the *A. testudineus* contained a high carbohydrate content of 2.8 mg/ml in comparison with lipids (1.2 mg/ml), and protein (0.9 mg/ml). In the estimates of the crude mucus of *C. striata*, the lipid (0.9 mg/ml) content was high in comparison with protein content (0.5 mg/ml) and carbohydrates (0.4 mg/ml) (Fig. 2a). In the mucus of *P. virens*, the carbohydrate was high (1.5 mg/ml) when compared to other lipids (0.8 mg/ ml) and protein (0.5 mg/ ml). In the *H. aspersa* crude mucus, the protein content was high (1.3 mg/ml) when compared to lipids (0.7 mg/ml) and carbohydrate (0.3 mg/ ml) content, respectively (Fig. 2b). In the fish mucus matrix, other than mucin production the delivery of molecules such as proteins has been reported.



Each bar represents mean ± S.D. of triplicates (P<0.01)

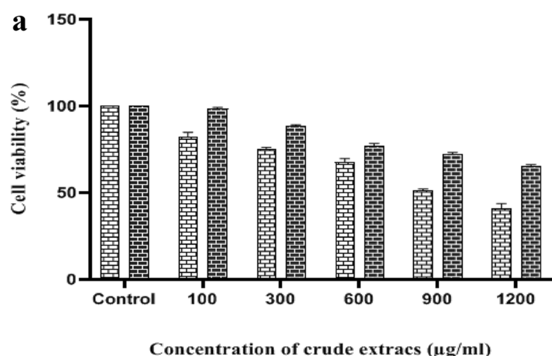


Each bar represents mean ± S.D. of triplicates (P<0.01)

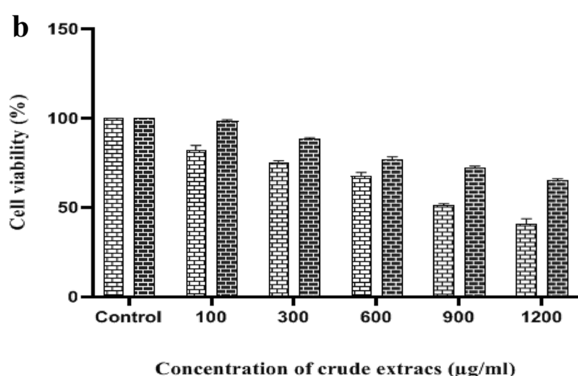
Fig. 2 **a** The biochemical constituents of crude mucus of *C. striata* and *A. testudineus*, **b** the biochemical constituents of crude mucus of *P. virens* and *H. aspersa*

Cytotoxicity and cell viability

The cell viability and cytotoxicity assays were analysed for HT 29 and Vero Cell lines (passage number 2) following MTT method. Upon treatment with Vero cells, the cells showed 80% viability at 800 µg/ml incubation for 24 h. Hence, we presume that the fish and gastropod crude mucus can be used on standard cell lines. The antitumour of mucus of *C. striata*, *A. testudineus*, *P. virens*, and *H. aspersa* was analysed upon human being colon cancer cell line of HT-29 cells. Figure 3a and b represents a reduction in the cell feasibility at concentrations values from 100–1200 µg/ml of treated cells where the lowest apoptosis was observed. The inhibition concentration (IC_{50}) value was 100 µg/ml of crude mucus at 24-h gestation (Fig. 3a, b). The Vero cells were insignificantly affected by the crude mucus. However, the HT-29 Cells were significantly reduced upon treatment with crude mucus.



The values are Mean ± S.D. of triplicates (P<0.01)



The values are Mean ± S.D. of triplicates (P<0.01)

Fig. 3 **a** Cell viability assessment of crude mucus from *C. striata* and *A. testudineus* on the HT-29 cells, **b** cell viability assessment of crude mucus from *P. virens* and *H. aspersa* on the HT-29 cells

Morphological observations

Morphological changes were noted in HT-29 cells in control and treated cells with rudimentary mucus (Fig. 4a and b). The control cells displayed asymmetrical confluent combinations with round cell morphology. After 24-h incubation, the treated cells were polygonal cells which began to shrivel and appear spherical.

Propidium iodide staining

The damage of nuclear cells was observed under fluorescent microscope with the propidium iodide staining method. We observed that in the control cells, a lesser amount of cells was stained with propidium iodide whereas the cells that were treated with 100 μg/ml of crude mucus showed a gradual increase in propidium iodide stained fluorescence in the nucleus upon 24-h incubation. The propidium iodide marked bright fluorescence was present in the condensed nuclear cells which was detected at the maximum concentration of 1200 μg/ml (Fig. 4a and b). The fluorescent red colour indicated that the stain binds to the nucleic acid of dead cells HT cells. The apoptotic cell also began to show morphological changes which eventually led to DNA damage.

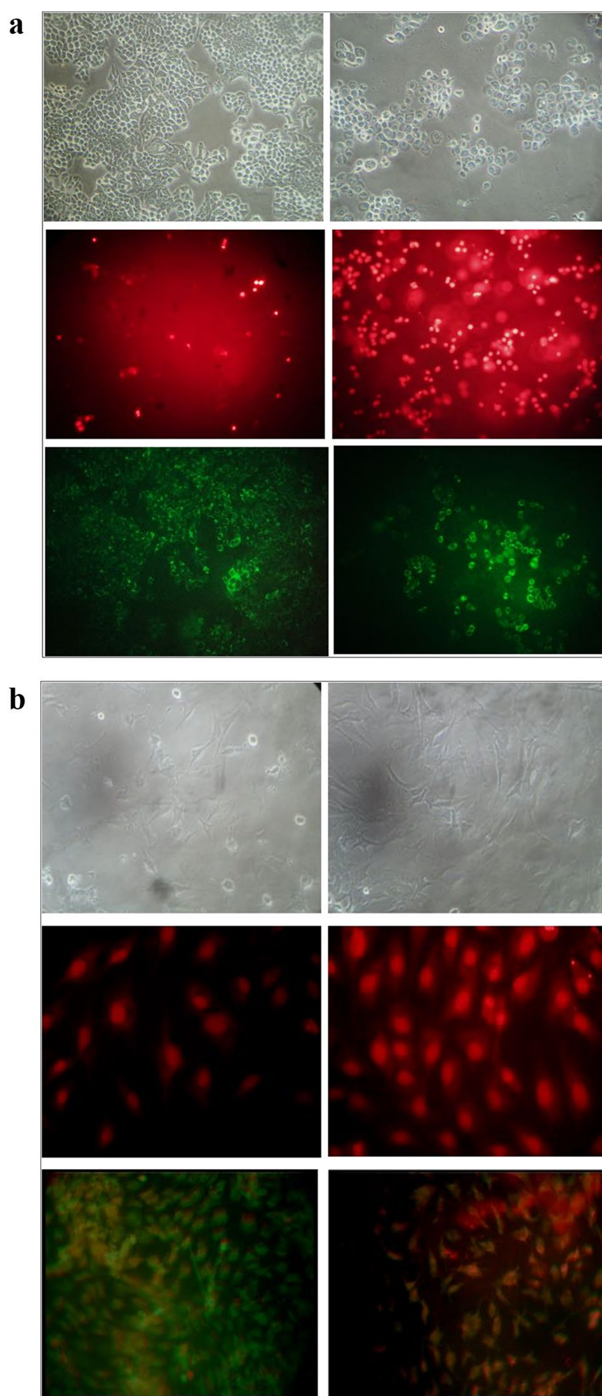


Fig. 4 **a** Morphological observation and nuclear morphological observation of HT-29 cells treated with crude mucus extract of *C. striata* and *A. testudineus*, **b** morphological observation and nuclear morphological observation of HT-29 cells treated with crude mucus extract of *P. virens* and *H. aspersa*

DNA fragmentation assay

The DNA fragmentation assay was employed to confirm whether the cytotoxic effects induced by extracted fish epidermal crude mucus from *C. striata, A. testudineus, P. virens*, and *H. aspersa* involve nuclear damage leading to apoptotic changes. Treatment with the crude mucus protein (Fig. 5a & b) revealed distinct DNA bands in the control group of HT-29 cells, which remained intact, while the treated cells exhibited fragmented patterns and streaking.

These observations suggest that crude mucus has the capability to induce apoptosis in HT-29 cells, with the degree of fragmentation increasing with higher concentrations of mucus. Moreover, a higher concentration of mucus resulted in more pronounced fragmented streaking compared to the IC50 value.

Discussion

Marine and freshwater organisms are taxonomically diverse, with thousands of new compounds derived from marine natural sources entering clinical trials for human benefit each year. Coastal and inland water invertebrates, which develop in environments distinct from those of terrestrial animals, are sources of a wide range of pharmacological substances. These organisms either express these substances constitutively or their expression is induced by exposure to pathogens (Sri Kumaran et al. 2011). Due to these unique properties, marine organisms, particularly invertebrates, are of significant pharmaceutical interest (Keivan et al. 2007). Marine environments are a rich source of therapeutic compounds, with mollusks and fish species producing a variety of drugs that exhibit antibiotic, antiviral, antiparasitic, analgesic, and anticancer properties. The proteins can be conveyed to the ichthyofauna mucus film by cellular organelle like rough endoplasmic reticulum (EPR) and then transported to the membrane of cell over the Golgi apparatus (Brinchmann, 2016, Kim et al. 1985).

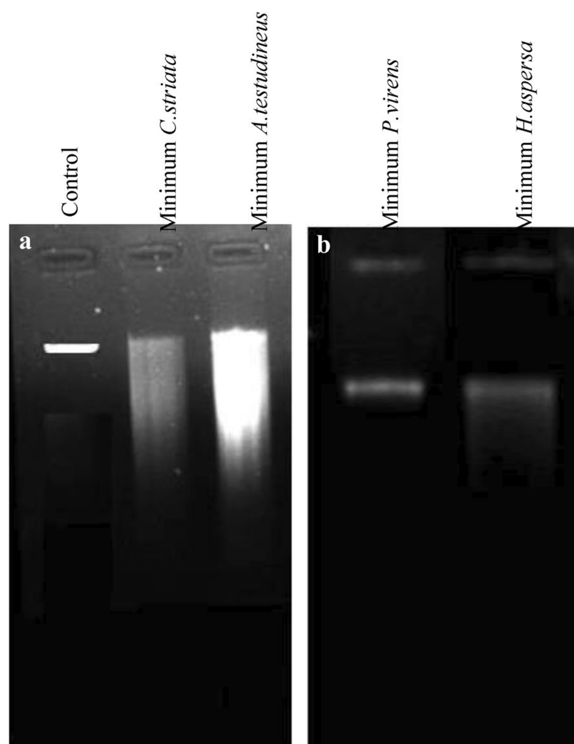


Fig. 5 **a** DNA fragmentation of HT-29 cell line treated with crude mucus extract from *C. striata* and *A. testudineus*; **b** DNA fragmentation of HT-29 cell line treated with crude mucus extract from *P. virens* and *H. aspersa*

The molecules are present in organisms that produce biomolecule compounds to ward off predators, including sponges, corals, and ascidians (Rosenfeld and Shai, 2006). Fish processing wastes and other biologically active compounds derived from marine animal by products are regarded as a safe, high-protein substance with a good pattern of essential amino acids and other nutritional advantages. Additionally, because fish proteins are a rich source of bioactive peptides, they have promise as functional foods in ingredients (Rasmussen et al. 2007). Therefore, that skin by product that covers the skin had a good amount of protein and was inhibiting the growth of colon cancer cells. Antimicrobial molecules such as pore-forming enzymes and glycoproteins (Ebran et al. 2000; Fuochi et al. and crinotoxins (Sugiyama et al. 2005) have been reported in fish mucus. The mucus of the snail also possesses different biological properties, namely antimicrobial, anticancer, and anti-inflammation (Mane et al. 2021). Cytotoxicity and cell viability could be due to stimulating factors of cell death which can basis mechanical damage and mitochondrial dysfunction of cells (Marchetti et al. 1996). The present study, after 24-h incubation the treated cells were polygonal cells which began to shrivel and appear spherical. The cell viability and cytotoxicity assays were analysed for HT 29 and Vero Cell lines following MTT method. This implied that there was DNA damage to the cells which led to apoptosis (Venkatachalam et al. 1993; Walker et al. 1997).

Vero cells, which were unable to impact nearby healthy cells, were used to investigate the cytotoxicity of skin extract. The doses for HT 29 cells were set between 100 and 1000 µg/ml because there was no discernible cell death at the maximal dosage. An IC $_{50}$ value of 600 µg/ml was found when we used a human colon cancer cell line to analyse the cell viability of the crude skin ex tract. Data analysis revealed that the crude skin extract significantly inhibits human colon cancer cells. Through the removal of old, superfluous, and harmful cells, apoptosis a process of programmed

necrosis plays a crucial role in the development and maintenance of the body's health. Blebbing, shrinkage, nuclear fragmentation, chromatin condensation, poly nucleosomal DNA fragmentation, global mRNA decay, and eventually the fragmentation of cells into apoptotic bodies are some of the distinctive cell morphologies that occur during various stages of apoptosis (Kavitha et al. 2020; Degterev and Yuan, 2008).

The current observations suggest that crude mucus has the capability to induce apoptosis in HT-29 cells, with the degree of fragmentation increasing with higher concentrations of mucus. Moreover, a higher concentration of mucus resulted in more pronounced fragmented streaking compared to the IC_{50} value. We examined the *C. striata*, *A. testudineus, P. virens* and *H. aspersa* mucus and tested its anticancer activity. Thus, the crude mucus can be considered an efficient and safe novel substitute. However, it should be purified and structurally identified. Due to their effectiveness, they can be considered as health care products and an eco-friendly compound with modifications of natural products. Similarly, Rahman et al. (2018a, 2018b) stated that *C. striata* species is mainly used for food, therapeutic and traditional medicinal properties including antibacterial, anti-inflammatory and antitumour properties for clinical trials in Korea. Resemblance to the present findings, Najm et al. (2021) characterizing the anticancer activities from *A. testudineus* antimicrobial peptides of mucus fractions showed toxicity against MCF7 and MDA MB-231 cell lines. Anticancer properties of haemocyanins derived from gastropod *H. aspersa* induced chemotherapeutic effects decreased cancer cell growth without toxicity effects in a cancer cell model (Moltedo et al., 2006 and Georgeiva et al., 2023). In addition, Gayathri et al., (2020) conducted the cytotoxicity assay of *P. virens* methanolic extract was investigated for cytotoxicity effects on Vero cell lines showed 20% of maximum toxicity observed at 1000 µg/ml concentration at different time periods.

Conclusion

The earlier literature and present findings revealed that the edible fish mucus and gastropods mucus have been good sources in the treatment of various diseases and are noteworthy animal for the improvement of antitumour medicines.

Abbreviations

DMEM Dulbecco's modified Eagle medium
ANOVA Analysis of variance

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Author contributions

D.E and C.A conceptualized, designed the research, carried out the experiments and drafted the manuscript, and H.M, R.K, S.G, M.S, N.V, E.A.S and R.M contributed to reviewing, editing and formal analysis.

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Data availability

All data generated during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interest.

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