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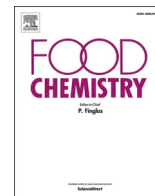
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Review

Health-promoting properties of bioactive proteins and peptides of garlic (*Allium sativum*)

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ABSTRACT

Garlic is a popular food spice with diverse and well-established medicinal properties. Many research interests have been directed toward the biological activities of the phytochemical constituents of garlic. However, prospects of its bioactive proteins and peptides have been understudied to date. With the advances in food proteomics/peptide research, a review of studies on garlic bioactive proteins and peptides, especially on their nature, extraction, and biological activities, is timely. Garlic has been reported to express several proteins, endogenous and protein-derived peptides with interesting bioactivities, including antioxidant, anti-inflammatory, antibacterial, antifungal, anti-proliferative, antiviral, anti-hypertensive and immunomodulatory activities, suggesting their therapeutic and pharmacological potentials. Compared to legumes, the low protein contents of garlic bulbs and their low stability are possible limitations that would hinder future applications. We suggest adopting heterologous expression systems for peptide overproduction and stability enhancement. Therefore, we recommend increased scientific interest in the bioactive peptides of garlic and other spice plants.

1. Introduction

Advances in the field of medicinal and food chemistry have continuously unravelled the health-promoting potentials of natural products (Santini & Cicero, 2020). Garlic (*Allium sativum*), a popular culinary spice of global significance, has attracted particular interest for several decades, especially due to the vast biological activities of its extracts and phytochemical constituents (Ezeorba et al., 2022). Apart from its content of over 200 valuable phytochemicals, garlic has been reported as the source of bioactive proteins and peptides with diverse pharmacological and therapeutic potentials as well as prospects as functional food ingredients (Kovarović et al., 2019).

A recent study on the proximate composition of several varieties of garlic reported their protein contents to be 6.38–9.5 g/100 g garlic (Tahir et al., 2022). The extraction of garlic protein is similar to the conventional methods used for protein extraction from plant materials. Generally, the lipid contents are removed first before protein extraction. A few studies have discussed, in detail, the workflow of several common and energy-assisted extraction methods, as well as the pros and cons of

achieving purified plant protein components (Bar et al., 2022; Li et al., 2022). Furthermore, extracted or purified protein components (consisting of long-chain polypeptides) are hydrolysed enzymatically by analytical grade proteases or microbial fermentation to yield hydrolysates (a mixture of small-chain peptides). Finally, the bioactive peptides within the hydrolysates can be further fractionated, purified, and characterised (Cruz-Casas et al., 2021; Okagu, Ezeorba et al., 2022).

Interestingly, garlic hydrolysates or peptides are uniquely rich in sulfur-containing amino acids (SCAA) or their derivatives, such as S-allyl cysteine (Amino et al., 2018; Valle-Rodríguez et al., 2017). Sulfur-containing amino acids (SCAA) contribute enormously to the biological activities of their inherent protein or peptides. Although SCAA are mostly non-polar and hydrophobic, the sulfhydryl group are easily ionisable and function in balancing the cellular redox potential and the detoxification of toxicant. More so, the sulfhydryl group could also foster distortion of the membrane potentials of microbes, promoting their inherent proteins' antimicrobial properties (Xi et al., 2018). Recent studies have demonstrated the biofunctional roles of garlic bioactive protein and peptide (GBP), especially as antioxidant, antimicrobial, anti-

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inflammatory, anti-cancer, and immunomodulatory agents (Fig. 1). Compared to the large body of literature on the bioactivities of garlic extracts and phytochemicals, there are limited studies on the bifunctional roles of the protein constituents, including hydrolysates and bioactive peptides. This review discusses available literature on this topic and highlights the need for increased scientific interest in the underutilised potentials of garlic bioactive proteins and peptides, especially their emerging roles as functional food ingredients and nutraceuticals. The review also highlights some limiting factors and potential solutions for the application of garlic peptides in nutraceutical and functional food formulations.

2. Methodology

This review followed the recommended guideline for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to retrieve relevant and focused literature. Related published studies on garlic bioactive proteins and peptides were retrieved from the following databases – Scopus, PubMed, and Google Scholar using specific key terms and Boolean connectors (AND or OR). Specifically, the input strings (“bioactive protein” OR “bioactive peptide” OR “hydrolysate” OR “food-derived peptide” OR “plant-derived peptide”) AND (“garlic” OR “*Allium sativum*”) were searched across the title, abstract, and keywords. Posters, abstracts, conference proceedings, and book chapters were excluded during the search.

2.1. Data extraction and management

First, a summary of all papers from the different databases was collated in an Excel sheet, and duplicated studies were removed. A preliminary screening was performed using the title and abstract to ascertain suitability and ensure that selected papers were focused on the central theme of the study. Afterward, the full text of selected papers was screened based on the inclusion and exclusion criteria. All reviews, non-English, and papers without a specific focus on the key subjects were excluded. The final selected papers were collated, and relevant data were extracted, such as first author, year of publication, method of bioactive peptide extraction, specific bioactive peptide sequences, and methods and outcomes of biological activities.

2.2. Data synthesis

This study adopted a narrative synthesis method in discussing the therapeutic and pharmacological potentials of garlic bioactive proteins and peptides. Although there was no graded system, the confidence and reliability of the accumulated knowledge were assessed qualitatively.

2.3. Results

Forty-five (45) papers were obtained after searching the databases and eliminating duplicates. After thoroughly scrutinising the title and

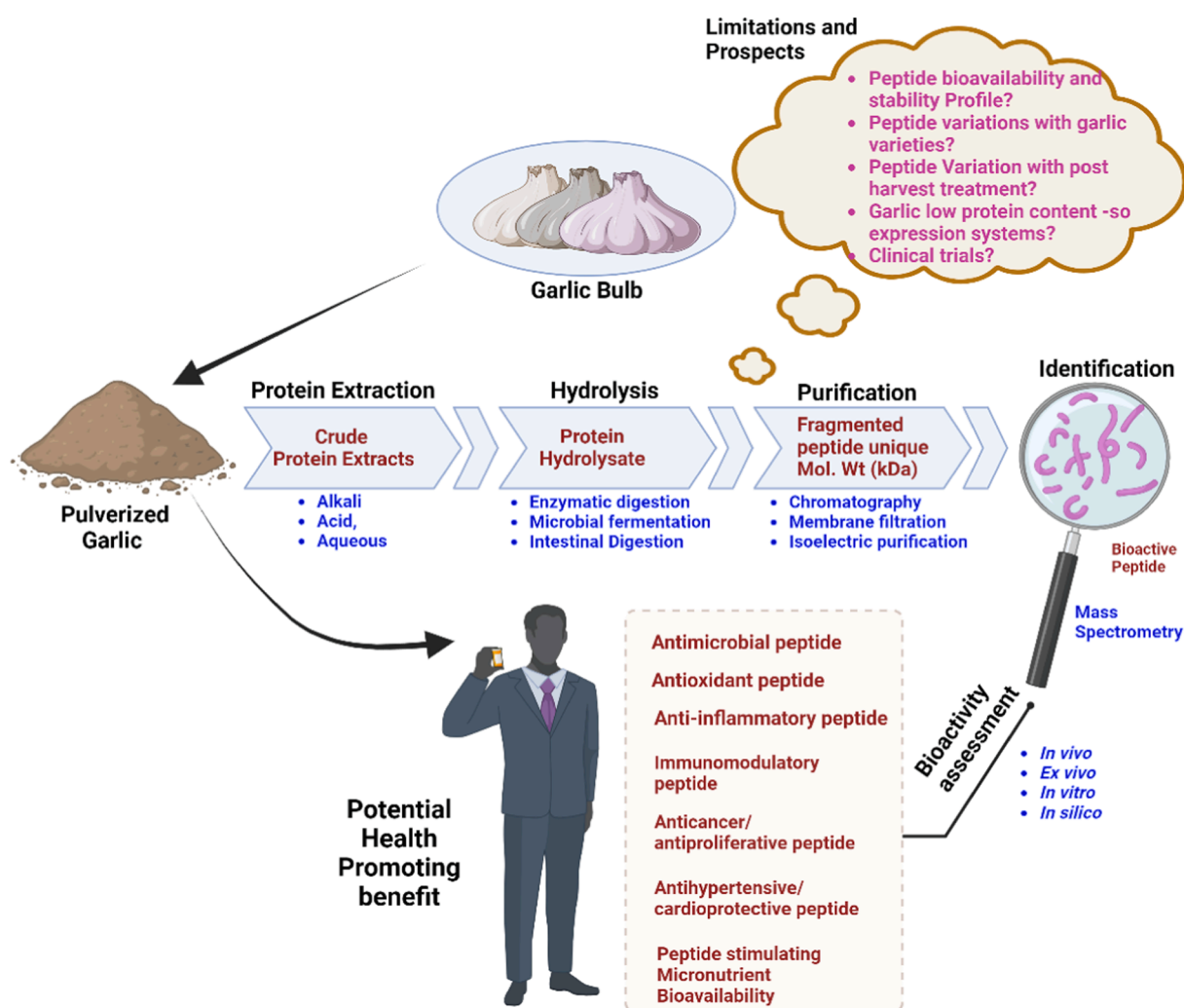


Fig. 1. A concise illustration highlighting the isolation process for obtaining garlic bioactive peptides, their biological activities for health promoting potentials and current limitation and prospects for their real-life applications.

abstract, three documents comprising one review document and two non-English papers were eliminated. After a final check on the full text of each manuscript, an additional five papers not directly linked to the focus of the review article were eliminated. Although all 36 papers were scrutinised regardless of the year of publication, particular attention was given to papers published after 2010. Hence, 20 articles formed the review's core, discussing the past and present progress and future prospects of bioactive proteins or peptides from garlic (Figs. 2 and 3).

3. Nature and composition of garlic bioactive proteins and peptides (GBP)

Different cultivars of garlic reportedly comprise about 6.3–9.5 % protein contents and the potential to be hydrolysed into peptides with many health-promoting properties, including antioxidant, anti-cancer, anti-hypertensive, and anti-obesity properties (Gao et al., 2019; Li et al., 2022; Petropoulos et al., 2018; Sasi et al., 2021). In addition, these proteins, via enzymatic hydrolysis or fermentation, can be converted to bioactive peptides (Gao et al., 2019; Li et al., 2022). Bioactive peptides are protein fragments of 2–20 amino acid residues that can serve as functional ingredients in pharmaceutical preparations and health-promoting functional foods and nutraceuticals (Rasaratnam et al.,

2021; Subroto et al., 2021). Although not well experimentally established, the component of the bioactive peptide and protein of garlic may vary across different garlic cultivars as well as their level of maturity. Many studies highlighted in this paper (Table 1) used fresh matured garlic obtained from their local markets. However, a few studies have shown that interesting peptides can be isolated from aged matured garlic (kept in frozen condition for many months, 5–12 before bioactive peptide isolation) as well as vinegar-preserved garlic (Laba garlic). Future studies could seek to establish the correlation between the age, maturity and treatment of varieties of garlic to their protein contents and bioactivity.

Naturally occurring bioactive dipeptides known to be present in whole and intact garlic extract include gamma-glutamyl-S-alk(enyl)-L-cysteines (which are biosynthetic precursors of cysteine sulfoxides), S-(2-carboxypropyl)glutathione, gamma-glutamyl-S-(trans-1-propenyl)-L-cysteine, gamma-glutamyl-S-(trans-1-propenyl)-L-cysteine, and gamma-glutamyl-S-allyl-mercapto-L-cysteine (Kodera et al., 2017; Amagase et al., 2001; Sasi et al., 2021). A garlic bulb has about 0.9 % γ -glutamylcysteine, which naturally undergoes hydrolysis and oxidation to form S-allyl cysteine (Milner, 2001; Shang et al., 2019).

Moreover, Nakamoto et al. (2018) isolated and identified three novel sulfur-containing compounds, including γ -glutamyl- γ -glutamyl-S-

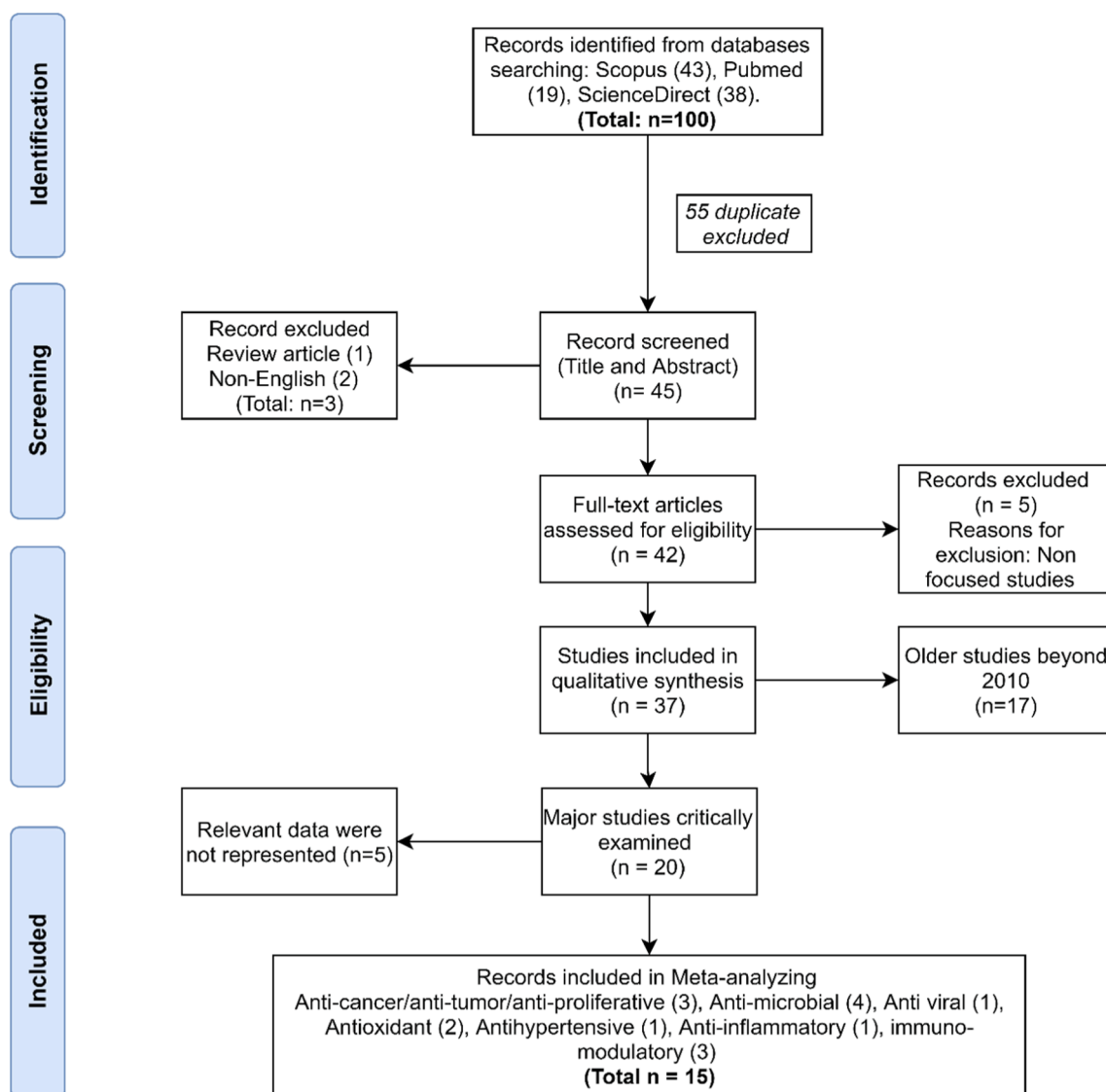


Fig. 2. PRISMA diagram showing the study selection process.

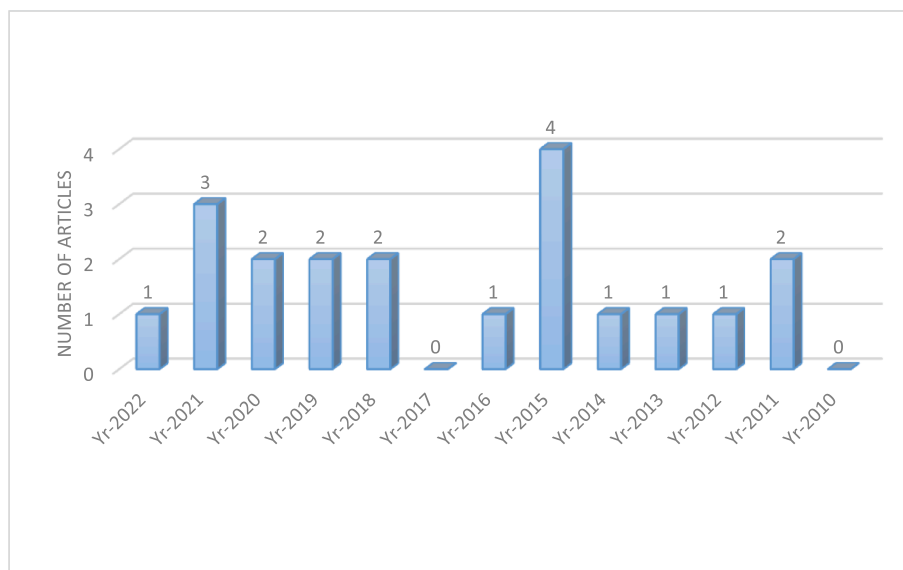


Fig. 3. Number of retrieved studies/articles on Garlic Bioactive Peptides/proteins published between 2010 and 2022.

methyl cysteine (GGSMC), γ -glutamyl- γ -glutamyl-S-allyl cysteine (GGSAC) and γ -glutamyl- γ -glutamyl-S-1-propenyl-cysteine (GGS1PC) by post-column HPLC and NMR/LC-MS analysis. The novel compounds were reported to occur in trace amounts in raw and fresh garlic, and their concentrations increased with the ageing process. Finally, peptide production was significantly inhibited in the presence of γ -glutamyl transpeptidase (GGT) inhibitors. Hence, GGT is central to the garlic peptide syntheses or metabolisms (Nakamoto et al., 2018). Water-soluble γ -L-glutamyl-S-(trans-1-propenyl)-L-cysteine and γ -glutamyl-S-(2-propenyl)-L-cysteine are garlic's two most abundant peptides. The abundance of these peptides depends on the source of the garlic (Yu et al., 2020).

Different studies have shown garlic contains other bioactive peptides derived from protein hydrolysis. For example, Gao et al. (2019) isolated three antimicrobial peptides from laba garlic (a clove of aged garlic preserved in vinegar) after hydrolysis with pepsin and trypsin and identified the peptides as WPTSFT, YNHNF, and AVDRAV. AVDRAV, which is amphipathic, contains 25.8 % α -helix and 19.7 % β -strands with 21.2 % turns and 33.3 % unordered conformation. Although the study structurally characterised the peptides, there was no report of their biological activities (Gao et al., 2019). Conversely, the protein extract of garlic digested with pepsin yielded a novel peptide, VKLRSLCS (VS-9), identified after a series of purification steps. Peptide (VS-9) was reported to have minimal inhibitory activity against normal human peripheral blood mononuclear cells (PBMCs) and anti-proliferative solid activity against MOLT-4 and K562 leukemic cell lines. Hence, the VS-9 peptides could have prospects as anti-cancer therapy with the potential of high selectivity for normal cells (Rasaratnam et al., 2021).

Garlic proteins with bioactivity have also been reported. The major protein in garlic bulbs includes agglutinins (25 kDa), allinase, glycoprotein agglutinin (110 kDa), alliumin (13 kDa), allivin (13 kDa) (Clement et al., 2010; Ebrahimi et al., 2013). Two different isoforms of agglutinins (lectins) with molecular weights of 25 and 110 kDa were isolated from garlic by Gupta and Sandhu (1997). This protein functions as an antibody or a sugar-binding protein, biologically promoting signalling pathways and intercellular interactions (Padiyappa et al., 2022). One of the isoforms (ASA₂₅) is a dimeric protein containing two subunits of 12.5 and 13.0 kDa. In contrast, the second isoform (ASA₁₁₀) is a glycoprotein of two identical subunits with a molecular weight of 47 kDa. This isoform predominantly contains glycine, aspartic acid, leucine, and serine but has low contents of methionine and cysteine

(Gupta & Sandhu, 1997).

The protein content, composition and properties could vary among different varieties of garlic, as well as post-harvest treatment. Li et al. (2022) analysed and compared the protein composition of Laba garlic and white garlic and reported that the processed Laba garlic protein had a lower isoelectric point (pI) and larger particle size, decreased dominant α -helix structure forming a random coil, and reduced surface hydrophobicity compared to the unprocessed white garlic protein. The molecular mass distribution of proteins in laba and white garlic ranged from 10–25 kDa and 10–80 kDa, respectively. White garlic has many proteins, including vital allinase, which was absent in laba garlic (Li et al., 2022). The variety of proteins occurring in different garlic varieties provides an attractive template for producing structurally diverse peptides with the potential to exhibit bioactivity.

Alliumin, a 13-kDa protein, was also isolated from multiple clove garlic bulbs (Xia & Ng, 2005). The protein was purified from garlic cloves through ion exchange chromatography, affinity chromatography, and gel filtration, and its N-terminal sequence was reported to be similar to a partial sequence of glucanase. Alliumin showed several bioactivities as it exhibited antifungal activities against *Mycosphaerella arachidicola*, antibacterial activities against *Pseudomonas fluorescens* as well as anti-proliferative activities in mouse lymphocytic leukemia (L1210) cells. Similarly, three different proteins with molecular weights of 12–14 kDa were isolated and purified from aged garlic extract and were shown to possess immunomodulatory, mitogenic, and mannose-binding activities as confirm by hemagglutination analysis (Chandrashekar & Venkatesh, 2009). Daneshmandi et al. (2011) also isolated two protein fractions with molecular weights of 14 and 47 kDa from aged garlic extract and showed that the proteins regulated the production of macrophages. Marzouki et al. (2005) isolated and characterised a monomeric 36.5-kDa protein exhibiting peroxidase activity at an optimum temperature of 25–40 °C and pH of 5.0. Other garlic proteins with promising bioactivities have also been reported and discussed in depth in Section 5 (Sun et al., 2019)(Yoshimoto et al., 2015).

4. Methods of production, extraction, purification, and characterisation of garlic-derived bioactive peptides

Garlic-derived bioactive peptides and proteins have been shown to exhibit health-promoting properties due to their pharmacological and nutritional values (Espinoza et al., 2020). Therefore, different processes are used to separate the biologically active peptides from garlic bulbs

Table 1
Biological activities of garlic bioactive protein and peptides.

Biological activities	Garlic Source (specific variety/maturity)	Peptides Sequence or Protein characteristic kDa	Quantity/ amount protein and peptides	Protease	<i>in vitro</i> /in vivo/ Ex vivo	Experimental Model/cell line/analytical method	Treatment/Dosage	Biological activity	References
Anti-cancer	Fresh Matured Garlic bulb (Salaya, Thailand)	VKLRLLCS (VS-9) from < 3 kDa fraction (fresh garlic)	3.73 mg protein/ g of raw garlic 12.13 % Garlic protein hydrolysate	Pepsin	<i>Ex vivo</i> <i>In silico</i>	MOLT-4 and K562 leukemic cell lines Molecular peptide-protein docking (VS-9 peptide vs. Bcl-2 protein)	Treatment with cytotoxic IC50 concentration - (IC50 0.84 mM)	↓Cell proliferation ↑Apoptosis ↑mRNA levels of caspase 3, 8, and 9, as well as Bax ↓Bcl-2, Bcl-xL, and Bcl-w	(Rasaratnam et al., 2021)
	Fresh Matured Garlic bulbs (India)	<i>A. sativum</i> lectin 50 kDa (ASL ₅₀)	19 mg/ml from 50 g of dried garlic bulb- ASL ₅₀ conc. 1.4 mg/ml (7.37 % of the protein)	Isolated and purified by gel chromatography on Sephacryl S-200 column	Ex vivo	a) Oral carcinoma KB cells b) Human Erythrocyte cells group A and Bc) normal human embryonic kidney cells (HEK 293 cells)	a) MTT dye reduction assay (anti-proliferative assay) a) Annexin-V Binding Assay (Apoptosis) b) Hemolytic assay (hemolytic) Apo-Glo™ assay (caspase activity assay)	*Non-cytotoxic to human erythrocyte cells (at conc of 700 µg/ml of protein) while gentamicin and fluconazole (+ve control showed 36 and 88 % hemolysis)*Non-cytotoxic to HEK293 cells (conc 0–500 µg/ml)* Anti-proliferative activity on KB cells at IC50 of 36 µg/ml in a DDM*Increase apoptotic activity on KB cells by 67 and 80 times by 30 and 60 µg/ml protein after 48 h* Caspase 3/7 expression increase by 1.5–2.5 fold at 30 & 60 µg/ml protein.	(Kumar et al., 2015)
Antimicrobial (antibacterial/ antifungal)	Fresh matured garlic bulb (Hamadan, Western Iran)	Garlic protein (R10 fraction) containing 3 single polypeptides (~10–13 kDa)	-	-	In vivo	Breast transplanted tumor in BALB/c Mice Model	20 mg/kg of R10 into the lesion up to 7 days	↑ significant CD8 + subpopulation of T lymphocytes ↓ Tumor sizes	(Ebrahimi et al., 2013)
		34 amino acid peptide (AsR416) 3799.52 Da			In vitro In vivo	<i>Bacillus Subtilis</i> and <i>Rhizoctonia solani</i>	100 mg/ml	↓bacteria growth ↓sclerotia formation ↑O ₂ formation in hyphae and mycelia death ↓ cellulase (the microbial virulence factor)	(Kong et al., 2018; Nassimi et al., 2021)
	Fresh matured garlic bulb (Podmoskovnyi cultivar)	Novel peptide (4392 Da)		Trypsin	In vitro	Magnaporthe grisea Bipolaris sorokiniana	agar-diffusion method Treatment with 100 µl of the peptide at 26oC for 3 days	No significant microbial inhibition (<i>in vitro</i>) Prevent pathogen attack (<i>in vivo</i>)	(Kulikova et al., 2016)
	Laba Garlic (vinegar-preserved garlic) (Tianjin, China)	AVDRAV (F3-3-c) from < 4 kDa fraction	100 g of crude protein from 1 kg Laba Garlic – 23.5 g of < 4 kDa fraction and 6.5 g peptide	Pepsin and Trypsin	In vitro	<i>E. coli</i> and <i>S. aureus</i>	Micro-diffusion assay. 2-fold dilution of 12.5–1600 µM peptide conc in 5 × 10 ⁵ CFU mL – 1 for 37oC for 16–20 h	MIC of 100 µM	(Gao et al., 2019)
Matured Garlic bulbs (India)	<i>A. sativum</i> lectin 50 kDa	19 mg/ml from 50 g of dried garlic bulb- ASL ₅₀ conc. 1.4 mg/ml (7.37 % of the protein)	Isolated and purified by gel chromatography on Sephacryl S-200 column	In vitro	- Clinical Pseudomonas aeruginosa - Clinical Candida isolates	Agar well diffusion assay	MIC (Bacteria) – 10–80 µg/ml after 18 h incubation MIC (fungi) – 10–40 µg/ml after 24 h incubation	(Kumar et al., 2015)	

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Table 1 (continued)

Biological activities	Garlic Source (specific variety/ maturity)	Peptides Sequence or Protein characteristic kDa	Quantity/ amount protein and peptides	Protease	<i>in vitro</i> / <i>in vivo</i> / Ex vivo	Experimental Model/cell line/analytical method	Treatment/Dosage	Biological activity	References
Antiviral	Hairy garlic bulb (<i>Allium subhirsutum</i> L.) (Barazan, Saudi Arabia)	Asn-Asn-AsnHis-Phe-GlnGln-His-PheThr-Leu-TrpGln-Phe-Tyr (Hairy Garlic)	-	-	In silico	Molecular docking of the peptide against S-protein, hACE2, and furin of SARS-CoV-2. And proinflammatory targets such as Nlk, PLA2, IRAK-4, and COX2	Pharmacophore, drug-likeness, and ADMET	Binding affinities ranged from 8.2 to 10.5 kcal/mol	(Snoussi et al., 2022)
Antioxidant	Fresh Garlic (Tianjin, China)	Garlic and garlic protein hydrolysate	-	Pepsin and Trypsin	In vitro	- DPPH radical scavenging - FRP assay - LPI assay	0.1–0.5 mg/ml (DPPH)1.19–5.95 mg/ml (FRP)s 2.5–12.5 mg/ml (LPI)	DPPH IC50 – 14.18–14.83 µg/ml FRP – at conc of 5.95 µg/ml, the FRP was 0.192–0.252 LPI IC50 – 10.63 µg/ml	(Gao et al., 2020)
	- Matured fresh garlic (Purple scales variety)	Garlic glycoprotein (N-linked to galactose)MW – 55.7 kDa (GPC) and 13.2 kDa (SDS PAGE)	– 2.122 g of crude glycoprotein from 300 g of garlic	-	In vitro	- DPPH radical scavenging -LPI assay	DPPH – 1–25 mg/ml LPI – 1–20 mg/ml	A dose-dependent increase in DPPH radical scavenging and PUFA peroxidation	(Y. Wang et al., 2016)
Anti-hypertensive	Fresh Garlic (Tianjin, China)	Garlic and garlic protein hydrolysate	-	Pepsin and Trypsin	In vitro and in vivo	ACE inhibition (<i>in vitro</i>) DBP and SBP (<i>in vivo</i>)	0.5–2.5 mg/ml (ACE) Orally administer 50 mg/kg to SHR and DBP, and SBP measured from 0–24 h	ACE inhibition – IC50 – 0.87–0.99 mg/ml Decrease in DBP 16.7–23.33 mm Hg (Positive control – 17 mm Hg decrease) after 4 h Decrease in SBP after 8 h by 32 to 40 mm Hg	(Gao et al., 2020)
Anti-inflammatory	Dried Garlic bulb	Garlic 14-kDa protein	-	Isolated by NH4SO4 precipitation and purified by gel chromatographic	Ex vivo	J774A.1 macrophages cells induced with LPS (1 µg/ml) for proinflammatory production Western blot assay for inducible NO synthase, COX-2, and 14-kDa protein	Treatment of induced cells with 5–40 µg/ml of Garlic 14-kDa protein	↓ NO ↓ PGE, ↓ TNF-α, ↓ IL-1β Zero cytotoxic effects ↓ inducible NO synthase expression ↓ Activity and expression of NF-κB transcription factor protein.	(Rabe et al., 2015)
Immuno-modulatory	Dried and aged matured Garlic bulb (kept for 6 months at –20 °C (Hamadan, Iran)	Garlic 47 kDa protein from Aged Garlic extract	-	Isolated and purified by NH4SO4 precipitation and gel filtration and SDS-PAGE	in vivo	Dendritic cells (DC) purified from the spleen of BALB/c mice and expressed surface markers assessed by flow cytometry after treatment with protein	Treatment of 2 × 10 ⁵ DCs with 5–20 µg/ml protein	↓ DC maturation makers, such as ↓CD40 (47 %–41 %) ↓CD86 (91 %–84 %) ↓ MHC-II (90–83 %)	(Ahmadabad et al., 2012)
	Dried and aged matured Garlic bulb (kept for 6 months at –20 °C (Hamadan, Iran)	Garlic 14 kDa protein from Aged Garlic extract	-	Isolated and purified by NH4SO4 precipitation and gel filtration and SDS-PAGE	Ex vivo	Dendritic cells (DC) purified from the spleen of BALB/c mice and expressed surface markers assessed by flow cytometry after treatment with protein	Treatment of 2 × 10 ⁵ DCs with 5–20 µg/ml protein	↑CD40, No effect on CD86, and No effect on MHC-II	(Ahmadabad et al., 2011)
	Dried and aged matured Garlic bulb (kept for 12 months	Garlic 14 kDa and 47 kDa	-	Isolated and purified by NH4SO4 precipitation	Ex vivo	Peritoneal macrophage (PM) lavaged from mice to a microtitre plate.	Treatment of 3 × 10 ⁵ PM with 5–20 µg/ml protein Measurement of NO	↓ NO Maintained viability after treatment by MTT assay ↑TNF-α	(Daneshmandi et al., 2011)

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Table 1 (continued)

Biological activities	Garlic Source (specific variety/maturity)	Peptides Sequence or Protein characteristic kDa	Quantity/ amount protein and peptides	Protease	<i>in vitro</i> / <i>in vivo</i> / Ex <i>in vivo</i>	Experimental Model/cell line/analytical method	Treatment/Dosage	Biological activity	References
	at -20 °C (Hamadan, Iran)			and gel filtration and SDS-PAGE			conc MTT reduction assay Measurement of TNF-α bioactivity		
	Fresh matured garlic bulb (Hamadan, Western Iran)	Garlic protein (14 kDa fraction)	-	-	In <i>in vivo</i>	Breast transplanted tumor in BALB/c Mice Model (8-10 wks old)	20 mg/kg of R10 administered intraperitoneally for 5 days	↑ hyperplasia and hypertrophy of spleen and lymph node ↑ Delayed type hypersensitivity ↑ Natural killer cell activities	(Gharzafar et al., 2002; Hassan et al., 2003)

NIK - NF-κB-inducing kinase; PLA2 - phospholipase A2; IRAK-4 - interleukin-1 receptor-associated kinase 4; COX2 - cyclooxygenase 2; DBP - Diastolic blood pressure; SBP - systolic blood pressure; ACE - angiotensin-converting enzyme; LPI - Lipid peroxidase inhibition; FRP - Ferric reducing power assay; GFP - Garlic protein hydrolysate; SHR - spontaneously hypertensive rats; GPC - Gel permeation chromatography; DPPH-1,1-diphenyl-2-picrylhydrazil; DDM - Dose-dependent manner.

(Fig. 1). Extraction and purification of bioactive proteins involve several approaches which adopt differential solubility properties of proteins in aqueous and organic solvents, elimination of cell debris, and purification (Zaky et al., 2021). There are limited studies on extracting and purifying garlic bioactive peptides intended for human consumption. Also, no studies have reported the techniques for industrial-scale production of garlic peptides.

The most widely used chromatographic methods for protein and peptide separations include size exclusion, ion exchange, and affinity chromatography. However, the use of chromatography in large-scale separation remains a challenge due to the high cost of resins, limited sample handling capacity, and slow processing time (Yu et al., 2020). Using chromatographic techniques usually leads to a very dilute product due to the large solvent used against the small sample amount. Hydrophobic interaction chromatography has been used to extract and purify bioactive peptides from garlic. The principle involves increasing the polarity of the stationary phase and solute while decreasing the polarity of the organic solvent (Jia et al., 2021).

Using a single chromatographic method presents the difficulties of long analysis time, complex sample preparation, limited number and type of detection for bioactive peptides, and low sensitivity (Jia et al., 2021). These issues have led to the pursuit of alternative techniques for purifying bioactive peptides, such as salting-out/pH-driven precipitation of peptides and ultrafiltration membranes designed to separate peptides based on molecular mass cutoff (Yu et al., 2020).

Different methods, including physical, chemical, and enzyme-assisted, are used in protein isolation from plants (Zaky et al., 2021). The method applied depends on the nature of the protein and its location in the plant. The physical method involves homogenisation to break open the cell walls, allowing for the release of proteins/peptides from garlic bulbs. However, this method results in a lower extraction yield than the chemical and enzymatic methods. Micellisation, also known as the salt removal method, is another plant protein extraction method. This method involves the addition of salt concentrations that denature the proteins at a particular ionic strength. The dilute solution formed is centrifuged and dried to precipitate the protein. The precipitation process requires NaCl to dissolve in ice water.

Ultrasound, which works by generating bubble cavitation in biological matrices, is also a suitable extraction technology for the mass production of bioactive proteins and peptides (Jia et al., 2021; Ma et al., 2015). The alkaline-based plant protein extraction followed by precipitation has resulted in a high product yield (Zhang et al., 2015). Extraction of plant proteins by the alkaline method is widely used due to its cost-effectiveness. However, this method requires membrane ultrafiltration and salting-out processes, which adds significantly to the processing cost (Zaky et al., 2021). Proteins are usually soluble in alkaline solutions and precipitate at their isoelectric point, typically at acidic pH (Zhang et al., 2015).

The enzyme-assisted extraction of proteins involves using enzymes like pectinases, cellulases, lignocelluloses, xylanases, and phytases to break open the plant cell walls, break down carbohydrates, and break down carbohydrates and some structural proteins, releasing the protein of interest. Achieving specific hydrolysis of the structural proteins could be a major challenge of this method. Proteases such as trypsin, pepsin, papain, and chymotrypsin are used for partial hydrolysis of proteins to form peptides, improving the functionality and application of the proteins. Nevertheless, peptides obtained using this approach usually have lower molecular weight and secondary structure content than the native ones (Zaky et al., 2021).

Protein extraction from garlic bulbs usually uses Tris buffer containing 1 M NaCl at pH 8.2. The homogenates are filtered and stirred for five h. Phenylmethylsulfonyl fluoride and polyvinyl pyrrolidone are added to the homogenates to prevent protein digestion by proteases (Shamsi et al., 2016). The homogenate is centrifuged for 1 h at 9000 rpm to remove the cell debris. After ammonium sulfate precipitation, the mixture is centrifuged at 9000 rpm for 1 h. The pellet is solubilised in

Tris buffer and dialysed in the same buffer using cellulose tubing for 24 h. The dialysate is subjected to ion-exchange chromatography pre-equilibrated with Tris buffer (pH 8.2). The unbound proteins are washed, whereas the bound proteins are eluted with a Tris-buffer solution containing 0.1 to 1 M NaCl. The bioactive eluents are further concentrated using an Amicon filter (Shamsi et al., 2016).

Purification and identification of bioactive peptides from garlic bulbs follow the same approach used for other plants (Zaky et al., 2021). Biologically active peptides are purified using ultrafiltration, reversed-phase high-performance liquid chromatography, gel filtration chromatography, and ion exchange chromatography. After purification, several analytical methods are routinely applied to identify the bioactive peptides and proteins, including matrix-assisted laser desorption/ionisation time-of-flight/MS, electrospray ionisation/MS, mass spectrometry (MS), hydrophilic interaction liquid chromatography (HILIC) and liquid chromatography-MS/MS (Jia et al., 2021; Zaky et al., 2021). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), which operates based on the charge and size of biological molecules, can also be used to separate the bioactive peptides. Ferreras et al. (2021) separated a 15-kDa bioactive protein from plant extract using SDS-PAGE. Bipolar membrane electro dialysis, a new and effective technique that uses bipolar membranes to generate ions, has also been used to extract bioactive peptides. This process uses monovalent cation-selective permeation membranes and anion exchange membranes to separate ions (Jia et al., 2021). This technique provides a sustainable alternative to the chemical and enzymatic hydrolysis methods because it does not involve the use of chemicals.

Other highly efficient bioactive peptide extraction techniques are being developed, such as the magnetic solid-phase extraction approach, which was recently used for garlic bioactive γ -glutamyl peptide purification (Yu et al., 2020).

5. Biological and pharmacological roles of proteins and peptides from garlic

Peptide drugs are currently used in managing and treating various diseases due to their high specificity, low toxicity, easy synthesis, and rate of degradation and clearance in the body (Gao et al., 2019). The amino acid sequence generally governs the biological functions of proteins and peptides. In contrast, bioactivity sometimes depends on the relative ratio of a specific amino acid or group of amino acids. For instance, the amino acid composition, hydrophobicity, molecular weight, chain length, and type of residues at the C- and N-terminals influence peptides' functional properties and bioactivities (Guha & Majumder, 2018). This section discusses GBP's biological and pharmacological roles, including antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, anti-cancer, anti-proliferative, and other activities (Table 1 and Fig. 1).

5.1. Antimicrobial activity of proteins and peptides from garlic

Fresh garlic has been consumed for its antimicrobial (antibacterial, antifungal, and antiparasitic) activities (Horita et al., 2016; Kulikova et al., 2016). This has been a shifting focus from synthetic to natural antimicrobial agents, including plant extracts, oligosaccharides, and peptides, for human and animal consumption (Gao et al., 2019). Antimicrobial peptides (AMPs) are peptides with less than 50 amino acids found in fungi, bacteria, insects, amphibians, marine vertebrates, mammals, and plants (Nassimi et al., 2021). Plants-derived AMPs have exhibited inhibitory activity against human pathogens and phytopathogens (Nassimi et al., 2021). They exert antimicrobial effects by binding to cell membranes, destroying the integrity of cell membranes, inhibiting DNA and RNA biosynthesis, as well as enzymes involved in linking cell wall structural proteins and disrupting cell membrane integrity (Gao et al., 2019; Nassimi et al., 2021). The two main mechanisms of AMPs on the membrane are the "barrel-stave model" and the "carpet model".

Because AMPs are amphiphilic, in the barrel-stave model, the hydrophobic end of the AMPs can be inserted into the cell membrane to form holes, destroying the membrane structure. However, in the carpet model, AMPs bind to the membrane surface through their hydrophobic amino acid residues and cover it like a carpet, causing membrane damage and leakage of cellular contents (Gao et al., 2019).

In a recent study, three peptides were isolated from Loba garlic designated as F3-3-a, F3-3-b, and F3-3-c peptides with molecular weights of 693.72 Da, 737.80 Da, and 629.79 Da, respectively. F3-3-a was identified as a pentapeptide Tyr-Asn-His-Asn-Phe (YNHNF), F3-3-b is the hexapeptide Trp-Pro-Thr-Ser-Phe-Thr (WPTSFT), and F3-3-c is the hexapeptide Ala-Val-Asp-Arg-Ala-Val (AVDRAV) (Gao et al., 2019). The antimicrobial activity of the three peptides against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis*, and *Bacillus subtilis* showed that F3-3-b and F3-3-c significantly inhibited the growth of the four bacteria, especially F3-3-c. The difference in amino acid composition and conformation may account for the observed difference in the antimicrobial activity of the peptides. The composition of hydrophobic amino acids in the peptides was 20, 50, and 67 % for F3-3-a, F3-3-b, and F3-3-c peptides, respectively. The presence of hydrophobic amino acids (Val and Ala) and basic amino acid Arg in F3-3-c, which had the highest antimicrobial activity, corroborates with previous findings that Val/Arg residues enhance the antibacterial activity of peptides (Gao et al., 2019). The bactericidal action of F3-3-c was demonstrated to be by causing physical damage to the bacteria cell membrane, thereby initiating leakage of cellular contents.

Furthermore, a new AMP isolated from garlic, AsR416, with a molecular weight of 3799.52 Da, was found to contain cysteine disulfide, α -helix and β -sheet structures, 1-aspartine, 1-histidine, *n*-acetyl-D-glucosamine 6 phosphates, N1-acetyl spermidine, analine, and 1-urogenate (Xi et al., 2018). AsR416 demonstrated antibacterial activity against Gram-negative bacteria, including *Agrobacterium tumefaciens*, *E. coli* DE3, and *Xanthomonas campestris* pv. *oryzicola*, *Ralstonia solanacearum*, and Gram-positive bacteria such as *Bacillus anthrax*, *Bacillus cereus*, *Bacillus subtilis*, *Clavibacter fangii*, and *Clavibacter michiganensis* (Xi et al., 2018). In another study, the antifungal activity of AsR416 against *Rhizoctonia solani* (AG1-IA), a phytopathogen that reduces annual rice yield by up to 50 % as well as those of other crops such as soybean, bean, and corn, was reported (Nassimi et al., 2021). The authors demonstrated that AsR416 inhibited vegetative growth, virulence, and survival of *R. solani* via changing membrane permeability in *R. solani* hyphal cells, thereby decreasing the activity of cell wall degrading enzymes, inhibiting the production of xylitol required for sclerotia production and preventing rice-sheath blight disease. Moreover, the antifungal activity of allivin, a 13-kDa protein against *Botrytis cinerea*, *Mycosphaerella ardhidicola*, and *Physalospora piricola*, was reported to be higher than those of proteins isolated from other plants (Wang & Ng, 2001). In summary, several garlic peptides are potent agents of different microbial species affecting human and plant health.

5.2. Antioxidant activity of proteins and peptides from garlic

Usually, physiological processes produce reactive oxygen species (ROS) to mediate various cellular functions and homeostasis (Sharifi-Rad et al., 2020). However, excessive generation of ROS overwhelms the body's endogenous antioxidant defence mechanisms, leading to oxidative stress, which is implicated in ageing, diabetes, cancer, cardiovascular disease, and neurological disorders (Chukwuma et al., 2023). To avert the health consequences of oxidative stress, the consumption of dietary antioxidants, such as food rich in polyphenols and antioxidant bioactive peptides, is highly encouraged (Okagu et al., 2021).

Garlic protein hydrolysates produced with pepsin (GPH-P) and Trypsin (GPH-T) exhibited antioxidant activity in three *in vitro* assays; DPPH radical scavenging, ferric reducing power, and lipid peroxidation inhibition. DPPH scavenging capacity of the hydrolysate might be due to the high concentration of Met, Tyr, Val, Ile, His, Leu, Gly, Phe, and Trp,

which are all hydrophobic amino acids (Gao et al., 2020). Moreover, in food, protein-derived bioactive peptides, certain properties such as a terminal methionine residue, the presence of sulfur-containing amino acids, and amino acid hydrophobicity have been reported to enhance DPPH and FRAP. In contrast, the presence of Lys residues and a high content of cationic amino acids in protein hydrolysates are thought to impair their FRAP potential donor (Nwachukwu et al., 2021). Another study reported that a 55.7-kDa garlic glycoprotein dose-dependently scavenged DPPH radical and inhibited lipid peroxidation (Wang et al., 2016). Tyr and His at the C-terminal position have phenyl and imidazole groups, respectively, which could scavenge the hydroxyl radical produced by the metal ion-induced oxidation, whereas the thiol group of Cys could serve as an electron donor to form a disulfide (Nwachukwu et al., 2021). Inhibition of lipid peroxidation by garlic protein hydrolysates will prevent cross-linking of malondialdehyde (an end product of lipid oxidation) with the amino acid-containing protein target (Gao et al., 2020).

Tan et al. (2015) also reported the anti-glycation activities of γ -glutamyl-S-allyl-cysteine (GSAC) peptide isolated from fresh garlic scales in a bovine serum albumin (BSA)/glucose system. The increased browning fluorescence intensity at 440 nm was inhibited, and the free lysine chain reacted in the BSA/glucose system. It was reported that the antioxidative effect of the peptide, specifically the radical scavenging (>70 % at 160 μ g/mL GSAC) and metal chelating activities (>90 % at 160 μ g/mL GSAC), accounted for their ability to prevent glycation (Tan et al., 2015). The antioxidative potential of the garlic hydrolysates and glycoproteins from recent experimental evidence is a pointer to the vast functional application of the peptides for health, nutrition, and pharmaceutical preparations.

5.3. Anti-inflammatory activity of proteins and peptides from garlic

Inflammation is a physiological response to activating and recruiting mediators (such as nitric oxide (NO), prostaglandins (PG), thromboxanes, interleukins and TNF- α) to eliminate harmful stimuli (Chukwuma et al., 2022). Excessive release and activation of these mediators amplify inflammatory responses, leading to loss of immune function and tissue damage. Chronic inflammation is implicated in the aetiology and pathogenesis of diseases such as arthritis, CVD, diabetes, and inflammatory bowel disease (Guha & Majumder, 2018). Considering the cost and side effects associated with prolonged usage of synthetic anti-inflammatory agents, there is a need for an alternative remedy from natural sources such as plant-derived peptides.

Generally, interest in plant-derived peptides is increasing partly due to their effectiveness in modulating inflammatory responses, mainly through modulation of signalling pathways, including mitogen-activated protein kinase (MAPK) pathway and nuclear factor-kappa light-chain-enhancer of activated B cells (NF- κ B) pathway, and reduction of the production of NO, cyclooxygenase-2 (COX-2) and TNF- α (Majumder et al., 2016). Rabe et al. (2015) demonstrated the anti-inflammatory effects of a 14-kDa garlic protein on lipopolysaccharide (LPS)-stimulated J774A.1 macrophage and the underlying mechanisms of action on release and expression of inflammatory mediators and genes. The authors reported that treatment with the garlic protein increased cell viability and decreased the levels of NO, TNF- α , PGE2, and IL-1 β secreted by the macrophage. Additionally, western blot analysis revealed a significant reduction in the expression of COX-2 and iNOS via the inactivation of NF- κ B p65 expression. Overproduction of PGE2 and NO is implicated in cancer, neuronal diseases, and chronic inflammation, thus, their reduction is a key factor for an agent to be considered a potential drug for inflammatory complications. Despite the prospects, the intact garlic protein will not survive the hydrolytic activity of proteases and peptidases in the gastrointestinal tract when taken orally as functional food or nutraceutical, affecting its bioavailability and physiological anti-inflammatory effects.

Similarly, Daneshmandi et al. (2011) reported that a 14-kDa garlic

protein prevented the production of NO from cultured peritoneal macrophages via inhibition of iNOS mRNA and protein expression in the activated macrophages. iNOS is responsible for NO production from L-arginine and oxygen during inflammation. Hence, the downregulation of iNOS suppresses NO production. The inhibition of NO production and antioxidant effects of garlic 14-kDa protein present a tremendous potential for multifunctional effects in reducing the risk of several health complications, such as stroke, cancer, unhealthy ageing, cardiovascular diseases, and neurological disorders.

5.4. Immunomodulatory and hypersensitivity activities

Immunomodulatory actions of garlic proteins have been reported, although it is not clear whether the immunomodulatory effects are attributable to a specific protein or mixtures or linked to their interactions with potentially co-isolated organosulfur compounds. Immunomodulatory effects on dendritic cells from the spleen of BALB/c mice have been reported for a 14-kDa protein from aged garlic extract. The proteins were profiled using gel filtration on Sephadex G50 and SDS-PAGE. The purified garlic protein elevated the expression of CD40 molecules on the dendritic cells. Interestingly, the CD40 cells are key players in the signalling pathway for the functional roles of other immune cells, including the B-cells, monocytes and dendritic cells (Ahmadabad et al., 2011). In a similar experiment, a 47-kDa protein was isolated from aged garlic extract and afterwards inoculated into the mouse dendritic cell medium, downregulated the expression of the markers of cell maturation, which are indicators for the generation of tolerogenic dendritic cells *in vitro* (Ahmadabad et al., 2012). This is vital in the therapeutic management of autoimmune disorders. Furthermore, immunomodulatory protein isolates of 14 kDa and 47 kDa fractions of aged garlic extract were purified by ammonium sulfate, gel chromatography, and SDS-PAGE. Both fractions suppressed nitric oxide production from the peritoneal macrophage cells and thus attenuated the tumoricidal tendencies of the macrophages (Daneshmandi et al., 2011). Agglutinins, proteins from garlic bulbs, have been reported to exhibit significantly higher agglutination in rabbit erythrocytes than in human erythrocytes. Agglutinin is known for its mimicry roles as antibodies that foster the aggregation of antigens for immunological clearance (Gupta & Sandhu, 1997). According to Chandrashekar and Venkatesh (2009), proteins isolated from garlic with a molecular mass ranging from 11 to 14 kDa exhibited immunomodulatory activity. The purified protein components QA-1, QA-2, and QA-3 display mitogenic activity. Protein isolates from QA-2 and QA-1 contained garlic lectins ASA I and ASA II, respectively, with strong hemagglutination activity. Three single polypeptides, approximately 10–13 kDa, were isolated from garlic. From the study, the protein fractions from garlic injected into mice augmented the delay type hypersensitivity response against intra-tumour injected in mice and significantly suppressed the tumour growth by augmenting CD8 + T-cell infiltration into the tumour site, thereby causing cytotoxic actions (Ebrahimi et al., 2013).

5.5. Anti-cancer and anti-proliferative activities

Recently, bioactive peptides from garlic and other natural resources have gained attention in cancer research due to their abundance as promising ingredients in the formulation of functional foods and pharmaceuticals and their low toxicity and minimal side effects. A novel peptide, VKLRSLCS (VS-9), from aged garlic extract exhibited anti-cancer activity by suppressing the expansion of MOLT-4 and K562 leukemic cell lines after treatment for 24 and 48 h, with IC₅₀ of 0.84 mM for MOLT-4 cells and 1.57 mM for K562 cells. The study suggested that the VS-9 peptide has anti-cancer potential due to its apoptotic actions against leukemic cell lines via the anti-apoptotic Bcl-2 protein family (Rasaratnam et al., 2021). VS-9 is amphiphilic, which promotes its anti-cancer actions through the destabilisation of the membrane and cytotoxicity of cancer cells. Furthermore, serine-containing peptides have

been reported to arrest cell cycle propagation and cause the death of colon cancer cells (Luna-Vital et al., 2015). Moreover, a garlic protein fraction containing three polypeptides was reported to attenuate the expression of the cellular immune response against breast-induced tumours in BALA/c mice experiments (Ebrahimi et al., 2013). Lectin 50 (50 kDa) isolated from aged garlic bulbs and further purified using gel filtration chromatography on Sephacryl S-200 column showed a strong anti-proliferative action on oral carcinoma KB cells with an IC₅₀ of 36 µg/mL and induced cancer cell apoptosis by triggering the activity of caspase enzyme (Kumar et al., 2015). A protein, alliumin (13 kDa), isolated from multiple-clove garlic bulbs and purified using ion exchange chromatography, affinity chromatography, and gel filtration exerted anti-proliferative activity on leukaemia L1210 cells but not on hepatoma Hep G2 cells (Xia & Ng, 2005). This suggests potential differences in cell surface receptors or bioactivity mechanisms in the different cells.

Furthermore, Saini et al. (2021) reported that a 10-kDa fresh garlic extract protein induced cell death at 300 µg/mL in INT-407 intestinal epithelial cells, unlike the boiled garlic extract. At a dose of 300 µg/mL, the cell exhibited a distorted cell elongation followed by enlargement of the cell, rounding up, and fragmentation of the cell, which are indications of cell apoptosis as verified using Hoechst 33342 nuclear staining and flow cytometry. The inactivity of boiled garlic extract suggests that the bioactive garlic proteins are heat-labile and, therefore, require moderate cooking before consumption to reduce the potential cytotoxicity in the early stage of cancer of the intestine.

5.6. Anti-hypertensive and cardioprotective activities

The global prevalence of cardiovascular diseases is steadily on the rise and has raised global concerns and interest in research for its treatment and management (Okagu, Ezeorba et al., 2022). An old study has shown the anti-hypertensive activities of Garlic peptides and their activities on angiotensin 1-converting enzymes (ACE), a key cardiovascular enzyme. Peptides with potential ACE were isolated from garlic using Dowex 50 W and Sephadex G-25 chromatography. Seven dipeptides with ACE inhibitory activities were identified as Ser-Tyr, Gly-Tyr, Phe-Tyr, Asn-Tyr, Ser-Phe, Gly-Phe, and Asn-Phe with either tyrosine or phenylalanine residue at the C terminus. In contrast, dipeptides with Tyr exhibited more potency than those with Phe. Administration of 200 mg/kg at a single dose of the various dipeptides at different time intervals to hypertensive rats drastically lowered the systolic blood pressure (SBP) (Suetsuna, 1998). This potential biological function of garlic peptide has remained unexplored in recent research. More research focus could delve into the search for new garlic peptides with cardioprotection and their possible mechanisms of action.

5.7. Other biofunctional roles of garlic proteins and peptides

Bioactive proteins or peptides from garlic have been reported to enhance the bioavailability of trace minerals for human health benefits. Bai et al. (2014) isolated and characterised two dipeptides ((SC2R2C7)-γ-L-glutamyl-S-allyl-L-cysteine and (SC2R2C7)-γ-L-glutamyl-S-propyl-L-cysteine) from fresh garlic scales by ion-exchange chromatography, HPLC-MS, CD, ¹H NMR, and ¹³C NMR. Both dipeptides improved the bioavailability of the micronutrients in soybean and mung beans in a simulated gastrointestinal system. Adding the garlic peptides at a concentration of 0.01 mmol/5 g legume increased the bioavailability of iron from soybeans from 1.88 % to 6.73 % and 4.42 % and for mung beans from 2.52 % to 12.04 % and 9.38 %, respectively. Similarly, the bioavailability of zinc also increased from 13.37 to 23.95 % and 20.58 % in soybeans and for mung beans from 15.98 % to 28.44 % and 27.05 %, respectively (Bai et al., 2014). Despite this strong potential of the garlic peptides, additional studies are needed to unravel the myriad of possibilities around improving micronutrient bioavailability *in vivo* as well as the associated mechanisms.

Garlic leaf proteins were also shown to exhibit insecticidal activities and plant protection against the homopteran group of polyphagous-sucking insect pests. Dutta et al. (2005) expressed the coding sequences of a mannose-binding 25-kDa homodimeric protein from garlic leave (ASAL) in a tobacco plant using an agrobacterium transformation system and cauliflower mosaic virus (CaMV) 35S promoter. Transformed plants were reported to produce 0.68–2 % soluble ASAL protein, and this expression fostered the decrease in Aphid (*Myzus persicae*) survival by 16–20 % in To and T1 progeny compared to 75 % survival in the untransformed tobacco after 144 h incubation (Dutta et al., 2005). The future assay could consider investigating the potentials of the isolated protein, *in vitro* or *ex vivo*, in inhibiting the survival of various plant pests and disease-spreading pathogens.

6. Potential limitations and future prospects

Substantial research efforts have been directed toward unravelling the biofunctional potentials of bioactive proteins and peptides from different sources (Okoye et al., 2022). Due to their availability and high protein content, leguminous plants such as soybean, mung bean, cowpea, and others have been studied in-depth as potential sources of bioactive peptides. However, little interest has been directed toward spices or medical plants as sources of bioactive peptides. With the rapid increase in the understanding of the diverse structural functionality and bioactivity of several novel peptides, there is a need to direct research efforts to seeking sustainable and alternative sources of potent bioactive peptides (Ejike et al., 2017; Okagu, Aham, et al., 2022).

Although garlic as a culinary and medical plant has been well studied, there is a paucity of up-to-date knowledge and understanding of its bioactive proteins and peptides. This is possible because of its lower protein content than other conventional sources such as legumes and milk. Hence, it would be difficult to achieve a significant amount of specific garlic bioactive peptides for practical applications in functional food and nutraceutical formation (Munir et al., 2021). Future studies should explore the overproduction of well-established garlic peptides with known biological activities using heterologous expression systems.

The advent of advanced biotechnological approaches has paved the way for more high-throughput systems, which promise better yields of the bioactive peptides.

Another limitation is the low stability of garlic proteins and peptides and their susceptibility to degradation by proteases. An older study reported the spontaneous conversion of γ-glutamyl peptides from garlic to S-alk(en)yl-L-cysteine sulphoxides and asserted that the peptides were part of the intermediates of the biosynthetic pathway of favouring agents (Lancaster & Shaw, 1989). Moreover, other evidence of its poor stability can be deduced from the numerous proteases reported *in situ* in garlic cloves (Halmi et al., 2014; Malik et al., 2004). Future research may consider investigating and seeking safe garlic protease inhibitors to bring under control the *in situ* protein degradative system (Shamsi et al., 2016).

Finally, the few available studies have failed to establish the correlation between the garlic variety/cultivar, their age/maturity as well as their post-harvest treatment to their protein/biopeptide contents and bioactivities. Moreover, despite the interesting biological activities being reported so far from garlic protein and peptides, there are sparingly available data or information on their bioavailability and stability *in vivo*, possibly limiting their real-life application as nutraceutical and for human health. Therefore, future studies could reveal the stability and bioavailability profiles of some of these interesting peptides from garlic with excellent bioactivities.

7. Conclusions

Despite the widespread awareness of the therapeutic significance of garlic, reported in countless published studies on the bioactivity of its phytochemicals, little attention has been given to the potential of its

bioactive proteins or peptides. This review discussed available literature on garlic bioactive proteins and peptides, focusing on its nature, extraction, and biological activities. Some studies showed garlic proteins and peptides' antioxidative, antimicrobial, anti-cancer, and other pharmacological potentials. However, the practical application of bioactive proteins for health and therapeutic benefits is far from actualisation at the time of this review. Available reports on this subject were based on *in vitro* and *ex vivo* experimental models, with sparse animal studies and no human clinical trials recorded.

Moreover, studies on the stability and bioavailability of the peptides are missing. Specifically, studies on the ability of the garlic peptides to resist gastrointestinal proteases and p-glycoprotein efflux are needed, as well as other parameters to ascertain their bioavailability. Finally, there is a dearth of information on the safety of various purified garlic peptides, which is crucial in ensuring no adverse effects at therapeutic doses. Future studies should be directed to fill the lacuna in knowledge and promote the practical application of garlic bioactive proteins and peptides.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- Ahmadabad, H. N., Hassan, Z. M., Safari, E., Bozorgmehr, M., Ghazanfari, T., & Moazzeni, S. M. (2011). Evaluation of the immunomodulatory effect of the 14kDa protein isolated from aged garlic extract on dendritic cells. *Cellular Immunology*, 269(2), 90–95. <https://doi.org/10.1016/j.cellimm.2011.02.005>
- Ahmadabad, H. N., Zuhair, M. H., Safari, E., Bozorgmehr, M., & Moazzeni, S. M. (2012). Evaluation of the effect of the 47 kDa protein isolated from aged garlic extract on dendritic cells. *Iranian Journal of Basic Medical Sciences*, 15(2), 745–751.
- Amino, Y., Wakabayashi, H., Akashi, S., & Ishiwatari, Y. (2018). Structural analysis and taste evaluation of γ -glutamyl peptides comprising sulfur-containing amino acids. *Bioscience, Biotechnology, and Biochemistry*, 82(3), 383–394. <https://doi.org/10.1080/09168451.2018.1436433>
- Bai, B., Chen, L. L., Li, Q. L., Duan, Y. Q., Liu, L., Tan, D. H., & Ji, S. J. (2014). Preparation and functional exploration of cysteine peptides from fresh garlic scales for improving bioavailability of food legume iron and zinc. *Chinese Journal of Analytical Chemistry*, 42(10), 1507–1512. [https://doi.org/10.1016/S1872-2040\(14\)60776-3](https://doi.org/10.1016/S1872-2040(14)60776-3)
- Bar, M., Binduga, U. E., & Szychowski, K. A. (2022). Methods of Isolation of Active Substances from Garlic (*Allium sativum* L.) and its impact on the composition and biological properties of garlic extracts. *Antioxidants*, 11(7), 1345. doi: 10.3390/ANTIOX11071345.
- Chandrashekar, P. M., & Venkatesh, Y. P. (2009). Identification of the protein components displaying immunomodulatory activity in aged garlic extract. *Journal of Ethnopharmacology*, 124(3), 384–390. <https://doi.org/10.1016/j.jep.2009.05.030>
- Chukwuma, I. F., Apeh, V. O., Nwora, F. N., Nkwocha, C. C., Mba, S. E., & Ossai, E. C. (2023). Phytochemical profiling and antioxidative potential of phenolic-rich extract of *Cola acuminata* nuts. *Biointerface Research in Applied Chemistry*, 13(1), 29–39.
- Chukwuma, I. F., Apeh, V. O., Nworah, F. N., Nkwocha, C. C., Emaimo, J., Uchenna, L., Ezeanyika, S., & Ogunua, V. N. (2022). Inhibition of phospholipase A2 and prostaglandin synthase activities as possible mechanistic insight into the anti-inflammatory activity of *Brenania bryei* methanol and chloroform fractions. *Thai Journal of Pharmaceutical Sciences*, 46(1), 75–84.
- Clement, F., Pramod, S. N., & Venkatesh, Y. P. (2010). Identity of the immunomodulatory proteins from garlic (*Allium sativum*) with the major garlic lectins or agglutinins. *International Immunopharmacology*, 10(3), 316–324. <https://doi.org/10.1016/j.intimp.2009.12.002>
- Cruz-Casas, D. E., Aguilar, C. N., Ascacio-Valdés, J. A., Rodríguez-Herrera, R., Chávez-González, M. L., & Flores-Gallegos, A. C. (2021). Enzymatic hydrolysis and microbial fermentation: The most favorable biotechnological methods for the release of bioactive peptides. *Food Chemistry: Molecular Sciences*, 3, Article 100047. <https://doi.org/10.1016/J.FOCHMS.2021.100047>
- Daneshmandi, S., Hajimoradi, M., Ahmadabad, H. N., Hassan, Z. M., Roudbary, M., & Ghazanfari, T. (2011). Effect of 14-kDa and 47-kDa protein molecules of aged garlic extract on peritoneal macrophages. *Immunopharmacology and Immunotoxicology*, 33(1), 21–27. <https://doi.org/10.3109/08923971003690041>
- Dutta, I., Saha, P., Majumder, P., Sarkar, A., Chakraborti, D., Banerjee, S., & Das, S. (2005). The efficacy of a novel insecticidal protein, *Allium sativum* leaf lectin (ASAL), against homopteran insects monitored in transgenic tobacco. *Plant Biotechnology Journal*, 3(6), 601–611. <https://doi.org/10.1111/J.1467-7652.2005.00151.X>
- Ebrahimi, M., Hassan, Z. M., Mostafaie, A., Mehrjardi, N. Z., & Ghazanfari, T. (2013). Purified protein fraction of garlic extract modulates cellular immune response against breast transplanted tumors in BALB/c mice model. *Cell Journal*, 15(1), 65–74.
- Ejike, C. E. C. C., Collins, S. A., Balasuriya, N., Swanson, A. K., Mason, B., & Udenigwe, C. C. (2017). Prospects of microalgae proteins in producing peptide-based functional foods for promoting cardiovascular health. *Trends in Food Science & Technology*, 59, 30–36. <https://doi.org/10.1016/J.TIFS.2016.10.026>
- Espinoza, T., Valencia, E., Albarrán, M., Díaz, D., Quevedo, R., Díaz, O., & Bastías, J. (2020). Garlic (*Allium sativum* L) and its beneficial properties for health: A review. *Agroindustrial Science*, 10(1), 103–115. <https://doi.org/10.17268/agroind.sci.2020.01.14>
- Ezeorba, P. T. C., Chukwudozie, I. K., Ezema, C. A., Anaduaka, E. G., & Nweze, E. (2022). Potentials for health and therapeutic benefits of garlic essential oils: Recent findings and future prospects Pharmacological Research - Modern Chinese Medicine Potentials for health and therapeutic benefits of garlic essential oils: Recent findings and. *Pharmacological Research - Modern Chinese Medicine*, 3(March), Article 100075. <https://doi.org/10.1016/j.prcm.2022.100075>
- Ferreras, J. M., Clemencia, M. C. M., Hizon-Fradejas, A., Uy, L. Y., & Torio, M. A. (2021). Isolation, purification and characterisation of proteins in 'señorita' banana (*Musa acuminata* (aaa) 'senorita') pulp with bioactive peptides exhibiting anti-hypertensive and antioxidant activities. *Applied Sciences (Switzerland)*, 11(5), 1–13. <https://doi.org/10.3390/app11052190>
- Gao, X., Chen, Y., Chen, Z., Xue, Z., Jia, Y., Guo, Q., Ma, Q., Zhang, M., & Chen, H. (2019). Identification and antimicrobial activity evaluation of three peptides from laba garlic and the related mechanism. *Food & Function*, 10(8), 4486–4496. <https://pubs.rsc.org/en/content/articlehtml/2019/fo/c9fo00236g>
- Gao, X., Xue, Z., Ma, Q., Guo, Q., Xing, L., Santhanam, R. K., Zhang, M., & Chen, H. (2020). Antioxidant and anti-hypertensive effects of garlic protein and its hydrolysates and the related mechanism. *Journal of Food Biochemistry*, 44(2), 1–12. <https://pubmed.ncbi.nlm.nih.gov/31877235/>
- Ghazanfari, T., Hassan, Z. M., & Ebrahimi, M. (2002). Immunomodulatory activity of a protein isolated from garlic extract on delayed type hypersensitivity. *International Immunopharmacology*, 2, 1541–1549.
- Guha, S., & Majumder, K. (2018). Structural-features of food-derived bioactive peptides with anti-inflammatory activity: A brief review. *Journal of Food Biochemistry*, 43, e12531.
- Gupta, A., & Sandhu, R. S. (1997). A new high molecular weight agglutinin from garlic. pdf. In *Molecular and cellular biochemistry* (Issue 166, pp. 1–9).
- Halmi, M. I. E., Sakeh, N. S. M., Masdor, N. A., Gunasekaran, B., Wasoh, H., Syed, M. A., Johari, W. L. W., & Shukur, M. Y. (2014). The application of plant proteases from garlic (*Allium sativum*) for biomonitoring of heavy metals in the environment. *Asian Journal of Plant Biology*, 2(2), 53–59. [10.54987/AJPB.V2I2.182](https://doi.org/10.54987/AJPB.V2I2.182)
- Hassan, Z. M., Yaraee, R., Zare, N., Ghazanfari, T., Nejad, A. H. S., & Nozari, B. (2003). Immunomodulatory effect of R10 fraction of garlic extract on natural killer activity. *International Immunopharmacology*, 3(10–11), 1483–1489. [https://doi.org/10.1016/S1567-5769\(03\)00161-9](https://doi.org/10.1016/S1567-5769(03)00161-9)
- Horita, C. N., Farias-Campomanes, A. M., Barbosa, T. S., Esmerino, E. A., da Cruz, A. G., Bolini, H. M. A., Meireles, M. A. A., & Pollonio, M. A. R. (2016). The antimicrobial, antioxidant and sensory properties of garlic and its derivatives in Brazilian low-sodium frankfurters along shelf-life. *Food Research International*, 84, 1–8. <https://doi.org/10.1016/j.foodres.2016.02.006>
- Jia, L., Wang, L., Liu, C., Liang, Y., & Lin, Q. (2021). Bioactive peptides from foods: Production, function, and application. *Food and Function*, 12(16), 7108–7125. <https://doi.org/10.1039/d1fo01265g>
- Kodera, Y., Ushijima, M., Amano, H., Suzuki, J. I., & Matsutomo, T. (2017). Chemical and biological properties of s-1-propenyl-L-cysteine in aged garlic extract. *Molecules*, 22(4), 1–18. <https://doi.org/10.3390/molecules22040570>
- Kong, X., Yang, M., Abbas, H. M. K., Wu, J., Li, M., & Dong, W. (2018). Antimicrobial genes from *Allium sativum* and *Pinellia ternata* revealed by a *Bacillus subtilis* expression system. *Scientific Reports*, 8(1), 1–12. doi: 10.1038/s41598-018-32852-x.
- Kovarović, J., Bystrická, J., Vollmannová, A., Tóth, T., & Brindza, J. (2019). Biologically valuable substances in garlic (*Allium sativum* L.) – A review. *Journal of Central European Agriculture*, 20(1), 292–304. <https://doi.org/10.5513/JCEA01/20.1.2304>
- Kulikova, O. G., Mal'tsev, D. I., Kartashov, M. I., Il'Ina, A. P., Yamskova, V. P., & Yamskov, I. A. (2016). Proteins from garlic *Allium sativum* L. Which are active against fungal pathogens of barley, wheat and rice, and can stimulate seeds in garlic. *Sel'skokhozyaystvennaya Biologiya*, 51(5), 705–713. [10.15389/AGROBIOLOGY.2016.5.705ENG](https://doi.org/10.15389/AGROBIOLOGY.2016.5.705ENG)
- Kumar, S., Jitendra, K., Singh, K., Kapoor, V., Sinha, M., Xess, I., Das, S. N., Sharma, S., Singh, T. P., & Dey, S. (2015). Biological properties and characterisation of ASL50 protein from Aged *Allium sativum* bulbs. *Applied Biochemistry and Biotechnology*, 176(7), 1914–1927. <https://pubmed.ncbi.nlm.nih.gov/26043852/>
- Lancaster, J. E., & Shaw, M. L. (1989). γ -Glutamyl peptides in the biosynthesis of S-alk(enyl)-L-cysteine sulphoxides (flavour precursors) in *Allium*. *Phytochemistry*, 28(2), 455–460. [https://doi.org/10.1016/0031-9422\(89\)80031-7](https://doi.org/10.1016/0031-9422(89)80031-7)
- Li, S., Li, N., Wang, Y., Wang, Q., Li, R., Zhang, M., Panichayupakaranant, P., Gao, Y., & Chen, H. (2022). Structural, functional and proteomic differences of proteins extracted from white garlic and Laba garlic. *Food Research International*, 155 (February), Article 111047.
- Luna-Vital, D. A., Mojica, L., González de Mejía, E., Mendoza, S., & Loarca-Piña, G. (2015). Biological potential of protein hydrolysates and peptides from common bean (*Phaseolus vulgaris* L.): A review. *Food Research International*, 76(P1), 39–50. <https://doi.org/10.1016/j.foodres.2014.11.024>

- Ma, H., Huang, L., Peng, L., Wang, Z., & Yang, Q. (2015). Pretreatment of garlic powder using sweep frequency ultrasound and single frequency countercurrent ultrasound: Optimisation and comparison for ACE inhibitory activities. *Ultrasonics Sonochemistry*, 23, 109–115. <https://doi.org/10.1016/j.ULTSONCH.2014.10.020>
- Majumder, K., Mine, Y., & Wu, J. (2016). The potential of food protein derived anti-inflammatory peptides against various chronic inflammatory diseases. *Journal of the Science of Food and Agriculture*, 96(7), 2303–2311.
- Malik, M. N., Spivack, W. D., Sheikh, A. M., & Fenko, M. D. (2004). The 26S proteasome in garlic (*Allium sativum*): Purification and partial characterisation. *Journal of Agricultural and Food Chemistry*, 52(11), 3350–3355. <https://doi.org/10.1021/JF035309R>
- Marzouki, S., Limam, F., & Smaali, M. (2005). A new thermostable peroxidase from garlic *Allium sativum*. *Applied Biochemistry*, 127, 201–214.
- Milner, J. A. (2001). Recent advances on the nutritional effects associated with the use of garlic as a supplement a historical perspective on garlic and cancer. *Journal of Nutrition*, 1, 1027–1031.
- Munir, N., Khilji, S. A., Shabir, M., & Sajid, Z. A. (2021). Exogenous application of ascorbic acid enhances the antimicrobial and antioxidant potential of *Ocimum sanctum* L. grown under salt stress. *Journal of Food Quality*, 2021. doi: 10.1155/2021/4977410.
- Nakamoto, M., Fujii, T., Matsutomo, T., & Kodera, Y. (2018). Isolation and identification of three γ -Glutamyl tripeptides and their putative production mechanism in aged garlic extract. *Journal of Agricultural and Food Chemistry*, 66(11), 2891–2899. <https://doi.org/10.1021/ACS.JAFC.7B05480>
- Nassimi, Z., Taheri, P., Kong, X., Dong, W., & Tarighi, S. (2021). The antimicrobial peptide AsR416 can inhibit the growth, sclerotium formation and virulence of *Rhizoctonia solani* AG1-IA. *European Journal of Plant Pathology*, 160(2), 469–485. <https://doi.org/10.1007/s10658-021-02257-0>
- Nwachukwu, I. D., Sarteshtnizi, R. A., Udenigwe, C. C., & Aluko, R. E. (2021). A concise review of current in vitro chemical and cell-based antioxidant assay methods. *Molecules*, 26(16). <https://doi.org/10.3390/molecules26164865>
- Okagu, I. U., Aham, E. C., Ezeorba, T. P. C., Ndefo, J. C., Aguchem, R. N., & Udenigwe, C. C. (2022). Osteo-modulatory dietary proteins and peptides: A concise review. *Journal of Food Biochemistry*, 46(10), e14365.
- Okagu, I. U., Ezeorba, T. P. C., Aham, E. C., Aguchem, R. N., & Nechi, R. N. (2022). Recent findings on the cellular and molecular mechanisms of action of novel food-derived anti-hypertensive peptides. *Food Chemistry: Molecular Sciences*, 4, Article 100078. <https://doi.org/10.1016/j.fochms.2022.100078>
- Okagu, I. U., Ndefo, J. C., Aham, E. C., Obeme-Nmom, J. I., Agboinghale, P. E., Aguchem, R. N., Nechi, R. N., & Lammi, C. (2021). Lupin-derived bioactive peptides: Intestinal transport, bioavailability and health benefits. *Nutrients*, 13(9). <https://doi.org/10.3390/nu13093266>
- Okoye, C. O., Ezeorba, T. P. C., Okeke, E. S., & Okagu, I. U. (2022). Recent findings on the isolation, identification and quantification of bioactive peptides. *Applied Food Research*, 2(1), Article 100065. <https://doi.org/10.1016/J.AFRES.2022.100065>
- Padiyappa, S. D., Avalappa, H., Somegowda, M., Sridhara, S., Venkatesh, Y. P., Prabhakar, B. T., Pramod, S. N., Almajaydil, M. S., Shokrala, S., Abdelbacki, A. M. M., Elansary, H. O., El-Sabrou, A. M., & Mahmoud, E. A. (2022). Immunoadjuvant and humoral immune responses of garlic (*Allium sativum* L.) lectins upon systemic and mucosal administration in BALB/c mice. *Molecules*, 27(4). <https://doi.org/10.3390/molecules27041375>
- Petropoulos, S. A., Fernandes, A., Ntatsi, G., Petrotos, K., Barros, L., & Ferreira, I. C. F. R. (2018). Nutritional value, chemical characterisation and bulb morphology of Greek Garlic landraces. *Molecules*, 23(2), 1–14. <https://doi.org/10.3390/molecules23020319>
- Rabe, S. Z. T., Ghazanfari, T., Siadat, Z., Rastin, M., Rabe, S. Z. T., & Mahmoudi, M. (2015). Anti-inflammatory effect of garlic 14-kDa protein on LPS-stimulated-J774A.1 macrophages. *Immunopharmacology and Immunotoxicology*, 37(2), 158–164. <https://doi.org/10.3109/08923973.2015.1005229>
- Rasaratnam, K., Nantasenamat, C., Phaonakrop, N., Roytrakul, S., & Tanyong, D. (2021). A novel peptide isolated from garlic shows anti-cancer effect against leukemic cell lines via interaction with Bcl-2 family proteins. *Chemical Biology & Drug Design*, 97(5), 1017–1028. <https://doi.org/10.1111/cbdd.13831>
- Saini, N., Kadian, M., Khera, A., Aggarwal, A., & Kumar, A. (2021). Therapeutic potential of *Allium sativum* against the β (1–40)-induced oxidative stress and mitochondrial dysfunction in the Wistar rats. *American Journal of Neurodegenerative Disease*, 10(2), 13–27.
- Santini, A., & Cicero, N. (2020). Development of food chemistry, natural products, and nutrition research: Targeting new frontiers. *Foods*, 9(4), 482. <https://doi.org/10.3390/FOODS9040482>
- Sasi, M., Kumar, S., Kumar, M., Thapa, S., Prajapati, U., Tak, Y., Changan, S., Saurabh, V., Kumari, S., Kumar, A., Hasan, M., Chandran, D., Radha, Bangar, S. P., Dhupal, S., Senapathy, M., Thiyagarajan, A., Alhariri, A., Dey, A., ... Mekhemar, M. (2021). Garlic (*Allium sativum* L.) bioactives and its role in alleviating oral pathologies. *Antioxidants*, 10(11). <https://doi.org/10.3390/antiox10111847>
- Shamsi, T. N., Parveen, R., Amir, M., Baig, M. A., Irfan Qureshi, M., Ali, S., & Fatima, S. (2016). *Allium sativum* protease inhibitor: A novel kunitz trypsin inhibitor from garlic is a new comrade of the serpin family. *PLoS One*, 11(11), 1–16. <https://doi.org/10.1371/journal.pone.0165572>
- Shang, A., Cao, S. Y., Xu, X. Y., Gan, R. Y., Tang, G. Y., Corke, H., Mavumengwana, V., & Li, H. B. (2019). Bioactive compounds and biological functions of garlic (*Allium sativum* L.). *Foods*, 8(7), 1–31. <https://doi.org/10.3390/foods8070246>
- Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., ... Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694. <https://doi.org/10.3389/fphys.2020.00694/BIBTEX>
- Snoussi, M., Noumi, E., Mosbah, A., Redissi, A., Saeed, M., Tasleem, M., Alreshidi, M., Adnan, M., Al-Rashidi, A., Siddiqui, A. J., Aouadi, K., De Feo, V., & Kadri, A. (2022). Tripeptides from *Allium subhirsutum* L. extracts: Pharmacokinetics properties, toxicity prediction and in silico study against SARS-CoV-2 enzymes and pro-inflammatory proteins. *Cellular and Molecular Biology (Noisy-Le-Grand, France)*, 67(4), 143–162. <https://doi.org/10.14715/CMB/2021.67.4.17>
- Subroto, E., Cahyana, Y., Tensiska, M., Filianty, F., Lembong, E., Wulandari, E., Kurniati, D., Saputra, R. A., & Faturachman, F. (2021). Bioactive compounds in garlic (*Allium sativum* L.) as a source of antioxidants and its potential to improve the immune system: A review. *Food Research*, 5(6), 1–11. [https://doi.org/10.26656/FR.2017.5\(6\).042](https://doi.org/10.26656/FR.2017.5(6).042)
- Suetsuna, K. (1998). Isolation and characterisation of angiotensin I-converting enzyme inhibitor dipeptides derived from *Allium sativum* L. (garlic). *Journal of Nutritional Biochemistry*, 9(7), 415–419. [https://doi.org/10.1016/S0955-2863\(98\)00036-9](https://doi.org/10.1016/S0955-2863(98)00036-9)
- Sun, Y., Hu, J., Wang, W., Zhang, B., & Shen, Y. (2019). Characterisation of γ -glutamyltranspeptidases from dormant garlic and onion bulbs. *Food Science and Nutrition*, 7(2), 499–505. <https://doi.org/10.1002/fsn3.820>
- Tahir, Z., Saeed, F., Nosheen, F., Ahmed, A., & Anjum, F. M. (2022). Comparative study of nutritional properties and antioxidant activity of raw and fermented (black) garlic. *International Journal of Food Properties*, 25(1), 116–127. <https://doi.org/10.1080/10942912.2022.2026954>
- Tan, D., Zhang, Y., Chen, L., Liu, L., Zhang, X., Wu, Z., Bai, B., & Ji, S. (2015). Decreased glycation and structural protection properties of γ -glutamyl-S-allyl-cysteine peptide isolated from fresh garlic scales (*Allium sativum* L.). *Natural Product Research*, 29(23), 2219–2222. <https://doi.org/10.1080/14786419.2014.1003065>
- Valle-Rodríguez, C., Valdés-Rodríguez, S., Vasco-Méndez, N., Garcidueñas-Piña, C., Soria-Guerra, R., & Morales-Domínguez, J. (2017). Biochemical and molecular evidences of the presence of a plant natriuretic peptide in garlic (*Allium sativum* L.). *Phyton*, 86(1), 143–150. <https://doi.org/10.32604/phyton.2017.86.143>
- Wang, H. X., & Ng, T. B. (2001). Purification of allivin, a novel antifungal protein from bulbs of the round-cloved garlic. *Life Sciences*, 70(3), 357–365. [https://doi.org/10.1016/S0024-3205\(01\)01399-6](https://doi.org/10.1016/S0024-3205(01)01399-6)
- Wang, Y., Zou, T., Xiang, M., Jin, C., Zhang, X., Chen, Y., Jiang, Q., & Hu, Y. (2016). Purification and characterisation of a soluble glycoprotein from garlic (*Allium sativum*) and its in vitro bioactivity. *Preparative Biochemistry & Biotechnology*, 46(7), 709–716. <https://doi.org/10.1080/10826068.2015.1135454>
- Xi, K., Yang, M., Abbas, H. M. K., Wu, J., Li, M., & Dong, W. (2018). Antimicrobial genes from *Allium sativum* and *Pinellia ternata* revealed by *Bacillus subtilis* expression system. *Scientific Reports*, 8, 14514.
- Xia, L., & Ng, T. B. (2005). Isolation of alliumin, a novel protein with antimicrobial and anti-proliferative activities from multiple-cloved garlic bulbs. *Peptides*, 26(2), 177–183. <https://doi.org/10.1016/j.peptides.2004.09.019>
- Yoshimoto, N., Yabe, A., Sugino, Y., Murakami, S., Sai-Ngum, N., Sumi, S. I., Tsuneyoshi, T., & Saito, K. (2015). Garlic γ -glutamyl transpeptidases that catalyse deglutamylation of biosynthetic intermediate of alliin. *Frontiers Plant Science*, 5 (JAN), 1–12. <https://doi.org/10.3389/fpls.2014.00758>
- Yu, X., Lim, C. Y. X., Dong, B., & Hadinoto, K. (2020). Development of magnetic solid phase extraction platform for the purification of bioactive γ -glutamyl peptides from garlic (*Allium sativum*). *LWT*, 127(October 2019). <https://doi.org/10.1016/j.lwt.2020.109410>
- Zaky, A. A., Shim, J. H., & Abd El-Aty, A. M. (2021). A review on extraction, characterisation, and applications of bioactive peptides from pressed black cumin seed cake. *Frontiers in Nutrition*, 8(September), 1–11. <https://doi.org/10.3389/fnut.2021.743909>
- Zhang, C., Sanders, J. P. M., Xiao, T. T., & Bruins, M. E. (2015). How does alkali aid protein extraction in green tea leaf residue: A basis for integrated biorefinery of leaves. *PLoS One*, 10(7), 1–14. <https://doi.org/10.1371/journal.pone.0133046>