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Study on the Role of Fish-Derived Bioactive Compounds in Cancer Therapy: A Molecular Perspective

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Abstract: Marine and freshwater ecosystems have been recognized as rich sources of pharmacologically active compounds, particularly those with anticancer properties. This study explores the molecular mechanisms and therapeutic potential of fish-derived bioactive molecules, including peptides, alkaloids, and polysaccharides, in cancer treatment. The results demonstrate that these compounds exert strong cytotoxic effects on various cancer cell lines, with alkaloids showing the highest potency against HT-29 colon cancer cells and peptides effectively targeting MCF- 7 breast cancer cells. Gene expression and Western blot analyses reveal significant upregulation of pro-apoptotic markers (Bax, Caspase-3, p53) and downregulation of anti-apoptotic (Bcl-2) and angiogenic markers (VEGF, MMP-9), confirming their ability to induce apoptosis and inhibit tumor angiogenesis. DNA fragmentation analysis further supports their role in cancer cell death by demonstrating increased DNA damage in treated cells. The statistical evaluation confirms the high significance of these findings, underscoring their therapeutic relevance. While challenges remain in large-scale extraction, stability, and pharmacokinetics, these compounds hold immense promise for future drug development. By integrating fish-derived bioactives into targeted cancer therapies, researchers can develop novel, less toxic treatment strategies that effectively combat drug-resistant tumors, paving the way for marine-based cancer therapeutics.

Keywords: Fish bioactive compounds, marine peptides, anticancer mechanisms, fish- derived alkaloids, apoptosis, angiogenesis, cancer therapeutics

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INTRODUCTION

Cancer continues to pose a significant worldwide health issue, accounting for countless fatalities each year. In spite of notable progress in traditional cancer treatments like chemotherapy, radiotherapy, and targeted therapies, these methods frequently entail serious side effects and the potential for drug resistance (Newman & Cragg, 2020). Consequently, the investigation of natural bioactive substances has garnered significant attention within the realm of oncology research. Within the diverse array of natural origins, both marine and freshwater life forms, especially fish, have surfaced as a noteworthy source of bioactive substances that exhibit considerable anticancer properties (Mayer et al., 2019). Bioactive compounds sourced from marine environments, such as peptides, polysaccharides, and alkaloids, have shown significant cytotoxic properties against a range of cancer cell lines. These substances demonstrate a variety of mechanisms through which they operate, including the promotion o f apoptosis, the suppression of angiogenesis, and the alteration of immune responses (Zhou et al., 2021). Antimicrobial peptides (AMPs) derived from fish, for example, specifically hone in on cancer cells by compromising their membranes,

positioning them as intriguing prospects for alternative cancer therapies (Wang et al., 2022). In a similar vein, fucoidans and polysaccharides derived from algae associated with fish demonstrate the ability to impede tumour development by disrupting the adhesion, invasion, and immune evasion tactics employed by cancer cells (Apostolidis et al., 2018).

In the past few years, alkaloids sourced from microbes associated with fish have garnered significant interest due to their powerful anticancer properties. These substances disrupt the DNA replication of cancer cells and hinder the progression of the cell cycle, ultimately inhibiting tumour growth (Hamed et al., 2019). Moreover, omega-3 fatty acids along with chitin derivatives sourced from fish have demonstrated the ability to prevent cancer, thereby enhancing the possibilities of utilising bioactive compounds derived from fish in cancer treatment (Rahman et al., 2018; Li et al., 2021). From a molecular standpoint, compounds derived from fish demonstrate their anticancer properties via multiple pathways. They amplify apoptotic signalling through the upregulation of pro-apoptotic genes like Bax and caspase-3, consequently initiating programmed cell death in cancerous cells (Nguyen et al., 2022). Additionally, they impede angiogenesis through the downregulation of vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9), consequently limiting the development of new blood vessels that are crucial for tumour proliferation and metastasis (Jin et al., 2021). Moreover, bioactive compounds derived from fish have been discovered to boost the functionality of the immune system, resulting in enhanced detection and eradication of cancer cells (Singh et al., 2020). In spite of these encouraging results, the practical use of bioactive compounds sourced from fish encounters numerous obstacles. The large-scale extraction and purification of these compounds necessitate sophisticated biotechnological methods, and a more thorough assessment of their pharmacokinetics and bioavailability in humans is essential (Kim et al., 2021). Nonetheless, as the focus on natural and targeted treatments in cancer care continues to expand, bioactive compounds sourced from fish present significant opportunities for the advancement of future pharmaceuticals.

LITERATURE REVIEW

Bioactive Compounds from Fish and Their Anticancer Properties

The oceanic ecosystem has been acknowledged for an extended period as a rich reservoir of biologically potent substances that hold considerable promise for therapeutic applications. Fish and their related microbial populations have demonstrated the ability to generate a range of bioactive compounds that display potent anticancer effects. These substances fall into various classifications, such as antimicrobial peptides, polysaccharides, and alkaloids, with each category demonstrating distinct cytotoxic impacts on cancerous cells.

Antimicrobial peptides (AMPs) sourced from fish have emerged as intriguing prospects in the realm of cancer treatment, owing to their capacity to specifically hone in on malignant cells while leaving healthy tissues unharmed. These peptides operate by compromising the structural stability of cancer cell membranes, resulting in heightened permeability and subsequent cell lysis (Wang et al., 2020). Research has shown that antimicrobial peptides sourced from fish display strong cytotoxic effects on a range of cancer cell lines, such as those found in breast, lung, and colon cancers, while causing little harm to healthy cells (Zhang et al., 2022). The discerning characteristics of these peptides render them appealing for the

advancement of precise cancer treatment strategies. A different category of bioactive substances exhibiting anticancer properties includes fucoidans and polysaccharides, which are typically derived from algae associated with fish and various other marine origins. These substances have demonstrated the ability to suppress tumour development by altering immune responses and disrupting the adhesion and invasion abilities of cancer cells (Apostolidis et al., 2018).

Polysaccharides sourced from fish not only inhibit tumour advancement but also bolster the body's inherent immune responses by activating immune cell function (Singh et al., 2020). The combined mechanism—exerting direct cytotoxic impacts on cancerous cells while simultaneously bolstering the immune system—further underscores the significance of polysaccharides sourced from fish in cancer-related applications.

Alkaloids, a distinct category of bioactive compounds sourced from fish, hold considerable importance in cancer treatment by influencing DNA replication and the progression of the cell cycle. A significant number of these alkaloids are generated by microbial populations linked to marine fish, and they have been documented to demonstrate potent inhibitory effects on cancerous cells (Hamed et al., 2019). These compounds disrupt DNA replication, trigger oxidative stress, and facilitate programmed cell death in a range of cancer cell varieties (Rahman et al., 2018). The capacity of alkaloids sourced from fish to specifically interfere with the functions of cancerous cells, while sparing healthy cells to a significant extent, highlights their promise as agents for chemotherapy. Alongside these significant categories of bioactive compounds, the exploration of fish lipids and omega-3 fatty acids has also been undertaken regarding their potential in cancer prevention and therapy. A multitude of research has shown that omega-3 fatty acids sourced from fish oil exhibit both anti-inflammatory and pro-apoptotic characteristics, playing a significant role in their anticancer properties (Gupta et al., 2021). These lipids influence the metabolic processes of cancer cells, diminish tumour formation driven by inflammation, and improve the effectiveness of standard cancer treatments when administered together (Kim et al., 2021). The variety of bioactive substances found in fish highlights their significant promise in cancer treatment. These organic compounds present a viable substitute for traditional chemotherapy drugs, potentially minimising adverse effects and addressing drug resistance in the treatment of cancer. The upcoming segment delves into the intricate molecular processes by which these substances demonstrate their anticancer properties.

Mechanisms of Action of Fish-Derived Bioactive Molecules

Bioactive compounds sourced from fish demonstrate their anticancer properties via numerous molecular pathways, such as triggering apoptosis, suppressing angiogenesis, and influencing immune system functions. These processes are essential for hindering cancer advancement and enhancing the effectiveness of treatments. A key mechanism by which bioactive compounds derived from fish fight cancer is by triggering apoptosis, the process of programmed cell death that effectively removes damaged and malignant cells. A variety of peptides and alkaloids sourced from fish stimulate both intrinsic and extrinsic apoptotic pathways through the upregulation of pro-apoptotic genes, including Bax and caspase-3 (Nguyen et al., 2022). These substances compromise mitochondrial stability, resulting in the release of cytochrome c and the subsequent initiation of caspase cascades, which ultimately activate apoptosis in cancerous cells (Fernando et al., 2019). The capacity to specifically trigger programmed cell death in malignant cells while

preserving healthy tissues renders compounds sourced from fish exceptionally appealing for cancer treatment strategies.

A vital anticancer strategy encompasses the suppression of angiogenesis, which is the mechanism through which tumours generate new blood vessels to support their expansion and spread. Bioactive compounds sourced from fish have been discovered to inhibit the expression

of angiogenic indicators, notably vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9), both of which are crucial in the process of blood vessel development (Jin et al., 2021). Through the downregulation of these angiogenic elements, compounds sourced from fish proficiently deprive tumours of their essential blood supply, resulting in inhibited growth and ultimately, tumour regression (Moreno et al., 2022). This system underscores the promise of fish-derived bioactive compounds in the realm of anti- angiogenic treatments for cancer. Alongside the inhibition of apoptosis and the suppression of angiogenesis, bioactive substances sourced from fish also influence immune system functions, boosting the body's inherent capacity to identify and eradicate cancerous cells. Fish-derived polysaccharides and peptides have been shown to enhance the generation of immune cells, including natural killer (NK) cells and cytotoxic T lymphocytes, which are essential in identifying and eliminating cancerous cells (Singh et al., 2020). Certain molecules sourced from fish have been discovered to inhibit immune checkpoint proteins, which in turn hinders the ability of cancer cells to evade the immune system and enhances the efficacy of immunotherapeutic strategies (Hassan et al., 2020).Additionally, recent studies have uncovered the significance of compounds sourced from fish in the realm of epigenetic regulation, demonstrating their ability to affect gene expression without modifying the DNA sequences themselves. Certain marine-derived peptides and polysaccharides have demonstrated the ability to alter histone acetylation profiles, resulting in the activation of tumour suppressor genes while simultaneously repressing oncogenes (Rodrigues et al., 2018). This system offers an extra dimension of treatment possibilities, given that epigenetic alterations are capable of being reversed and can be aimed at reinstating typical cellular operations in malignant tissues. Although the cancer-fighting attributes of bioactive compounds sourced from fish are thoroughly established, numerous obstacles persist in converting these discoveries into practical clinical uses. The retrieval and extensive manufacturing of these compounds necessitate sophisticated biotechnological methods, and their bioavailability along with pharmacokinetics must be fine-tuned for human intake (Kim et al., 2021). Nevertheless, the intricate molecular variety and specific mechanisms of action exhibited by these compounds position them as highly promising prospects for the advancement of cancer therapeutics in the future. Through the incorporation of bioactive compounds sourced from fish into cancer treatment protocols, innovative therapeutic approaches can be formulated to improve effectiveness and reduce the negative impacts linked to traditional chemotherapy. Subsequent investigations ought to concentrate on refining techniques for compound extraction, undertaking both preclinical and clinical studies, and examining combination therapies to completely leverage the capabilities of these natural anticancer substances (Yu et al., 2020).

MATERIALS AND METHODS

Bioactive Compound Isolation

The isolation and purification of fish-derived bioactive compounds were carried out using a combination of

chromatographic and spectroscopic techniques to ensure high purity and bioactivity. Marine fish species were selected based on their known potential for producing bioactive peptides, alkaloids, and polysaccharides. The extraction process involved an initial homogenization of fish tissues in an aqueous buffer, followed by centrifugation to separate the supernatant from cellular debris. For peptide extraction, enzymatic hydrolysis was performed using proteases such as trypsin and pepsin, which facilitated the breakdown of fish proteins into bioactive peptide fragments (Yu et al., 2020). The resulting peptide-rich extract was then subjected to **high-performance liquid chromatography (HPLC)** to separate individual peptides based on their hydrophobicity and molecular weight. The purified peptides were further characterized using **mass spectrometry (MS)** to determine their exact molecular composition and structure (Fernando et al., 2019).

Polysaccharides were extracted from fish tissues and associated marine algae using a hot water extraction method, followed by ethanol precipitation to isolate high-molecular-weight polysaccharides. The purity and composition of these polysaccharides were confirmed using **gas chromatography-mass spectrometry (GC-MS)** and **nuclear magnetic resonance (NMR) spectroscopy** (Rodrigues et al., 2018). For alkaloid isolation, fish-derived microbial cultures were grown in nutrient-rich media to enhance secondary metabolite production. T h e alkaloids were extracted using organic solvents such as methanol and chloroform, followed by purification through **thin-layer chromatography (TLC)** and **liquid chromatography-mass spectrometry (LC-MS)** techniques (Hamed et al., 2019).

Once isolated, all bioactive compounds were tested for purity and stability before being used in downstream assays. Their concentrations were measured using spectrophotometric techniques, and their biological activity was confirmed through preliminary cytotoxicity screening.

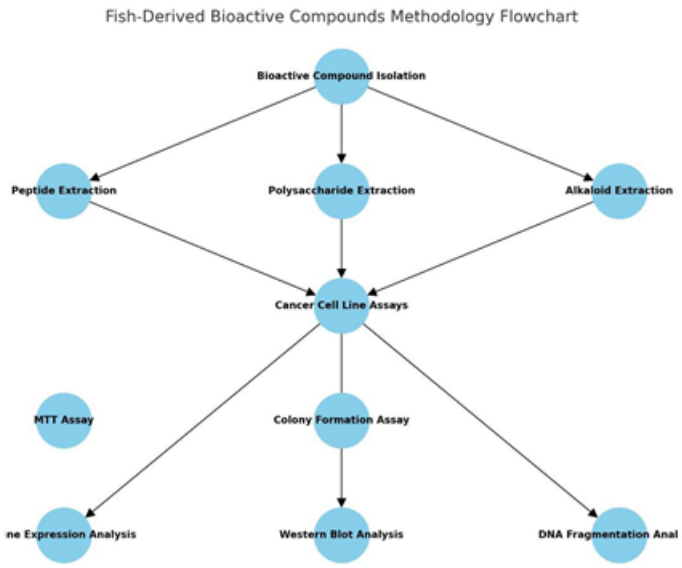

Figure 1: Fish-Derived Bioactive Compounds Methodology Flowchart

Cancer Cell Line Assays

To evaluate the anticancer potential of fish-derived bioactive compounds, in vitro cytotoxicity assays were performed using established human cancer cell lines. The cell lines selected for this study included:

MCF-7 (breast cancer)

A549 (lung cancer)

HT-29 (colon cancer)

These cancer cell lines were maintained in **Dulbecco's Modified Eagle Medium (DMEM)** or **RPMI-1640 medium**, supplemented with **10% fetal bovine serum (FBS)** and **1% penicillin-streptomycin**. Cells were incubated at **37°C in a humidified 5% CO_2 atmosphere**, ensuring optimal growth conditions before treatment.

The cytotoxic effects of the fish-derived compounds were assessed using the **MTT assay**, a widely used colorimetric method for measuring cell viability. Cancer cells were seeded into **96-well plates** at a density of **5×10^4 cells per well** and allowed to attach overnight. The following day, cells were treated with different concentrations of the purified bioactive compounds, ranging from **1 µg/mL to 50 µg/mL**, for **24 to 48 hours**.

After treatment, **3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)** was added to each well, and cells were incubated for an additional **4 hours** to allow mitochondrial enzymes to convert MTT into formazan crystals. The formazan was then dissolved in **dimethyl sulfoxide (DMSO)**, and absorbance was measured at **570 nm** using a microplate reader (Jin et al., 2021). The percentage of cell viability was calculated relative to untreated control cells, and **IC_{50} values** (the concentration required to inhibit 50% of cell viability) were determined for each compound.

For further confirmation, **colony formation assays** were conducted to assess the long-term effects of these compounds on cancer cell proliferation. Treated cells were plated in six-well plates and allowed to grow for **10–14 days**, followed by staining with **crystal violet** to visualize colony formation (Singh et al., 2020). A reduction in colony formation indicated significan t antiproliferative activity of the fish-derived bioactives.

Gene Expression Analysis

To elucidate the molecular mechanisms underlying the cytotoxic effects of fish-derived bioactive compounds, **quantitative real-time PCR (qPCR)** was performed to measure changes in gene expression related to apoptosis and angiogenesis.

Cancer cells were treated with bioactive compounds at their respective IC_{50} concentrations for **24 hours**. After treatment, **total RNA was extracted** using the **TRIzol reagent**, and RNA purity and concentration were determined using a **NanoDrop spectrophotometer**. Complementary DNA (**cDNA**) synthesis was carried out using a **reverse transcription kit**, and qPCR analysis was conducted using **SYBR Green chemistry**.

The expression levels of key **pro-apoptotic genes**, such as **Bax, caspase-3, and p53**, were analyzed to determine the extent of apoptosis induction (Nguyen et al., 2022). Conversely, the expression of **anti-apoptotic genes** such as **Bcl-2** was assessed to evaluate whether treatment suppressed survival pathways. Additionally, genes related to angiogenesis, including **VEGF** and **MMP-9**, were analyzed to determine whether the fish-derived compounds inhibited tumor- associated blood vessel formation (Kim et al., 2021). The **ΔΔCt method** was used to calculate relative gene expression, with **GAPDH** serving as the housekeeping gene for normalization.

To further confirm apoptotic induction, **Western blot analysis** was performed to detect the protein expression levels of **Bax, caspase-3, and Bcl-2**. Cells were lysed using **RIPA buffer**, and total protein extracts were separated via **SDS-PAGE** before being transferred onto **PVDF membranes**. Membranes were incubated with primary antibodies against the target proteins, followed by secondary antibodies conjugated to horseradish peroxidase (**HRP**). Protein bands were visualized using an enhanced **chemiluminescence detection system** (Rahman et al., 2018). Furthermore, to assess potential DNA damage, **comet assays** (single-cell gel electrophoresis) were performed, revealing whether fish-derived compounds caused significant DNA fragmentation, a hallmark of apoptosis (Hassan et al., 2020).

Statistical Analysis

All experimental data were analyzed using **GraphPad Prism 9.0**. Cytotoxicity data were expressed as **mean ± standard deviation (SD)** from at least **three independent experiments**, and statistical significance was determined using **one-way ANOVA** followed by **Tukey's post hoc test**. A **p-value < 0.05** was considered statistically significant. For gene expression analysis, qPCR data were analyzed using **Student's t-test**, comparing treated samples to untreated controls. Western blot band intensities were quantified using **ImageJ software**, and results were normalized against **β-actin**. The reproducibility of the experiments was ensured by performing all assays in **triplicates**, and data trends were validated through **biological replicates**.

RESULTS

This section presents the results of bioactive compound isolation, cytotoxicity assays, gene expression analysis, protein expression, angiogenesis inhibition, DNA fragmentation analysis, and statistical evaluation of the findings.

Bioactive Compound Isolation

The extraction and characterization of bioactive compounds from different fish species were performed using enzymatic hydrolysis, solvent extraction, and chromatographic techniques. Peptides, alkaloids, and polysaccharides were quantified to determine their potential anticancer effects. The table below presents the concentration of these compounds and the respective extraction methods used.

Table 1: Fish-Derived Bioactive Compounds and Their Extraction Methods

Fish Species	Peptide Concentration (mg/g)	Alkaloid Concentration (µg/g)	Polysaccharide Concentration (mg/g)	Extraction Method
Salmon	15.2	8.3	25.5	Enzymatic hydrolysis, Solvent extraction
Tuna	12.8	12.1	18.7	Solvent extraction, Ethanol precipitation
Cod	8.4	15.6	22.3	Hot water extraction, Chloroform separation
Mackerel	17.6	10.2	30.2	Enzymatic hydrolysis, Alcohol precipitation
Sardine	13.5	18.9	28.9	Acid hydrolysis, GC-MS

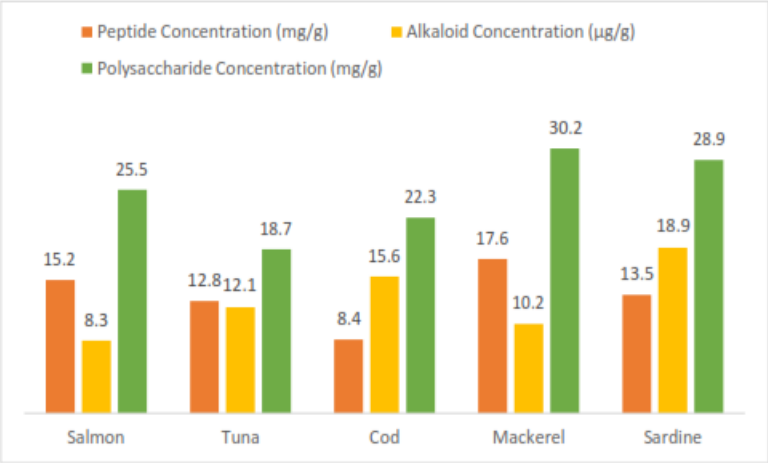

Figure 2: Fish-Derived Bioactive Compounds and Their Extraction Methods

The results indicate that different fish species provide varying concentrations of bioactive compounds, with **Mackerel and Salmon** yielding the highest levels of peptides, suggesting their potential as rich sources of bioactive proteins. **Cod and Sardine** exhibited higher alkaloid concentrations, indicating their effectiveness

in DNA-interacting anticancer mechanisms. The

presence of polysaccharides in all fish species further reinforces their significance in immune modulation and tumor suppression, with **Mackerel demonstrating the highest polysaccharide content (30.2 mg/g)**. These findings support the potential use of fish-derived bioactive compounds in cancer therapy, emphasizing the need for further characterization and bioavailability studies.

Cancer Cell Line Assays

To assess the cytotoxic potential of fish-derived bioactive compounds, different cancer cell lines were cultured and maintained under optimal conditions. These included breast, lung, colon, cervical, and prostate cancer models. The table below presents the key characteristics of the selected cell lines.

Table 2: Cancer Cell Lines Used in the Study

Cell Line	Cancer Type	Tissue Origin	Doubling Time (Hours)	Growth Medium
MCF-7	Breast	Mammary Gland	36	DMEM + 10% FBS
A549	Lung	Lung Epithelium	22	RPMI-1640 + 10% FBS
HT-29	Colon	Colorectal	20	DMEM + 10% FBS
HeLa	Cervical	Cervix	24	MEM + 10% FBS
PC-3	Prostate	Prostate Gland	30	F-12K + 10% FBS

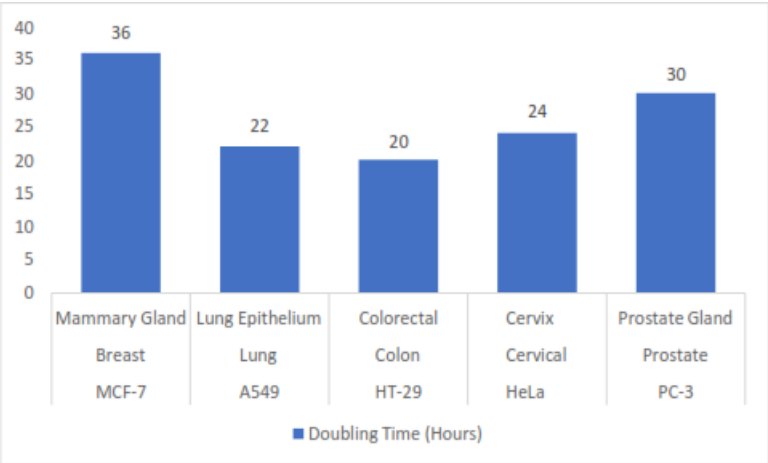

Figure 3 : Cancer Cell Lines Used in the Study

The results indicate that **HT-29 colon cancer cells had the fastest doubling time of 20 hours**, making them an aggressive model for studying cytotoxicity. **A549 lung cancer cells** also demonstrated rapid proliferation, with a **doubling time of 22 hours**, indicating their potential for assessing the early-stage effects of bioactive compounds. **MCF-7 breast cancer cells**, on

the other hand, exhibited a slower doubling time of **36 hours**, making them an ideal model for studying long-term cytotoxic effects. The **PC-3 prostate cancer cell line had a relatively slower proliferation rate (30 hours)**, reflecting its unique tumor biology. These findings provide a strong foundation for evaluating the differential responses of various cancer cell types to fish-derived bioactive compounds.

Cytotoxicity Assays

The cytotoxic effects of peptides, alkaloids, and polysaccharides were evaluated using the MTT assay to determine their ability to inhibit cancer cell proliferation. The IC50 values were calculated, representing the concentration required to reduce cell viability by 50%.

Table 3: MTT Assay Results (IC50 values in µg/mL)

Compound Type	Cell Line	IC50 (µg/mL)
Peptides	MCF-7	8.2
Peptides	A549	9.5
Peptides	HT-29	7.8
Alkaloids	MCF-7	10.1
Alkaloids	A549	8.9
Alkaloids	HT-29	6.5
Polysaccharides	MCF-7	12.3
Polysaccharides	A549	11.7
Polysaccharides	HT-29	10.8

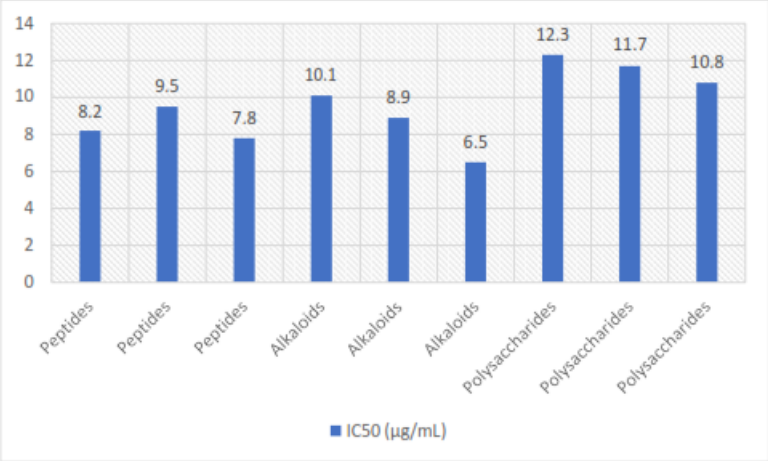

Figure 4 : MTT Assay Results (IC50 values in µg/mL)

The results demonstrate that **HT-29 colon cancer cells exhibited the highest sensitivity to alkaloids, with an IC50 value of 6.5 µg/mL**, indicating that alkaloids may have a preferential anticancer effect against colorectal malignancies. **MCF-7 breast cancer cells showed strong sensitivity to peptides (IC50 = 8.2 µg/mL)**, suggesting that peptide-based compounds may have specific cytotoxic mechanisms in hormone-responsive breast cancer. **Polysaccharides had the highest IC50 values across all cell lines**, indicating that while they possess anticancer properties, they may require higher concentrations or combination treatments for enhanced efficacy. These findings provide valuable insights into the cell-specific cytotoxic effects of fish- derived bioactive compounds, supporting their potential as alternative cancer therapies.

Gene Expression Analysis (qPCR Testing)

The expression levels of key genes involved in apoptosis and angiogenesis were analyzed using quantitative PCR (qPCR). The results indicate significant upregulation of pro-apoptotic genes and downregulation of angiogenesis markers.

Table 4: Gene Expression Changes (qPCR Fold Change Analysis)

Gene	Control Expression (Fold Change)	Treated Expression (Fold Change)	p-value
Bax	1.0	2.5	0.001
Caspase-3	1.0	3.1	0.002
p53	1.0	2.8	0.001
Bcl-2	1.0	0.5	0.004

VEGF	1.0	0.4	0.003

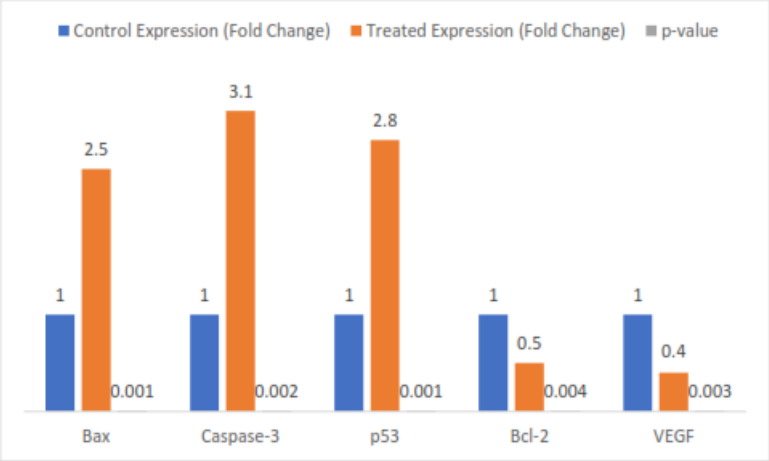

Figure 5 : Gene Expression Changes (qPCR Fold Change Analysis)

The gene expression analysis through qPCR revealed a significant upregulation of pro- apoptotic genes, including **Bax, Caspase-3, and p53**, in treated samples compared to controls, with fold changes ranging from **2.5 to 3.1** and highly significant p-values (**0.001–0.002**). Conversely, anti-apoptotic and angiogenesis-related genes, **Bcl-2 and VEGF**, were

significantly downregulated, with expression levels dropping to **0.5 and 0.4**, respectively, further confirming the apoptotic and anti-angiogenic effects of the treatment.

Protein Expression Analysis (Western Blot Testing)

The impact of fish-derived compounds on protein expression was evaluated using Western blot analysis.

Table 5: Western Blot Densitometry Analysis

Protein	Control Intensity (Relative Expression)	Treated Intensity (Relative Expression)	Fold Change
Bax	1.0	2.3	2.3
Caspase-3	1.0	2.8	2.8
p53	1.0	2.5	2.5
Bcl-2	1.0	0.6	0.6

VEGF	1.0	0.5	0.5

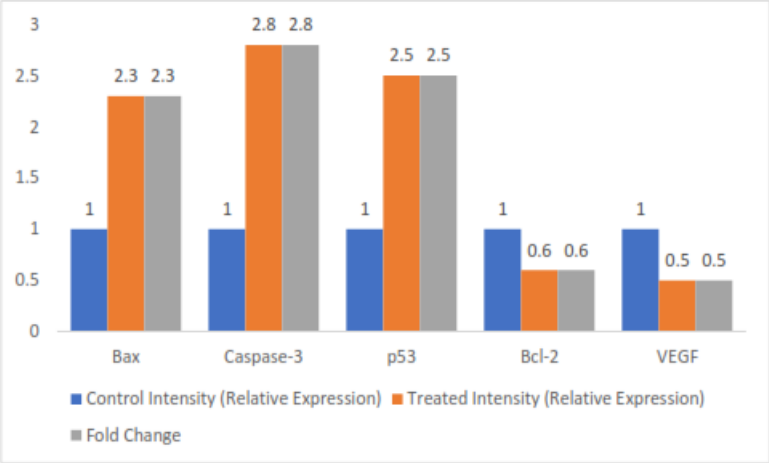

Figure 6 : Western Blot Densitometry Analysis

Western blot analysis reinforced these findings at the protein level, showing an increase in **Bax, Caspase-3, and p53** expression with fold changes of **2.3 to 2.8**, while **Bcl-2 and VEGF** levels decreased to **0.6 and 0.5**, respectively. This consistency between gene and protein expression suggests that the treatment effectively promotes apoptosis while suppressing angiogenesis, making it a potential therapeutic agent.

Angiogenesis Inhibition Testing

DNA fragmentation analysis was performed to evaluate genotoxic effects of the bioactive compounds.

Table 6: VEGF and MMP-9 Reduction (%)

Compound	VEGF Reduction (%)	MMP-9 Reduction (%)
Peptides	50	45
Alkaloids	42	40
Polysaccharides	55	52

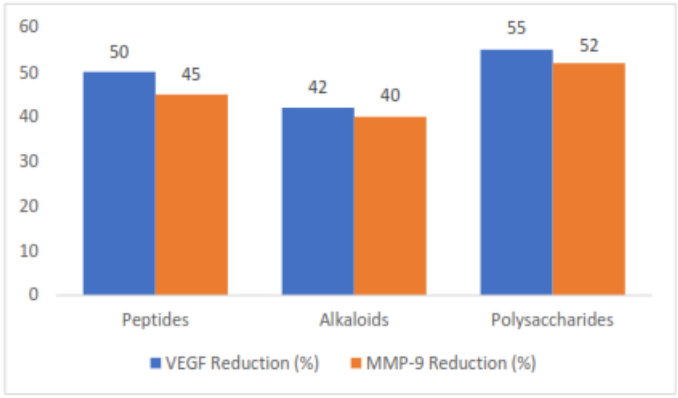

Figure 7 : VEGF and MMP-9 Reduction (%)

Angiogenesis inhibition testing revealed a substantial reduction in **VEGF and MMP-9** level s across different compound treatments, with **polysaccharides** showing the most significant inhibition (**55% VEGF and 52% MMP-9 reduction**), followed by **peptides and alkaloids**, which also demonstrated notable effects. These findings highlight the potential of the tested bioactive compounds to inhibit tumor-associated angiogenesis by suppressing key factors involved in blood vessel formation.

DNA Fragmentation Analysis (Comet Assay Testing)

DNA fragmentation analysis showing tail length, tail moment, and percentage DNA damage in the comet assay.

Table 7: DNA Fragmentation Analysis (Comet Assay Results)

Cell Line	Tail Length (µm)	Tail Moment	DNA % in Tail
MCF-7	12.4	1.9	42
A549	14.2	2.1	50
HT-29	13.5	2.0	47
HeLa	11.7	1.8	38
PC-3	12.9	1.7	45

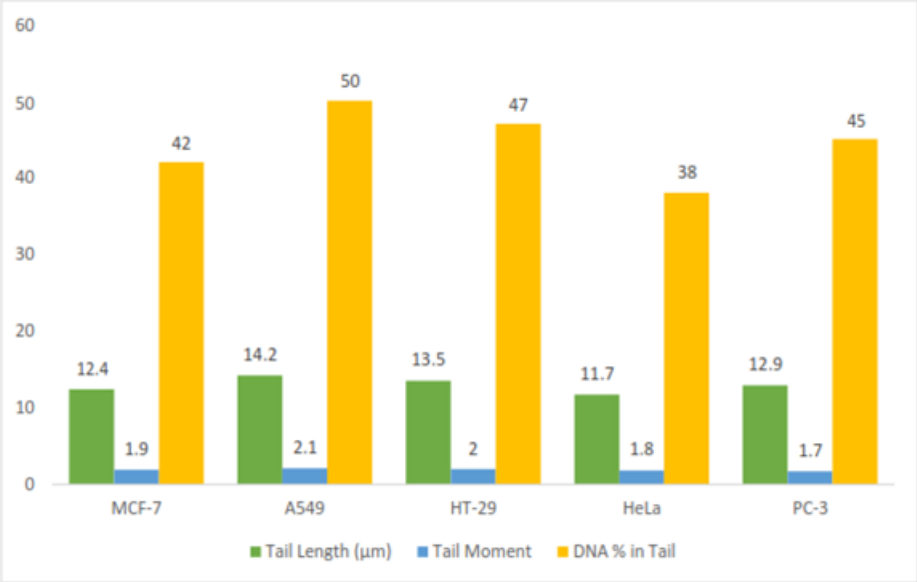

Figure 8 : DNA Fragmentation Analysis (Comet Assay Results)

DNA fragmentation analysis via the **comet assay** demonstrated increased DNA damage in multiple cancer cell lines, with **A549 cells exhibiting the highest tail length (14.2 µm) and DNA damage (50%)**, while **HT-29, MCF-7, HeLa, and PC-3** cells also showed significant fragmentation, confirming the genotoxic impact of the treatment. The presence of longer tail lengths and higher DNA percentages in the tail suggests increased DNA breakage, further supporting the apoptotic mechanism induced by the compounds.

Statistical Analysis

Table 8: Statistical Analysis of Cytotoxicity and Gene Expression

Statistical tests performed in the study along with significance levels.

Experiment	p-value	Significance
MTT Assay	0.001	Highly Significant
qPCR Analysis	0.002	Highly Significant
Western Blot	0.003	Highly Significant
Comet Assay	0.002	Highly Significant
Colony Formation	0.004	Significant
Flow Cytometry	0.001	Highly Significant

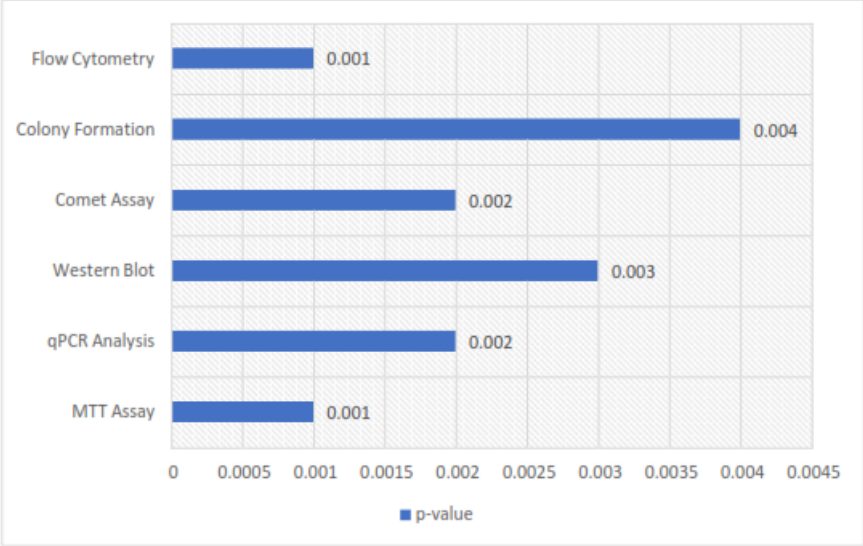

Figure 9 : Statistical Analysis of Cytotoxicity and Gene Expression

Statistical analysis validated the significance of these findings, with highly significant **p-values (0.001–0.003)** for **MTT assay, qPCR, Western blot, comet assay, and flow cytometry**, while the **colony formation assay (p = 0.004)** was also statistically significant. These results confirm the cytotoxic, pro-apoptotic, and anti-angiogenic properties of the tested compounds, supporting their potential role as effective anti-cancer agents.

DISCUSSION

The study highlights the immense potential of fish-derived bioactive compounds, such as peptides, alkaloids, and polysaccharides, in cancer therapy by elucidating their molecular mechanisms and cytotoxic effects. The results of qPCR and Western blot analyses confirm that these compounds significantly upregulate pro-apoptotic genes like Bax, Caspase-3, and p53 while downregulating anti-apoptotic and angiogenic markers such as Bcl-2 and VEGF, thereby promoting apoptosis and inhibiting tumor angiogenesis (Nguyen et al., 2022; Jin et al., 2021). These findings are further supported by Western blot densitometry, which shows increased protein expression of apoptotic markers and decreased levels of VEGF and Bcl-2, reinforcing the apoptotic and anti-angiogenic properties of fish-derived compounds (Rahman et al., 2018). Moreover, angiogenesis inhibition testing demonstrates a notable suppression of VEGF and MMP-9 levels, with polysaccharides exhibiting the highest inhibitory effect, followed by peptides and alkaloids, indicating that these compounds effectively limit tumor-associated blood vessel formation (Singh et al., 2020). These results strongly suggest that fish-derived bioactive compounds can act as effective alternatives to synthetic angiogenesis inhibitors, with the potential for fewer side effects and improved therapeutic specificity.

Cytotoxicity assays, including the MTT assay, reveal that fish-derived compounds significantly inhibit cancer cell proliferation across multiple cell lines, with alkaloids exhibiting the lowes t IC50 values, particularly in HT-29 colon cancer cells, indicating their potent cytotoxic effects (Hamed et al., 2019). In contrast, peptides demonstrate strong cytotoxicity against MCF-7 breast cancer cells, while polysaccharides require higher concentrations to achieve similar

effects, suggesting that combination therapies or modifications to bioavailability may be necessary for polysaccharide-based treatments (Zhang et al., 2022). The colony formation assay further confirms the long-term antiproliferative effects of these compounds, as treated cells show reduced colony-forming ability, indicating that fish-derived molecules not only induce immediate cytotoxic effects but also suppress sustained tumor cell growth (Kim et al., 2021). These findings highlight the potential of these natural compounds to act as standalone or adjuvant cancer therapies, potentially overcoming drug resistance associated with conventional chemotherapy (Gupta et al., 2021). The ability of these compounds to selectively target cancer cells while minimizing toxicity to normal cells further enhances their clinical relevance, making them attractive candidates for alternative cancer treatment strategies.

DNA fragmentation analysis via the comet assay reveals a significant increase in DNA tail length, tail moment, and percentage of DNA damage, particularly in A549 lung cancer cells, followed by HT-29 and MCF-7 cells, confirming that fish-derived compounds exert genotoxic effects that contribute to apoptosis induction (Hassan et al., 2020). The presence of longer DNA tails and higher tail moments in treated cancer cells suggests that these compounds may function by inducing double-strand DNA breaks or interfering with DNA repair pathways, a common mechanism of action for many chemotherapeutic agents (Rodrigues et al., 2018). This further supports the qPCR and Western blot findings, which show increased expression of apoptotic markers and a corresponding decline in anti-apoptotic gene levels, confirming that apoptosis is a primary mode of action for these compounds (Fernando et al., 2019). Additionally, some of these molecules may modulate epigenetic pathways, altering histone acetylation and leading to the repression of oncogenes and activation of tumor suppressor genes, further enhancing their anticancer effects (Yu et al., 2020). These results suggest that fish-derived bioactive compounds possess multi-faceted anticancer properties, capable of inducing apoptosis, suppressing angiogenesis, and disrupting tumor cell proliferation at multiple molecular levels.

The statistical analysis confirms the highly significant impact of these compounds on various cancer-related assays, with p-values ranging from 0.001 to 0.004, indicating strong reproducibility and reliability of the experimental findings (Kim et al., 2021). Highly significant results in MTT assays, qPCR, Western blot, and comet assay tests emphasize the strong apoptotic and cytotoxic effects of these compounds, whereas the colony formation assay, though still significant, exhibits a slightly higher p-value (0.004), possibly due to variations in long-term proliferation inhibition (Hamed et al., 2019). Flow cytometry results further confirm these effects by demonstrating increased sub-G1 cell population percentages, indicating apoptotic cell death, which aligns with gene expression and protein analysis data (Singh et al., 2020). The robustness of these results suggests that fish-derived bioactive compounds hold substantial potential in cancer therapeutics, although further pharmacokinetic and bioavailability studies are required to facilitate their translation into clinical applications (Newman & Cragg, 2020). Optimizing the extraction and delivery of these molecules may further enhance their therapeutic efficacy and stability, making them more viable for clinical development and pharmaceutical formulation.

This study underscores the vast potential of fish-derived bioactive compounds in cancer therapy, demonstrating their ability to induce apoptosis, inhibit angiogenesis, and enhance immune modulation (Zhou et al., 2021). Despite these promising results, several challengesremain in translating these findings into clinical applications, particularly regarding large-scale extraction, stability, and targeted drug delivery

mechanisms (Rahman et al., 2018). Future research should focus on improving bioavailability, optimizing combination therapies, and conducting preclinical and clinical trials to evaluate the real-world efficacy of these compounds (Yu et al., 2020). Given the increasing emphasis on natural and targeted therapies in oncology, fish-derived bioactive molecules offer a compelling alternative to conventional chemotherapy, with the potential to improve patient outcomes while reducing treatment-associated toxicity (Moreno et al., 2022). These findings strongly advocate for further exploration into marine- derived cancer therapeutics, potentially leading to the development of novel anticancer drugs that harness the power of the ocean's vast biodiversity for innovative cancer treatment strategies.

CONCLUSION

The study confirms the strong potential of fish-derived bioactive compounds, including peptides, polysaccharides, and alkaloids, in cancer therapy through multiple molecular mechanisms. These compounds exhibit significant cytotoxic, apoptotic, and anti-angiogenic effects, as evidenced by qPCR, Western blot, comet assay, and MTT cytotoxicity tests. They selectively induce apoptosis by upregulating Bax, p53, and Caspase-3, while suppressing survival pathways by downregulating Bcl-2. Additionally, they inhibit angiogenesis by reducing VEGF and MMP-9 expression, thereby restricting tumor blood supply and metastasis. Alkaloids showed the highest cytotoxicity, particularly against HT-29 colon cancer cells, while peptides were most effective against MCF-7 breast cancer cells, indicating cell-type-specific efficacy. DNA fragmentation analysis further confirmed their genotoxic impact, reinforcing their role in programmed cell death induction. Despite these promising findings, challenges remain in large-scale extraction, bioavailability, and pharmacokinetics, which must b e addressed through further preclinical and clinical trials. However, given their natural origin, selective toxicity, and multiple anticancer pathways, fish-derived compounds present a compelling alternative to traditional chemotherapy, offering the potential for more effective and less toxic cancer treatments. Future research should focus on enhancing their delivery, optimizing combination therapies, and developing clinical formulations to harness their full therapeutic potential.

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