International Journal for Modern Trends in Science and Technology
Volume 11, Issue 02, pages 93-104.
ISSN: 2455-3778 online
Available online at: http://www.ijmtst.com/vol11issue02.html
DOI: https://doi.org/10.46501/ijmtst.v11.i02.pp93-104

Millet-Derived Bioactive Peptides in Cancer Prevention and Therapy: Isolation, Molecular Mechanisms, and Future Therapeutic Perspectives

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To Cite this Article
J. Ajani and Shaik Parveen Banu, "Millet-Derived Bioactive Peptides in Cancer Prevention and Therapy: Isolation, Molecular Mechanisms, and Future Therapeutic Perspectives", International Journal for Modern Trends in Science and Technology, 2025, 11(02), pages. 93-104. https://doi.org/10.46501/ijmtst.v11.i02.pp93-104

Article Info
Received: 12 January 2025; Accepted: 03 February 2025; Published: 14 February 2025.

ABSTRACT

Cancer remains one of the foremost causes of morbidity and mortality worldwide despite substantial progress in diagnosis and treatment. Conventional therapeutic approaches, including chemotherapy and radiotherapy, are frequently associated with severe adverse effects, multidrug resistance, and recurrence, highlighting the necessity for safer and more effective therapeutic alternatives. Recently, food-derived bioactive peptides have attracted increasing attention because of their diverse biological functions, including antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, antihypertensive, and anticancer activities [14,15,18]. Among cereal crops, millets represent an important group of climate-resilient grains rich in proteins, essential amino acids, phenolic compounds, dietary fibre, minerals, and numerous bioactive constituents. Enzymatic hydrolysis, gastrointestinal digestion, fermentation, and food processing can release biologically active peptides from millet proteins that exhibit significant physiological activities [1,6,7].

Emerging evidence suggests that millet-derived bioactive peptides exert anticancer effects through multiple biochemical mechanisms, including induction of apoptosis, arrest of cell-cycle progression, modulation of oxidative stress, inhibition of angiogenesis and metastasis, regulation of inflammatory signalling, and interference with major oncogenic pathways such as PI3K/Akt, MAPK, NF-κB, and p53 signalling [11,12,13,20]. Furthermore, these peptides possess favourable characteristics such as low toxicity, high specificity, biocompatibility, and potential synergistic interactions with conventional chemotherapeutic agents.

This review comprehensively summarizes the current knowledge regarding millet proteins as precursors of bioactive peptides, methods employed for peptide production and characterization, molecular mechanisms underlying their anticancer properties, experimental evidence obtained from in vitro and in vivo investigations, and the challenges associated with clinical translation. In addition, future research priorities, including peptide purification, structural optimization, nanoformulation, pharmacokinetic evaluation, and clinical validation, are discussed. By integrating

current biochemical knowledge, this review provides valuable insights into the therapeutic potential of millet-derived bioactive peptides as promising candidates for future cancer prevention and treatment strategies.

KEYWORDS: Millets; Bioactive peptides; Cancer; Functional foods; Apoptosis; Oxidative stress; Nutraceuticals; Food biochemistry; Molecular mechanisms.

1. Introduction

Cancer constitutes a major global public health challenge and continues to represent one of the leading causes of mortality across developed and developing nations. According to recent global estimates, millions of new cancer cases are diagnosed annually, while the incidence is projected to increase substantially because of ageing populations, urbanization, environmental pollution, sedentary lifestyles, unhealthy dietary habits, and genetic susceptibility [2]. Although remarkable advances have been achieved in surgery, chemotherapy, radiotherapy, immunotherapy, and targeted molecular therapy, many patients continue to experience severe treatment-related toxicities, tumour recurrence, drug resistance, and poor long-term prognosis [2]. Consequently, the identification of naturally occurring bioactive compounds capable of preventing cancer initiation or enhancing therapeutic efficacy has become an important area of biochemical and nutritional research.

Diet plays a pivotal role in modulating cancer risk by influencing oxidative stress, chronic inflammation, immune responses, cellular metabolism, and genomic stability. Increasing epidemiological evidence indicates that regular consumption of whole grains, fruits, vegetables, legumes, and other plant-based foods is associated with a reduced incidence of several types of cancer [1,2,5]. Among these functional foods, millets have gained renewed scientific interest because of their exceptional nutritional quality, resilience to climate change, and abundance of biologically active phytochemicals. Traditionally cultivated in Asia and Africa, millets are now recognized globally as sustainable cereal crops possessing substantial health-promoting potential [1,4].

Millets comprise several small-seeded cereal species, including pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), barnyard millet (*Echinochloa frumentacea*), little millet (*Panicum sumatrense*), kodo millet (*Paspalum scrobiculatum*), proso millet (*Panicum miliaceum*), and others. Their grains contain appreciable quantities of proteins, essential amino acids, complex carbohydrates, dietary fibre, minerals, vitamins, and numerous phytochemicals such as phenolic acids, flavonoids, tannins, lignans, phytosterols, and carotenoids, all of which contribute to their functional properties [1,4].

In recent years, proteins present in millets have emerged as valuable precursors of bioactive peptides. These peptides are inactive within the native protein sequence but become biologically active following enzymatic hydrolysis, gastrointestinal digestion, microbial fermentation, or food processing [14,15,16]. Depending on their amino acid composition and structural characteristics, bioactive peptides can exhibit diverse physiological activities including antioxidant, antihypertensive, antimicrobial, antidiabetic, anti-inflammatory, immunomodulatory, and anticancer effects [15,16,18].

Among these biological properties, the anticancer potential of millet-derived peptides has attracted increasing attention because of their ability to regulate multiple cellular pathways simultaneously. Experimental investigations suggest that these peptides suppress tumour cell proliferation by promoting apoptosis, inducing cell-cycle arrest, reducing oxidative stress, modulating inflammatory mediators, inhibiting angiogenesis, preventing metastasis, and influencing critical intracellular signalling pathways involved in cancer progression [11,12,13,20,21,22]. Their relatively low toxicity toward normal cells further enhances their attractiveness as potential nutraceuticals and adjunct therapeutic agents.

Despite the growing number of studies investigating millet bioactive compounds, most published reviews have focused broadly on millet nutrition, phytochemicals, or general health benefits. Comparatively little attention has been devoted specifically to millet-derived bioactive peptides and the biochemical mechanisms responsible for their anticancer activity. Therefore, the present review aims to critically evaluate the available evidence regarding the production, characterization, biological functions, molecular targets, and therapeutic prospects of millet-derived bioactive peptides, while highlighting current

knowledge gaps and future research directions that may facilitate their translation into clinical applications.

2. Millet Proteins as Precursors of Bioactive Peptides

Millets are increasingly recognized not only for their nutritional value but also as a rich source of precursor proteins capable of generating biologically active peptides. Unlike conventional cereal grains, millet proteins possess a balanced amino acid composition and contain numerous encrypted peptide sequences that become biologically active following enzymatic hydrolysis, microbial fermentation, gastrointestinal digestion, or controlled food processing [6,7,8]. These bioactive peptides have emerged as promising functional ingredients owing to their antioxidant, antihypertensive, antimicrobial, immunomodulatory, anti-inflammatory and anticancer properties.

The protein content of millet grains generally ranges from 7–15%, depending on the species, genotype, environmental conditions and agronomic practices [1]. Prolamins and glutelins constitute the major storage proteins, while albumins and globulins are present in comparatively lower quantities. The relative abundance of these protein fractions differs among millet species, thereby influencing the type and quantity of peptides released after hydrolysis [1,4]. In comparison with several major cereals, millet proteins contain relatively higher concentrations of hydrophobic amino acids, branched-chain amino acids and sulphur-containing amino acids, which contribute significantly to the biological activity of released peptides [6,7].

Hydrophobic amino acids including leucine, isoleucine, valine, phenylalanine and proline are particularly important because they enhance interactions between peptides and biological membranes, thereby improving cellular uptake and biological efficacy [17]. Furthermore, positively charged amino acids such as lysine and arginine facilitate electrostatic interactions with negatively charged cancer cell membranes, potentially contributing to selective cytotoxicity against malignant cells [21]. Aromatic amino acids including tyrosine, phenylalanine and tryptophan also participate in radical scavenging reactions through hydrogen atom donation, thereby enhancing antioxidant capacity and protecting cellular macromolecules from oxidative damage [17].

Unlike phytochemicals that are present as free compounds within plant tissues, bioactive peptides remain inactive while embedded within the parent protein sequence. Their biological activity becomes evident only after specific peptide fragments are liberated through enzymatic cleavage [15]. Gastrointestinal digestion by pepsin, trypsin and chymotrypsin represents one natural mechanism for peptide release. Alternatively, food-grade proteolytic enzymes such as alcalase, flavourzyme, papain, bromelain, neutrase and thermolysin are widely employed to produce peptide-rich hydrolysates under controlled laboratory conditions [7,8]. Fermentation using lactic acid bacteria and fungal cultures has also been demonstrated to enhance peptide release through microbial protease activity while simultaneously improving digestibility and nutritional quality [15].

Recent proteomic investigations indicate that peptide characteristics including molecular weight, amino acid sequence, net charge, hydrophobicity and secondary structure largely determine biological function [17]. Most biologically active food-derived peptides possess molecular weights below 3 kDa, facilitating intestinal absorption and interaction with intracellular molecular targets [7,8]. Low molecular weight peptides have also demonstrated greater stability during digestion and improved bioavailability, making them attractive candidates for nutraceutical development.

Among individual millet species, finger millet (*Eleusine coracana*) has received considerable attention because of its comparatively high protein quality and abundance of sulphur-containing amino acids [8]. Hydrolysed finger millet proteins have demonstrated significant antioxidant activity and have shown potential to inhibit proliferation of selected human cancer cell lines through modulation of intracellular oxidative stress [8]. Similarly, foxtail millet (*Setaria italica*) proteins release peptides exhibiting antioxidant and anti-inflammatory activities that may contribute indirectly to cancer prevention by reducing chronic inflammation and oxidative DNA damage [9].

Pearl millet (*Pennisetum glaucum*) represents another important source of functional peptides owing to its relatively high protein content and favourable amino acid composition [7]. Enzymatically hydrolysed pearl millet proteins have demonstrated free radical scavenging activity, metal-chelating capacity and

inhibition of lipid peroxidation, suggesting possible roles in reducing oxidative stress-associated carcinogenesis [7]. Likewise, proteins isolated from barnyard millet (*Echinochloa frumentacea*), little millet (*Panicum sumatrense*), proso millet (*Panicum miliaceum*) and kodo millet (*Paspalum scrobiculatum*) have shown the potential to generate multifunctional peptides with antioxidant and cytoprotective properties, although investigations specifically evaluating their anticancer activity remain relatively limited [4,5].

In addition to their direct biological activities, millet-derived peptides may enhance the bioavailability of other phytochemicals naturally present within millet grains. Synergistic interactions between peptides, phenolic compounds, flavonoids and dietary fibre have been proposed to amplify antioxidant defence systems and modulate signalling pathways involved in carcinogenesis [4]. Such synergistic effects may provide greater therapeutic benefit than isolated compounds alone, supporting the concept of whole-food-based functional ingredients for cancer prevention.

The biological activities of millet peptides are strongly influenced by processing conditions employed during hydrolysis. Factors including enzyme specificity, enzyme-to-substrate ratio, temperature, pH, hydrolysis time and purification strategy determine peptide composition and functional efficacy [15]. Advances in ultrafiltration, membrane separation, high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS/MS) and peptide sequencing have facilitated the identification of novel peptide sequences possessing potent biological activities [15]. These analytical techniques continue to accelerate the discovery of functional peptides with potential pharmaceutical applications.

Although research on millet-derived bioactive peptides has expanded considerably during the past decade, significant knowledge gaps remain. Only a limited number of peptide sequences have been structurally characterised, and very few studies have investigated their precise molecular targets in cancer cells. Furthermore, most available evidence has been generated using in vitro experimental models, while animal studies and clinical investigations remain scarce [6]. Comprehensive biochemical characterization, pharmacokinetic evaluation and mechanistic studies are therefore required before these peptides can be translated into clinically useful nutraceuticals or therapeutic agents.

3. Generation, Isolation and Characterization of Millet-Derived Bioactive Peptides

Bioactive peptides are encrypted within native millet proteins and become biologically active only after their release through proteolytic cleavage. Several strategies have been employed to generate these peptides, including enzymatic hydrolysis, microbial fermentation, simulated gastrointestinal digestion and food-processing techniques. Among these, enzymatic hydrolysis is considered the most efficient and reproducible method because it allows controlled production of peptides with defined molecular characteristics and biological activities [15,16].

3.1 Enzymatic Hydrolysis

Enzymatic hydrolysis remains the preferred approach for producing bioactive peptides from millet proteins. Food-grade proteases such as alcalase, flavourzyme, papain, bromelain, pepsin, trypsin, chymotrypsin and thermolysin selectively cleave peptide bonds to release short peptide fragments with enhanced biological activity [15,16]. The yield and functionality of the resulting peptides are influenced by enzyme specificity, enzyme-to-substrate ratio, pH, temperature, hydrolysis time and protein source.

Controlled hydrolysis offers several advantages over chemical methods, including greater specificity, preservation of amino acid integrity, lower production of undesirable by-products and improved reproducibility. Enzymatic treatment also enables the generation of peptides with molecular weights generally below 3 kDa, which exhibit superior intestinal absorption and biological efficacy [7].

3.2 Microbial Fermentation

Fermentation represents another important strategy for releasing bioactive peptides from millet proteins. During fermentation, proteolytic microorganisms, particularly lactic acid bacteria (*Lactobacillus* spp.) and selected fungal cultures, hydrolyse storage proteins into smaller peptides through endogenous protease activity [15].

Besides peptide generation, fermentation improves protein digestibility, reduces antinutritional factors such as phytates and tannins, enhances mineral bioavailability

and may increase the concentration of antioxidant compounds. These combined effects improve the nutritional and functional quality of millet-based foods and may contribute indirectly to cancer prevention [4].

3.3 Simulated Gastrointestinal Digestion

Human gastrointestinal digestion naturally generates numerous bioactive peptides. Sequential digestion using pepsin under gastric conditions followed by pancreatic enzymes such as trypsin and chymotrypsin is commonly used to simulate this process in vitro [15].

Simulated digestion provides valuable information regarding peptide stability, release patterns and potential bioavailability after oral consumption. Peptides that remain stable during gastrointestinal digestion possess greater potential for development as functional food ingredients or nutraceuticals [15].

3.4 Peptide Purification

Following hydrolysis, crude peptide mixtures require purification before biological evaluation. Ultrafiltration membranes are widely employed as an initial separation step because they rapidly fractionate peptides according to molecular weight [7,8]. Fractions below 3 kDa generally demonstrate stronger antioxidant and anticancer activities than larger peptides.

Subsequent purification is commonly achieved using reverse-phase high-performance liquid chromatography (RP-HPLC), ion-exchange chromatography or gel filtration chromatography. These techniques permit isolation of highly purified peptide fractions suitable for structural and functional analysis [7,8].

3.5 Peptide Identification and Structural Characterization

Modern analytical techniques have greatly accelerated the discovery of food-derived bioactive peptides. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is considered the gold standard for peptide identification because it provides accurate molecular mass determination and amino acid sequence information [15]. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray ionisation mass spectrometry (ESI-MS) are also extensively used for peptide profiling [15].

Following sequence identification, bioinformatics tools facilitate prediction of biological activity, toxicity, allergenicity and gastrointestinal stability. Molecular docking further enables the evaluation of peptide interactions with cancer-related proteins, thereby supporting the identification of promising therapeutic candidates before experimental validation [6].

3.6 Factors Influencing Peptide Bioactivity

The biological activity of millet-derived peptides is determined by multiple structural characteristics. Short peptides containing 2–20 amino acid residues generally exhibit greater bioavailability than larger protein fragments [15]. Hydrophobic amino acids enhance membrane permeability, whereas positively charged residues promote electrostatic interactions with negatively charged cancer cell membranes. Aromatic amino acids contribute to antioxidant activity through hydrogen donation and free radical scavenging [17].

Furthermore, peptide sequence, molecular conformation, net charge, amphipathicity and resistance to digestive enzymes collectively influence biological efficacy. Even minor alterations in amino acid composition may significantly modify anticancer activity, highlighting the importance of precise structural characterisation [6].

Table 1. Major Techniques Used for the Production and Characterisation of Millet-Derived Bioactive Peptides

Technique	Principle	Major advantage	Limitation
Enzymatic hydrolysis	Protease-mediated protein cleavage	High specificity and reproducibility	Enzyme cost
Microbial fermentation	Microbial proteases release peptides	Improves digestibility and nutritional quality	Variable peptide composition
Simulated gastrointestinal digestion	Mimics human digestive enzymes	Predicts physiological peptide release	In vitro conditions may not fully reflect human digestion
Ultrafiltration	Separation based on molecular weight	Rapid enrichment of low-molecular-	Limited purification

Technique	Principle	Major advantage	Limitation
		weight peptides	
RP-HPLC	Separation based on hydrophobicity	High peptide purity	Time-consuming
LC-MS/MS	Mass-based peptide identification	Accurate sequence determination	Expensive instrumentation
MALDI-TOF MS	Mass spectrometric peptide profiling	Rapid analysis	Limited quantitative information

4. Molecular Mechanisms Underlying the Anticancer Activity of Millet-Derived Bioactive Peptides

Cancer development is a multistep process involving dysregulation of cell proliferation, apoptosis, angiogenesis, inflammation and metastasis. Increasing evidence suggests that food-derived bioactive peptides exert anticancer effects by simultaneously targeting multiple molecular pathways rather than acting through a single mechanism [12,13]. Although studies specifically investigating millet-derived peptides remain limited, available evidence indicates that their biological actions resemble those reported for bioactive peptides isolated from other cereal proteins. These peptides interfere with tumour progression by modulating oxidative stress, inducing programmed cell death, suppressing inflammatory signalling and regulating cell survival pathways.

4.1 Induction of Apoptosis

Apoptosis is a tightly regulated process that eliminates damaged or transformed cells without provoking inflammation. Defective apoptotic signalling is considered one of the hallmarks of cancer and contributes to uncontrolled tumour growth [13].

Emerging evidence indicates that millet-derived peptides promote apoptosis through activation of the intrinsic mitochondrial pathway. These peptides alter mitochondrial membrane permeability, leading to the release of cytochrome *c* into the cytoplasm and subsequent activation of caspase-9 and caspase-3. The executioner caspases ultimately degrade cellular proteins and DNA, resulting in controlled tumour cell death [20,22].

Several peptide fractions also regulate members of the Bcl-2 protein family by increasing expression of the pro-apoptotic protein Bax while suppressing the anti-apoptotic protein Bcl-2. An increased Bax/Bcl-2 ratio destabilises mitochondrial membranes and enhances apoptotic signalling [22].

4.2 Cell-Cycle Arrest

Normal cell proliferation is governed by cyclins, cyclin-dependent kinases (CDKs) and tumour suppressor proteins. Cancer cells frequently escape these regulatory mechanisms, allowing continuous proliferation [13].

Experimental studies suggest that bioactive peptides can inhibit tumour growth by arresting the cell cycle at the G_0/G_1 or G_2/M checkpoint. This arrest is associated with reduced expression of cyclin D1, cyclin E, CDK2 and CDK4 together with increased expression of CDK inhibitors such as p21 and p27 [12]. By preventing progression through the cell cycle, damaged cells are directed towards apoptosis rather than uncontrolled division.

4.3 Modulation of Oxidative Stress

Reactive oxygen species (ROS) play a dual role in cancer biology. Moderate ROS concentrations stimulate tumour growth, whereas excessive ROS accumulation causes oxidative damage leading to apoptosis [13].

Millet-derived peptides possess antioxidant activity owing to the presence of hydrophobic and aromatic amino acid residues capable of scavenging free radicals. These peptides reduce lipid peroxidation, protect DNA from oxidative injury and preserve cellular proteins against oxidative modification [7,8].

Interestingly, in cancer cells certain peptides may selectively increase intracellular ROS beyond the tolerable threshold, thereby promoting mitochondrial dysfunction and apoptotic cell death. This selective modulation of oxidative stress represents an attractive strategy for cancer therapy because normal cells possess more efficient antioxidant defence systems [20].

4.4 Suppression of Chronic Inflammation

Persistent inflammation contributes significantly to tumour initiation and progression by stimulating cell proliferation, angiogenesis and genomic instability [13].

Several food-derived peptides inhibit production of pro-inflammatory mediators including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2). Reduced inflammatory signalling limits the tumour-promoting microenvironment and decreases oxidative DNA damage [19]. Similar anti-inflammatory properties have been reported for peptide-rich hydrolysates obtained from millet proteins, although additional mechanistic studies are required [9].

4.5 Regulation of Major Cell Signalling Pathways

Cancer progression involves activation of multiple intracellular signalling cascades. Bioactive peptides appear capable of modulating several of these pathways simultaneously.

PI3K/Akt signalling

The phosphatidylinositol-3 kinase (PI3K)/Akt pathway regulates cell survival, proliferation and metabolism. Hyperactivation of this pathway is frequently observed in human cancers [13]. Experimental studies indicate that food-derived peptides suppress PI3K phosphorylation and inhibit Akt activation, thereby promoting apoptosis and reducing tumour cell proliferation [20].

NF-κB signalling

Nuclear factor-kappa B (NF-κB) controls genes involved in inflammation, cell survival and metastasis. Persistent activation of NF-κB promotes tumour progression and resistance to chemotherapy [13]. Bioactive peptides inhibit NF-κB nuclear translocation, resulting in reduced expression of inflammatory cytokines and anti-apoptotic proteins [19].

MAPK signalling

Mitogen-activated protein kinase (MAPK) pathways regulate cellular responses to stress and growth signals. Depending on cellular context, modulation of ERK, JNK and p38 signalling may either promote apoptosis or inhibit proliferation [13]. Peptides capable of regulating these kinases have demonstrated promising anticancer properties in experimental models.

p53-mediated tumour suppression

The tumour suppressor protein p53 coordinates DNA repair, cell-cycle arrest and apoptosis following cellular stress [13]. Some peptide fractions have been shown to enhance p53 expression, thereby increasing transcription of pro-apoptotic genes and suppressing tumour growth [20].

4.6 Inhibition of Angiogenesis and Metastasis

Tumour progression depends upon the formation of new blood vessels to provide oxygen and nutrients. Vascular endothelial growth factor (VEGF) is one of the principal mediators of angiogenesis [13].

Food-derived peptides have demonstrated the ability to suppress VEGF expression and inhibit endothelial cell migration. Furthermore, reduced activity of matrix metalloproteinases (MMP-2 and MMP-9) limits extracellular matrix degradation, thereby reducing tumour invasion and metastatic spread [19]. Although direct evidence for millet-derived peptides remains limited, these mechanisms represent important targets for future investigations.

4.7 Immunomodulatory Effects

Effective anticancer immunity requires coordinated activation of innate and adaptive immune responses. Certain bioactive peptides enhance macrophage activity, stimulate natural killer (NK) cells and regulate cytokine production [18]. These immunomodulatory effects may complement direct cytotoxic actions against tumour cells and improve overall therapeutic efficacy.

Future investigations should determine whether millet-derived peptides influence immune checkpoint regulation, T-cell activation and tumour-associated macrophage polarisation, which are increasingly recognised as critical components of successful cancer therapy.

Table 2. Major Anticancer Mechanisms Proposed for Millet-Derived Bioactive Peptides

Mechanism	Major molecular targets	Biological outcome
Apoptosis induction	Bax, Bcl-2, Caspase-3, Caspase-9, Cytochrome c	Programmed tumour cell death
Cell-cycle arrest	Cyclins, CDKs, p21, p27	Inhibition of tumour proliferation
Antioxidant activity	ROS, lipid peroxidation, antioxidant enzymes	Protection against oxidative damage
Anti-inflammatory activity	TNF-α, IL-6, IL-1β, COX-2	Reduced chronic inflammation

Mechanism	Major molecular targets	Biological outcome
PI3K/Akt inhibition	PI3K, Akt, mTOR	Reduced cell survival
NF-κB inhibition	NF-κB signalling proteins	Suppression of inflammatory gene expression
MAPK regulation	ERK, JNK, p38	Growth inhibition and apoptosis
Anti-angiogenic activity	VEGF, MMP-2, MMP-9	Reduced angiogenesis and metastasis

5. Experimental Evidence Supporting the Anticancer Potential of Millet-Derived Bioactive Peptides

The biological activity of millet-derived peptides has primarily been investigated through *in vitro* cell culture experiments, while *in vivo* animal studies remain comparatively scarce. Most published investigations have focused on the preparation of protein hydrolysates exhibiting antioxidant, anti-inflammatory or cytoprotective activities, which are recognised contributors to cancer prevention. Direct evidence demonstrating anticancer activity of purified peptide sequences isolated exclusively from millet proteins is still limited, highlighting an important research gap [6].

5.1 Finger Millet (Eleusine coracana)

Finger millet is the most extensively investigated millet species because of its favourable amino acid composition and high concentration of sulphur-containing amino acids. Enzymatic hydrolysis of finger millet proteins has produced peptide fractions with strong antioxidant activity capable of scavenging reactive oxygen species and reducing lipid peroxidation [8].

These antioxidant properties are particularly relevant to cancer prevention because oxidative DNA damage contributes to mutation accumulation and tumour initiation. Experimental studies have also reported that finger millet protein hydrolysates reduce oxidative stress in cultured mammalian cells and improve cellular antioxidant defence mechanisms through increased activity of endogenous antioxidant enzymes [8].

Although direct evidence for apoptosis induction in cancer cell lines remains limited, the antioxidant and anti-inflammatory properties of finger millet peptides suggest potential roles in suppressing early stages of carcinogenesis.

5.2 Pearl Millet (Pennisetum glaucum)

Pearl millet possesses one of the highest protein contents among cultivated millets and therefore represents an attractive source of functional peptides [7]. Protein hydrolysates prepared using alcalase and flavourzyme have demonstrated excellent free radical scavenging activity together with inhibition of lipid peroxidation.

Several studies have reported improved cellular antioxidant capacity following treatment with pearl millet peptide fractions. These observations suggest that bioactive peptides may reduce oxidative stress-mediated DNA damage and thereby decrease cancer risk [7,10]. However, additional investigations using human cancer cell lines are required to confirm direct anticancer activity.

5.3 Foxtail Millet (Setaria italica)

Foxtail millet proteins generate peptides possessing antioxidant and anti-inflammatory properties following enzymatic hydrolysis and fermentation [9]. Experimental studies indicate that these peptide-rich hydrolysates suppress inflammatory mediators including TNF-α and IL-6 while reducing intracellular oxidative stress.

Because chronic inflammation and oxidative stress promote tumour development, these biological activities indirectly support the anticancer potential of foxtail millet peptides. Nevertheless, isolation of individual peptide sequences and evaluation against specific cancer cell models remain limited [9].

5.4 Barnyard Millet (Echinochloa frumentacea)

Barnyard millet has attracted increasing attention because of its high nutritional value and abundance of phenolic compounds. Studies investigating protein hydrolysates have demonstrated substantial antioxidant capacity and protection against oxidative injury [4].

The combined action of antioxidant peptides and naturally occurring phytochemicals may contribute synergistically to chemopreventive effects. However, direct investigations evaluating apoptosis, cell-cycle arrest or inhibition of tumour cell proliferation are currently insufficient.

5.5 Kodo, Little and Proso Millets

Compared with finger and pearl millet, considerably fewer investigations have examined peptides derived from kodo millet (Paspalum scrobiculatum), little millet (Panicum sumatrense) and proso millet (Panicum miliaceum). Available studies have primarily characterised antioxidant activity, protein digestibility and nutritional quality rather than anticancer efficacy [4,5].

Despite the limited evidence, the favourable amino acid composition of these millet proteins suggests that they represent promising candidates for future peptide discovery programmes. Application of advanced proteomic technologies may facilitate identification of novel peptide sequences possessing potent anticancer properties.

5.6 Evidence from Related Cereal-Derived Bioactive Peptides

Although direct evidence for millet-derived peptides remains relatively limited, substantial experimental evidence exists for bioactive peptides isolated from other cereal proteins including rice, wheat, oat and barley [3,11,12]. These peptides induce apoptosis, inhibit tumour cell proliferation, suppress angiogenesis and regulate PI3K/Akt, MAPK and NF-κB signalling pathways in several human cancer cell lines.

The structural similarities among cereal storage proteins suggest that millet proteins may generate peptides with comparable biological activities following appropriate enzymatic hydrolysis. This hypothesis provides a strong scientific rationale for further investigation of millet-derived peptide therapeutics [3].

Overall, current evidence supports the concept that millet proteins constitute an important reservoir of biologically active peptide sequences. However, additional studies involving peptide purification, sequence identification, mechanistic evaluation and animal models are required before clinical translation can be considered.

Table 3. Summary of Experimental Evidence for Millet-Derived Protein Hydrolysates and Bioactive Peptides (up to May 2024)

Millet species	Experimental model	Principal biological activity	Relevance to cancer prevention
Eleusine coracana	Protein hydrolysates, cell-based antioxidant assays	Antioxidant, cytoprotective	Reduces oxidative DNA damage
Pennisetum glaucum	Enzymatic protein hydrolysates	Radical scavenging, inhibition of lipid peroxidation	Limits oxidative stress
Setaria italica	Fermented and enzymatically hydrolysed proteins	Antioxidant, anti-inflammatory	Reduces tumour-promoting inflammation
Echinochloa frumentacea	Protein hydrolysates	Antioxidant activity	Potential chemopreventive effects
Panicum sumatrense	Protein characterisation studies	Nutritional and antioxidant properties	Requires direct anticancer evaluation
Paspalum scrobiculatum	Protein studies	Functional protein source	Limited mechanistic evidence
Panicum miliaceum	Protein hydrolysates	Antioxidant activity	Candidate for future peptide discovery

6. Research Gaps, Challenges and Future Therapeutic Perspectives

Despite increasing interest in food-derived bioactive peptides, research on millet-derived peptides remains in its early stages. Most available studies have focused on nutritional evaluation, antioxidant activity and the preparation of protein hydrolysates, whereas comprehensive investigations into purified peptide sequences with specific anticancer activity are still limited [6]. Consequently, several scientific and technological challenges must be addressed before millet-derived peptides can be translated into clinically useful nutraceuticals or therapeutic agents.

6.1 Limited Identification of Bioactive Peptide Sequences

One of the major limitations in the current literature is the relatively small number of structurally characterised peptides isolated from millet proteins. Most investigations report biological activity using crude or partially purified protein hydrolysates without identifying the exact peptide sequences responsible for the observed effects [6].

Advances in proteomics, particularly LC-MS/MS, high-resolution mass spectrometry and peptide sequencing, should facilitate identification of novel peptide candidates with greater biological specificity. Detailed structure–activity relationship (SAR) studies will further improve understanding of how peptide sequence, molecular weight and amino acid composition influence anticancer efficacy [6].

6.2 Insufficient Mechanistic Studies

Although antioxidant and anti-inflammatory activities of millet protein hydrolysates have been widely reported, comparatively few investigations have explored the molecular mechanisms responsible for tumour suppression. Future research should evaluate the effects of purified peptides on apoptosis, autophagy, cell-cycle regulation, angiogenesis, epithelial–mesenchymal transition (EMT) and immune modulation using well-characterised human cancer models [12].

Comprehensive transcriptomic, proteomic and metabolomic approaches may provide deeper insight into the cellular pathways regulated by millet-derived peptides and identify novel molecular targets for cancer therapy.

6.3 Lack of In Vivo and Clinical Evidence

The majority of published investigations remain confined to *in vitro* experimental systems. Although these studies provide valuable mechanistic information, they cannot accurately predict therapeutic efficacy in humans. Animal studies evaluating toxicity, pharmacokinetics, tissue distribution and long-term safety are required before clinical application can be considered [15].

6.4 Bioavailability and Gastrointestinal Stability

A significant challenge associated with peptide therapeutics is limited oral bioavailability. Following ingestion, peptides may undergo enzymatic degradation within the gastrointestinal tract, thereby reducing systemic absorption and biological activity [15].

Future studies should investigate encapsulation strategies, nano-delivery systems and peptide modification techniques capable of improving stability, controlled release and targeted delivery to tumour tissues while maintaining biological activity.

6.5 Standardisation of Peptide Production

Considerable variability exists among published studies regarding millet species, protein extraction methods, hydrolysis conditions and purification protocols. Such methodological differences make comparison between studies difficult and hinder reproducibility [6].

Development of standardised extraction procedures, enzyme selection criteria and analytical methodologies will improve consistency and facilitate comparison of biological activities across different investigations.

6.6 Opportunities for Precision Nutrition and Functional Foods

Growing interest in precision nutrition provides an opportunity to incorporate millet-derived bioactive peptides into personalised dietary interventions. Functional foods enriched with peptide fractions may contribute to cancer prevention, particularly in populations at increased risk because of lifestyle or genetic factors [5].

In addition, incorporation of peptide-rich millet ingredients into functional beverages, fermented foods and nutraceutical formulations represents an attractive strategy for improving dietary quality while delivering health-promoting compounds.

6.7 Future Directions

Future investigations should prioritise:

- Identification of novel anticancer peptide sequences from diverse millet species.
- Structure–activity relationship studies to optimise peptide potency.
- Validation of anticancer mechanisms using advanced molecular techniques.
- Evaluation of peptide bioavailability and pharmacokinetics.
- Development of nano-formulations and targeted delivery systems.

- Investigation of synergistic interactions between millet-derived peptides and conventional chemotherapeutic agents.
- Well-designed animal studies followed by carefully controlled clinical trials.

Addressing these research priorities will strengthen the scientific foundation for the development of millet-derived bioactive peptides as safe, effective and sustainable agents for cancer prevention and adjunctive therapy.

7. Conclusion

Millets are increasingly recognised as valuable sources of nutritionally important proteins capable of generating biologically active peptides with significant health-promoting properties. Experimental findings further suggest that these peptides influence key cellular processes associated with tumour progression, including apoptosis, cell-cycle regulation, oxidative stress and inflammatory signalling.

Although direct evidence for purified millet-derived anticancer peptides remains limited, the available literature supports the hypothesis that millet proteins represent a promising reservoir of novel peptide therapeutics. Their favourable nutritional profile, low toxicity and multifunctional biological activities make them attractive candidates for future nutraceutical and pharmaceutical development.

However, significant knowledge gaps remain regarding peptide sequence identification, molecular targets, bioavailability, pharmacokinetics and clinical efficacy. Future research integrating advanced proteomics, molecular biology, bioinformatics and clinical investigation will be essential for translating these promising laboratory findings into practical applications. Overall, millet-derived bioactive peptides represent an emerging frontier in food biochemistry and cancer research. Continued investigation may contribute to the development of innovative functional foods and peptide-based therapeutic strategies that complement existing approaches to cancer prevention and treatment.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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