

Article

Anticancer Peptides Derived from Lupin (*Lupinus albus*) Conglutins Identified Through In Silico Enzymatic Hydrolysis and Computational Screening

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Abstract

Cancer remains a major global health challenge, and computational approaches can support the early-stage identification of peptide candidates for experimental evaluation. This study aimed to identify putative anticancer peptides (ACPs) encrypted within *Lupinus albus* β - and γ -conglutins using an in silico screening strategy. Protein sequences from two β -conglutins and two γ -conglutins were subjected to simulated enzymatic hydrolysis with pepsin, trypsin, and chymotrypsin. The resulting peptides were evaluated using PeptideRanker for bioactivity prediction, AntiCP 2.0 for anticancer peptide prediction, ToxinPred 3.0 for toxicity assessment, and ProtParam for physicochemical characterization. Simulated hydrolysis generated 1610 peptide fragments, of which 749 met the length criteria for downstream analysis. PeptideRanker identified 124 peptides with predicted bioactive potential, and AntiCP 2.0 classified 47 as putative ACPs. Toxicity screening reduced this number to 31 peptides predicted as non-toxic, resulting in 18 unique putative non-toxic ACP candidates after redundancy removal. Among these, 16 met the minimum sequence length required for complete ProtParam characterization. These findings indicate that *L. albus* conglutins may contain encrypted peptide sequences computationally prioritized as putative ACP candidates, whose activity, selectivity, toxicity, and safety require in vitro and/or in vivo validation.

Keywords: lupin; bioactive peptides; conglutins; anticancer; in silico



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1. Introduction

Cancer represents a major global health challenge due to its high incidence, mortality, and clinical complexity. In 2018, approximately 18.07 million new cancer cases and 9.56 million deaths were reported worldwide, increasing to 19.3 million cases and 10 million deaths in 2020. These figures highlight the sustained global burden of cancer and the need for continued research into improved prevention, diagnosis, and treatment strategies. Although chemotherapy, radiotherapy, and surgery remain widely used, their effectiveness can be limited by drug resistance, systemic toxicity, and low selectivity, which may result in damage to healthy cells. Consequently, there is increasing interest in more selective

therapeutic approaches, including targeted therapies, immunotherapy, and peptide-based strategies. Among these, anticancer peptides (ACPs) have attracted attention as molecules with potential antitumor activity and possible applications in future therapeutic development [1–4].

With scientific and technological advances, a large number of peptides with diverse bioactivities have been discovered, including peptides with antitumor properties, known as anticancer peptides (ACPs) [5]. Given that conventional cancer treatments present limitations such as toxicity, drug resistance, and low selectivity, ACPs have emerged as a promising alternative due to their greater specificity, lower toxicity, and both therapeutic and diagnostic potential, as well as their use in combination therapies to improve treatment efficacy [6]. ACPs are generally short peptides, commonly reported within broad length ranges of approximately 5 to 100 amino acids, although many described ACPs fall between 15 and 50 residues. Their activity is often associated not only with length, but also with sequence composition, net charge, hydrophobicity, and the presence of basic residues such as lysine and arginine, which favor interactions with negatively charged cancer cell membranes [7,8].

In this context, the production of bioactive peptides from food sources represents a relevant alternative; this process is primarily carried out through the hydrolysis of proteins by the action of proteolytic enzymes, as well as through chemical methods or microbial fermentation [9]. Accordingly, bioactive peptides derived from food proteins are considered key components in functional foods and nutraceuticals due to their ability to modulate physiological functions and contribute to the prevention of chronic diseases [10,11]. This has driven research into the production of bioactive peptides from underutilized food sources and protein by-products [12].

Given this, the growing interest in plant-based diets has driven the search for diverse protein sources, such as legumes, oilseeds, cereals, and pseudocereals. Among them, *Lupinus* species stand out due to their high protein content and their potential as a source of bioactive peptides, with conglutins being their main protein fraction, representing approximately 95% of the total [13–15]. Peptides derived from lupin constitute a promising source of sequences with biological activity, including anti-inflammatory, antioxidant, and neuroprotective effects, as well as inhibitory activities against angiotensin-converting enzyme (ACE) and dipeptidyl peptidase IV (DPP-IV), in addition to potential anticancer properties. However, studies focused specifically on anticancer peptides derived from lupin remain limited. Previous reports have described cancer-related effects for different lupin-derived materials, including lunasin or lunasin-like peptides, albumin and globulin fractions from *Lupinus albus*, and peptides identified from enzymatic hydrolysates, such as VVVVDE. In addition, lupin protein hydrolysates and peptide-containing fractions have been associated with anti-inflammatory and other bioactive effects [16–18]. Since conglutins constitute a major fraction of lupin seed proteins, these findings support the exploration of conglutin-derived peptides as putative bioactive candidates, although they do not confirm anticancer activity for the individual peptides identified in the present study.

To address these limitations and optimize the identification of compounds with biological potential, *in silico* tools have become established as key strategies in the characterization of bioactive peptides, as they enable the efficient prediction of their activity and mechanisms of action. In contrast to traditional sequential identification methods, bioinformatics approaches allow for peptide detection through virtual screening and the analysis of their structure–activity relationships. In this regard, various studies on lupin-derived peptides employing computational approaches have demonstrated their inhibitory potential against enzymes such as ACE, α -glucosidase, and α -amylase, as well as their ability to interact with

Fe²⁺, thereby facilitating the selection of compounds with nutraceutical and therapeutic applications [19–22].

Despite advances in the identification of bioactive peptides derived from lupin, limitations still exist in the systematic evaluation of their anticancer potential. In light of this issue, the present study aims to identify and prioritize putative anticancer peptide candidates derived from *Lupinus albus* conglutins through a computational approach integrating in silico enzymatic hydrolysis and virtual screening. This approach was intended to select peptide sequences with predicted bioactivity, anticancer potential, non-toxicity, and relevant physicochemical properties for subsequent biological testing, rather than to confirm their anticancer activity or therapeutic applicability.

2. Materials and Methods

2.1. Protein Sequence Retrieval

Sequences from *Lupinus albus* conglutins were obtained from the UniProt database [23] (<https://www.uniprot.org/>; accessed on 5 May 2026; UniProt release 2025_04). The canonical FASTA sequences available for γ -conglutin 1 (Q9FSH9), γ -conglutin 2 (Q9FEX1), β -conglutin 1 (Q53HY0), and β -conglutin 2 (Q6EBC1) were downloaded and used without manual modification. Therefore, any annotated regions present in the UniProt FASTA sequences, including signal peptides or propeptide regions, were retained. These four sequences were selected because they correspond to reviewed UniProtKB/Swiss-Prot entries for the β - and γ -conglutin families of *Lupinus albus*, which were the storage protein families targeted in this study as potential sources of encrypted bioactive peptides.

2.2. In Silico Hydrolysis

The selected protein sequences were subjected to in silico enzyme-specific hydrolysis using the PeptideCutter [24] tool available on the ExPASy server (https://web.expasy.org/peptide_cutter/; accessed on 5 May 2026). The digestion design consisted of independent single-enzyme simulations. Therefore, each enzyme was applied separately to the complete protein sequences to compare its individual ability to release peptide fragments from *L. albus* conglutins. The enzymes evaluated were pepsin at pH > 2, pepsin at pH 1.3, trypsin, and chymotrypsin under high- and low-specificity settings. For pepsin, both PeptideCutter settings were used: pH 1.3 and pH > 2. According to PeptideCutter, pepsin generally cleaves preferentially at Phe, Tyr, Trp, and Leu in position P1 or P1'. At pH 1.3, cleavage is more specific and occurs preferentially at Phe and Leu in position P1, whereas this specificity is lost at pH $\geq$ 2. Both settings were included to compare peptide release under the two pepsin cleavage specificities implemented by PeptideCutter. The resulting peptide fragments from each enzyme–protein combination were collected independently for downstream screening.

2.3. Prediction of General Peptide Bioactivity

The general bioactive potential of peptides derived from *Lupinus albus* conglutins was evaluated using the PeptideRanker platform [25] (<https://peptide.ucd.ie/peptideranker/>; accessed on 19 May 2026). Peptide sequences obtained after in silico hydrolysis were individually analyzed using the server's default prediction settings. Only peptides with lengths ranging from 4 to 50 amino acids were evaluated at this stage, as this range was required for the subsequent AntiCP 2.0 screening; therefore, smaller fragments were excluded from the analysis.

PeptideRanker employs a machine learning-based approach to estimate the probability of a peptide exhibiting bioactive properties, assigning a score ranging from 0 to 1, where higher values indicate a greater likelihood of general biological activity. In the

present study, a threshold value of ≥ 0.5 was used as an initial screening cutoff to retain peptides with predicted general bioactive potential. This threshold was selected to avoid the premature exclusion of potentially relevant sequences at an early stage of the workflow, since PeptideRanker was used as a preliminary bioactivity filter rather than as a final criterion for anticancer activity. Consequently, peptides with PeptideRanker scores ≥ 0.5 were considered to possess predicted bioactive potential and were therefore selected for subsequent analyses.

2.4. Anticancer Peptide Prediction

The potential anticancer activity of peptides derived from *Lupinus albus* conglutins was evaluated using the AntiCP 2.0 [26] platform (<https://webs.iiitd.edu.in/raghava/anticp2/index.html>; accessed on 3 June 2026). As a subsequent screening step following the PeptideRanker analysis, only peptides previously identified as having predicted bioactive potential (PeptideRanker score ≥ 0.5) were selected for anticancer peptide prediction. These peptide sequences were individually analyzed using the “Predict” module under prediction model 1, which was selected as a predefined AntiCP 2.0 classification option to maintain a standardized server-based workflow. Due to platform limitations, only peptides ranging from 4 to 50 amino acids in length were evaluated. Four-amino-acid peptides were not excluded at this stage because they were within the accepted input range of AntiCP 2.0 and had previously met the PeptideRanker bioactivity threshold. Therefore, these sequences were evaluated as putative candidates in the anticancer peptide prediction step. The default SVM threshold value provided by the server (0.45) was used for classification to avoid introducing an additional user-defined cutoff. Peptides predicted as “AntiCP” were considered potential candidates with anticancer activity and were selected for further analysis.

2.5. Toxicity Evaluation of Peptides

The toxicity of peptides generated from *Lupinus albus* conglutins was evaluated using the ToxinPred 3.0 [27] server (<https://webs.iiitd.edu.in/raghava/toxinpred3/>; accessed on 5 June 2026). As a subsequent screening step following the AntiCP 2.0 analysis, only peptides predicted as anticancer peptides (AntiCP) were selected for toxicity assessment. Peptide sequences were submitted in FASTA format and analyzed using the default prediction settings of the platform (threshold value 0.38). The ML+MERCI hybrid (ensemble) model, reported by the developers as the most accurate predictive approach available in the server, was employed for peptide classification. Peptides predicted as non-toxic were considered suitable candidates for further analyses, whereas peptides predicted as toxic were excluded from subsequent evaluation.

2.6. Physicochemical Characterization of Selected Peptides

The physicochemical properties of the peptides selected after the sequential screening process (predicted as anticancer and predicted as non-toxic) were analyzed using the ProtParam tool [24] available through ExPASy (<https://web.expasy.org/protparam/>; accessed on 8 June 2026). Each peptide sequence was individually submitted to estimate a set of sequence-derived physicochemical parameters. This analysis was conducted to provide a basic physicochemical characterization of the selected peptides as a complementary descriptive step within the computational workflow, rather than as a standalone assessment of anticancer potential. Peptides shorter than five amino acid residues were not subjected to complete ProtParam analysis because they did not meet the minimum sequence length required by the tool.

3. Results

3.1. Selected *Lupinus albus* Conglutin Sequences

Table 1 presents the *Lupinus albus* conglutin sequences selected for the in silico workflow, together with their UniProt entry identifiers and basic sequence characteristics, including protein name and sequence length. These protein sequences were subsequently used as substrates for the simulated enzymatic hydrolysis and downstream peptide screening analyses.

Table 1. Data of the protein collection.

Entry ID	Entry Name	Protein Name	Length (AA)
Q9FSH9	CONG1_LUPAL	γ -conglutin 1	452
Q9FEX1	CONG2_LUPAL	γ -conglutin 2	448
Q53HY0	CONB1_LUPAL	β -conglutin 1	531
Q6EBC1	CONB2_LUPAL	β -conglutin 2	533

3.2. In Silico Enzymatic Hydrolysis of *Lupinus albus* Conglutins

Simulated enzymatic hydrolysis of the selected *Lupinus albus* conglutins generated distinct numbers of sequence fragments depending on the enzyme and cleavage conditions employed (Table 2). Among the evaluated conditions, pepsin at pH > 2 produced the highest number of hydrolysis products for all analyzed proteins, generating between 121 and 122 fragments per sequence. In contrast, chymotrypsin under high-specificity conditions generated the lowest number of fragments, ranging from 34 to 44 products.

Table 2. Summary of total fragments generated.

Enzyme	Protein	Fragments Generated
Pepsin (pH > 2)	γ -conglutin 1	122
	γ -conglutin 2	122
	β -conglutin 1	122
	β -conglutin 2	121
Pepsin (pH 1.3)	γ -conglutin 1	102
	γ -conglutin 2	98
	β -conglutin 1	87
	β -conglutin 2	86
Chymotrypsin-high specificity	γ -conglutin 1	34
	γ -conglutin 2	39
	β -conglutin 1	44
	β -conglutin 2	43
Chymotrypsin-low specificity	γ -conglutin 1	105
	γ -conglutin 2	112
	β -conglutin 1	89
	β -conglutin 2	86
Trypsin	γ -conglutin 1	28
	γ -conglutin 2	27
	β -conglutin 1	71
	β -conglutin 2	72

Differences were also observed between enzymatic specificity conditions and pH-dependent cleavage patterns. Chymotrypsin with low specificity produced considerably higher numbers of fragments compared to the high-specificity setting, while pepsin at pH 1.3 generated fewer hydrolysis products than pepsin at pH > 2. Trypsin digestion also showed variability among proteins, producing between 27 and 72 fragments depending on the analyzed conglutin sequence. Although the initial hydrolysis output included

individual amino acid residues and short fragments, only eligible peptide sequences within the 4–50 amino acid range were retained for downstream prediction analyses. A detailed breakdown of the hydrolysis-derived fragments, including the fragments retained within the 4–50 amino acid range, unique sequences, and peptides retained after each prediction step for each protein–enzyme combination, is provided in the Supplementary Materials.

3.3. Screening of Bioactive Peptides Using PeptideRanker

A total of 1610 peptide fragments were generated through *in silico* enzymatic hydrolysis of the four selected lupin storage proteins. Following sequence length filtering, 749 peptides met the inclusion criterion of 4–50 amino acids required for subsequent computational analyses. These peptides were then evaluated using PeptideRanker to identify sequences with potential biological activity.

Based on the established threshold (score ≥ 0.5), 124 peptides were predicted as bioactive and selected for further analysis (Table 3). PeptideRanker scores among the retained sequences ranged from 0.505789 to 0.965648, indicating variability in predicted general bioactive potential. However, because PeptideRanker was used only as an initial bioactivity screening step, individual high-scoring peptides at this stage were not interpreted as final candidates unless they also passed the subsequent anticancer prediction and toxicity assessment steps. The identified peptides were subsequently subjected to anticancer activity prediction using AntiCP 2.0 to prioritize candidates with potential anticancer properties.

Table 3. Candidate bioactive peptides identified using PeptideRanker.

Protein	Enzyme	Sequence	Score
γ -conglutin 1	Pepsin (pH > 2)	SCAPTF	0.87312
		KRQFTMC	0.683221
		FCHSTQCSRANTHQCF	0.650569
		MAKNMAPIL	0.562057
		RHSIF	0.550742
		ARSRVGF	0.534916
	Pepsin (pH 1.3)	SCAPTF	0.87312
		KRQFTMC	0.683221
		HWGNI	0.679344
		FCHSTQCSRANTHQCF	0.650569
		MAKNMAPIL	0.562057
		RHSIF	0.550742
	Chymotrypsin-high specificity	ARSRVGF	0.534916
		QAPF	0.902425
		SCAPTF	0.87312
		GLCY	0.812574
		NTNSLKSHGKSCSNLF	0.550715
		CHSTQCSRANTHQCF	0.534683
	Chymotrypsin-low specificity	GLKRQF	0.516433
		QAPF	0.902425
		SCAPTF	0.87312
		APISLPNQL	0.662408
		APIL	0.642549
		KRQF	0.579856
	Trypsin	ARSRVGF	0.534916
		IPQFLFSCAPTFLTQK	0.794733
		SCSNLFDLNNP	0.604399
		AVGPFGLCYDTK	0.561262
		HLWVTCSQHYSSSTYQAPFCHSTQCSR	0.55468
		LHWGNILK	0.518162
		SDVVR	0.516968
		ISGGVPSVDLIMDK	0.505789

Table 3. *Cont.*

Protein	Enzyme	Sequence	Score
γ -conglutin 2	Pepsin (pH > 2)	FCHSTQCSRANSHHCFTCTDSATSRPGCHNNTCA	0.901253
		SCAPSF	0.829054
		KRQFAMC	0.726132
		HHSIF	0.569489
	Pepsin (pH 1.3)	FCHSTQCSRANSHHCFTCTDSATSRPGCHNNTCA	0.901253
		SCAPSF	0.829054
		KRQFAMC	0.726132
		HHSIF	0.569489
	Chymotrypsin-high specificity	QAPF	0.902425
		SCAPSF	0.829054
		GLCY	0.812574
		LSCSF	0.751547
		CHSTQCSRANSHHCF	0.650932
		NSNSLKSHGKTCANIF	0.630018
		MAKNMAQIFPF	0.562453
		AMCLSRYPTSNGAILF	0.544922
		GLKRQF	0.516433
		AQIFPF	0.95416
	Chymotrypsin-low specificity	QAPF	0.902425
		SCSF	0.872572
		SCAPSF	0.829054
		GDSRIGGAM	0.584379
		KRQF	0.579856
		GKTCANIF	0.533082
		AQKGLPNNVQGPL	0.515674
		QFAMCLSR	0.744774
		VLQYLFSCAPSFLAQK	0.727261
		LGPMVR	0.607089
	Trypsin	HLWVTCSYHYSSSTYQAPFCHSTQCSR	0.567814
β -conglutin 1	Pepsin (pH > 2)	PQQQQQSEKEGRRGRRGPISII	0.824977
		WQPRRQRPQSRREEREQEQQGSPSY	0.688377
		VGIRDQQRQQDEQEEEPVEVRRYSARL	0.666101
		MGKMRVRF	0.631814
		NSQRF	0.614205
		AIPINNPGY	0.607274
		LERF	0.57524
		NSKAIF	0.518454
	Pepsin (pH 1.3)	PQQQQQSEKEGRRGRRGPISII	0.824977
		VGIRDQQRQQDEQEEEPVEVRRYSARL	0.666101
		MGKMRVRF	0.631814
		NSQRF	0.614205
		AIPINNPGY	0.607274
		LERF	0.57524
		PHYNSKAIF	0.513703
	Chymotrypsin-high specificity	QPRRQRPQSRREEREQEQQGSPSYPRRQSGY	0.834804
		ANALPQQQQQSEKEGRRGRRGPISII	0.618305
		NSQRF	0.614205
		HERSEQREEREQEQQQGSPSY	0.613295
		NSKAIF	0.518454
		ELVGIRDQQRQQDEQEEEPVEVRRY	0.505495
	Chymotrypsin-low specificity	QPRRQRPQSRREEREQEQQGSPSYPRRQSGY	0.834804
		GIVF	0.691055
		VGIRDQQRQQDEQEEEPVEVRRY	0.627661
		ANALPQQQQQSEKEGRRGRRGPISII	0.618305
		NSQRF	0.614205
		AIPINNPGY	0.607274
		SEGDI	0.602674
		NSKAIF	0.518454
		RVRFPTL	0.509578

Table 3. Cont.

Protein	Enzyme	Sequence	Score
	Trypsin	FPTLVLVLGIVFLMAVSIGIAYGEK	0.965648
		DQQSYFSGFSR	0.707937
		DKPSQSGPFNLR	0.640216
		FDQR	0.614487
		NPYHFNSQR	0.517042
β -conglutin 2	Pepsin (pH > 2)	ANGQPQQQQQQQSEKEGRRGRRGSSL	0.759542
		SSQRF	0.688396
		WQPRRQRPQSRREEREQEQQGSPSY	0.688377
		MGKMRVRF	0.631814
		AIPINNPGY	0.607274
		LERF	0.57524
	Pepsin (pH 1.3)	ANGQPQQQQQQQSEKEGRRGRRGSSL	0.759542
		SSQRF	0.688396
		MGKMRVRF	0.631814
		AIPINNPGY	0.607274
		LERF	0.57524
		QPRRQRPQSRREEREQEQQGSPSYPRRQSGY	0.834804
	Chymotrypsin-high specificity	ANGQPQQQQQQQSEKEGRRGRRGSSLPF	0.821509
		SSQRF	0.688396
		HERSEQREEREQEQQGSPSY	0.613295
	Chymotrypsin-low specificity	QPRRQRPQSRREEREQEQQGSPSYPRRQSGY	0.834804
		ANGQPQQQQQQQSEKEGRRGRRGSSLPF	0.821509
		GIVF	0.691055
		SSQRF	0.688396
		AIPINNPGY	0.607274
		SEGDIF	0.602674
		EEQRRGQEQSDQDEGVIVIVSKKQIQKL	0.516968
		RVRFPTL	0.509578
		FPTLVLVLGIVFLMAVSIGIAYGEK	0.965648
	Trypsin	GSSLPF	0.86602
		DQQSYFSGFSR	0.707937
		DKPSDSGPFNLR	0.660803
		FDQR	0.614487

3.4. Prediction of Anticancer Peptides Using AntiCP 2.0

The 124 peptides previously identified as potentially bioactive by PeptideRanker were subsequently evaluated using AntiCP 2.0 to predict their anticancer potential. Based on the selected prediction criteria, 47 peptide entries were classified as anticancer peptides by AntiCP 2.0 and retained for further analyses. These entries corresponded to 28 unique peptide sequences, since some peptides were generated repeatedly from different source proteins or enzymatic hydrolysis conditions (Table 4).

The predicted anticancer peptides were derived from all four analyzed lupin storage proteins and were generated by different enzymatic hydrolysis conditions. AntiCP 2.0 scores ranged from 0.45 to 0.76. AntiCP 2.0 scores varied among the peptide entries classified as anticancer at this intermediate screening stage. However, high AntiCP 2.0 scores were not interpreted as sufficient to define final candidates, since peptide prioritization also required subsequent toxicity prediction.

Several peptides were identified repeatedly under different enzymatic hydrolysis conditions, including SCAPTF, KRQFAMC, NSQRF, NSKAIF, and SSQRF, indicating that these sequences can be released by multiple proteolytic enzymes. The anticancer peptide candidates identified in this stage were subsequently subjected to toxicity assessment using ToxinPred 3.0.

Table 4. Predicted Anticancer Peptides Identified by AntiCP 2.0.

Protein	Enzyme	Sequence	Score
γ -conglutin 1	Pepsin (pH > 2)	RHSIF	0.69
		SCAPTF	0.65
		KRQFTMC	0.54
	Pepsin (pH 1.3)	RHSIF	0.69
		HWGNI	0.67
		SCAPTF	0.65
		KRQFTMC	0.54
	Chymotrypsin-high specificity	SCAPTF	0.65
		NTNSLKSHGKSCSNLF	0.45
	Chymotrypsin-low specificity	SCAPTF	0.65
		APIL	0.54
		KRQF	0.54
	Trypsin	LHWGNILK	0.75
		IPQFLFSCAPTFILTQK	0.5
γ -conglutin 2	Pepsin (pH > 2)	KRQFAMC	0.76
	Pepsin (pH 1.3)	KRQFAMC	0.76
	Chymotrypsin-high specificity	NSNSLKSHGKTCANIF	0.45
	Chymotrypsin-low specificity	AQIFPF	0.62
		GKTCANIF	0.56
		KRQF	0.54
		GDSRIGGAM	0.5
		SCSF	0.48
		QFAMCLSR	0.69
		LGPMVR	0.63
	Trypsin	VLQYLFSCAPSFLAQK	0.51
β -conglutin 1	Pepsin (pH > 2)	NSKAIF	0.69
		NSQRF	0.63
	Pepsin (pH 1.3)	NSQRF	0.63
		PHYNSKAIF	0.59
	Chymotrypsin-high specificity	NSKAIF	0.69
		NSQRF	0.63
	Chymotrypsin-low specificity	NSKAIF	0.69
		NSQRF	0.63
		GIVF	0.56
		SEGDIF	0.5
		RVRFPTL	0.5
	Trypsin	FDQR	0.66
		NPYHFNSQR	0.49
β -conglutin 2	Pepsin (pH > 2)	SSQRF	0.64
	Pepsin (pH 1.3)	SSQRF	0.64
	Chymotrypsin-high specificity	SSQRF	0.64
	Chymotrypsin-low specificity	SSQRF	0.64
		GIVF	0.56
		SEGDIF	0.5
		RVRFPTL	0.5
	Trypsin	FDQR	0.66
		GSSLPF	0.54

3.5. Toxicity Prediction of Anticancer Peptides Using ToxinPred 3.0

The 47 peptides predicted as anticancer candidates by AntiCP 2.0 were subsequently evaluated using ToxinPred 3.0 to assess their potential toxicity. Based on the prediction results, 31 peptides were classified as “Non-Toxin”, whereas 16 peptides were predicted as “Toxin” (Table 5).

Table 5. Toxicity prediction of AntiCP-classified peptides from *Lupinus albus* storage proteins.

Protein	Enzyme	Sequence	Hybrid Score	Prediction
γ -conglutin 1	Pepsin (pH > 2)	KRQFTMC	0.625	Toxin
		SCAPTF	0.595	Toxin
		RHSIF	0.35	Non-Toxin
	Pepsin (pH 1.3)	KRQFTMC	0.625	Toxin
		SCAPTF	0.595	Toxin
		HWGNI	0.33	Non-Toxin
	Chymotrypsin-high specificity	RHSIF	0.35	Non-Toxin
		SCAPTF	0.595	Toxin
		NTNSLKSHGKSCSNLF	0.43	Toxin
	Chymotrypsin-low specificity	SCAPTF	0.595	Toxin
		KRQF	0.43	Toxin
		APIL	0.39	Toxin
	Trypsin	IPQFLFSCAPTFLTQK	0	Non-Toxin
		LHWGNILK	0	Non-Toxin
γ -conglutin 2	Pepsin (pH > 2)	KRQFAMC	0.73	Toxin
	Pepsin (pH 1.3)	KRQFAMC	0.73	Toxin
	Chymotrypsin-high specificity	NSNSLKSHGKTCANIF	0.43	Toxin
	Chymotrypsin-low specificity	SCSF	0.81	Toxin
		GKTCANIF	0.59	Toxin
		KRQF	0.43	Toxin
	Trypsin	AQIFPF	0.26	Non-Toxin
		GDSRIGGAM	0.205	Non-Toxin
		QFAMCLSR	0.6	Toxin
		VLQYLFSCAPSFLAQK	0.32	Non-Toxin
		LGPMVR	0.205	Non-Toxin
β -conglutin 1	Pepsin (pH > 2)	NSQRF	0.05	Non-Toxin
		NSKAIF	0	Non-Toxin
	Pepsin (pH 1.3)	NSQRF	0.05	Non-Toxin
		PHYNSKAIF	0	Non-Toxin
	Chymotrypsin-high specificity	NSQRF	0.05	Non-Toxin
		NSKAIF	0	Non-Toxin
	Chymotrypsin-low specificity	GIVF	0.3	Non-Toxin
		SEGDIF	0.195	Non-Toxin
		RVRFPTL	0.14	Non-Toxin
		NSQRF	0.05	Non-Toxin
		NSKAIF	0	Non-Toxin
	Trypsin	NPYHFNSQR	0.21	Non-Toxin
		FDQR	0.105	Non-Toxin
β -conglutin 2	Pepsin (pH > 2)	SSQRF	0.04	Non-Toxin
	Pepsin (pH 1.3)	SSQRF	0.04	Non-Toxin
	Chymotrypsin-high specificity	SSQRF	0.04	Non-Toxin
	Chymotrypsin-low specificity	GIVF	0.3	Non-Toxin
		SEGDIF	0.195	Non-Toxin
		RVRFPTL	0.14	Non-Toxin
		SSQRF	0.04	Non-Toxin
	Trypsin	FDQR	0.105	Non-Toxin
		GSSLPF	0	Non-Toxin

Peptides classified as “Non-Toxin” by ToxinPred 3.0 were identified in all four analyzed lupin storage proteins. Several peptide sequences derived from β -conglutins, including NSQRF, NSKAIF, FDQR, and SSQRF, were consistently classified as “Non-Toxin” under different enzymatic hydrolysis conditions. Likewise, peptides predicted as “Non-Toxin” were identified among γ -conglutin-derived sequences, including RHSIF, HWGNI, LHWGNILK, AQIFPF, GDSRIGGAM, VLQYLFSCAPSFLAQK, and LGPMVR.

3.6. Physicochemical Characterization of Selected Peptides Using ProtParam

The 31 peptide entries classified as “Non-Toxin” by ToxinPred 3.0 were selected for subsequent physicochemical characterization using ProtParam. However, several peptide sequences were identified repeatedly under different protein–enzyme combinations. Since physicochemical properties are determined by amino acid sequence rather than by protein source or hydrolysis condition, redundant entries with identical sequences were removed prior to ProtParam analysis. This redundancy-removal step reduced the 31 peptide entries to 18 unique anticancer peptide candidates classified as “Non-Toxin”. Among these 18 unique candidates, two sequences, GIVF and FDQR, contained only four amino acid residues and therefore did not meet the minimum sequence length required for complete ProtParam analysis. Consequently, only the remaining 16 peptides containing five or more amino acid residues were subjected to complete physicochemical characterization (Table 6). The two tetrapeptides, GIVF and FDQR, were retained in the final non-redundant list because they had fulfilled the previous bioactivity, anticancer prediction, and toxicity screening criteria; however, their physicochemical characterization was limited due to ProtParam restrictions.

Table 6. Physicochemical Properties of Unique Anticancer Peptide Candidates Classified as “Non-Toxin” by ToxinPred 3.0.

Peptide	Length (AA)	MW	pI	Instability Index	Aliphatic Index	GRAVY
IPQFLFSCAPTFLTQK	16	1841.20	8.22	50.31 (unstable)	79.38	0.531
VLQYLFSCAPSFLAQK	16	1815.16	8.17	69.49 (unstable)	103.75	0.744
NPYHFNSQR	9	1162.23	8.75	29.28 (stable)	0.00	−2.122
PHYNSKAIF	9	1076.22	9.01	57.71 (unstable)	54.44	−0.578
GDSRIGGAM	9	862.96	5.84	55.97 (unstable)	54.44	−0.200
LHWGNILK	8	980.18	8.76	49.96 (unstable)	146.25	0.025
RVRFPTL	7	888.08	12.00	36.09 (stable)	97.14	−0.071
AQIFPF	6	721.85	5.57	72.53 (unstable)	81.67	1.133
NSKAIF	6	678.79	8.75	8.33 (stable)	81.67	0.150
LGPMVR	6	671.86	9.75	−4.23 (stable)	113.33	0.567
SEGDI	6	666.69	3.67	40.43 (unstable)	65.00	−0.150
GSSLPF	6	606.68	5.52	104.63 (unstable)	65.00	0.500
RHSIF	5	658.76	9.76	46.52 (unstable)	78.00	−0.240
NSQRF	5	650.69	9.75	46.52 (unstable)	0.00	−1.900
HWGNI	5	625.68	6.74	52.40 (unstable)	78.00	−0.700
SSQRF	5	623.67	9.47	85.04 (unstable)	0.00	−1.360
FDQR	4	564.60	5.84	-	-	-
GIVF	4	434.54	5.52	-	-	-

Among the 18 unique predicted non-toxic AntiCP candidates retained after redundancy removal, 16 sequences contained five or more amino acid residues and were subjected to complete ProtParam characterization. These fully analyzed peptides ranged from 5 to 16 amino acids in length, with molecular weights ranging from 606.68 to 1841.20 Da. The two remaining AntiCP candidates, GIVF and FDQR, were tetrapeptides with molecular weights of 434.54 and 564.60 Da, respectively, but were not subjected to complete ProtParam analysis because they did not meet the minimum sequence length required by the tool. Among the 16 fully characterized peptides, theoretical isoelectric point (pI) values varied between 3.67 and 12.00, indicating substantial variability in peptide charge characteristics. Instability index values classified four peptides (LGPMVR, NSKAIF, RVRFPTL, and NPYHFNSQR) as stable, whereas the remaining fully analyzed peptides were classified as unstable. However, this classification should be interpreted cautiously because the instability index was originally developed for proteins and its predictive value may be limited for short peptide sequences, particularly those containing only five to nine amino

acid residues. Aliphatic index values ranged from 0.00 to 146.25, while GRAVY values ranged from -2.122 to 1.133 , reflecting differences in hydrophobicity among the selected peptide candidates.

4. Discussion

The identification of anticancer peptides (ACPs) derived from *Lupinus albus* conglutins through computational approaches addresses the growing need for novel therapeutic strategies with greater selectivity and lower toxicity than conventional cancer treatments [28,29]. In the present study, a stepwise in silico workflow integrating independent enzyme-specific hydrolysis, bioactivity prediction, anticancer peptide screening, toxicity prediction, and physicochemical characterization enabled the computational prioritization of 18 unique peptides predicted to have anticancer potential and low toxicity. These findings support the concept that food proteins may act as reservoirs of encrypted bioactive sequences that can be released through enzymatic hydrolysis and subsequently exert biological functions [18].

The effectiveness of the applied screening strategy is reflected in the progressive reduction in candidate sequences throughout the workflow. From 1610 peptide fragments generated by simulated enzymatic hydrolysis, only 124 peptides were predicted as potentially bioactive by PeptideRanker, 47 were subsequently classified as ACPs by AntiCP 2.0, and 31 were predicted as “Non-toxin” by ToxinPred 3.0, resulting in 18 unique peptide candidates after redundancy removal. Such progressive filtering highlights the value of integrating multiple predictive tools to prioritize peptides with both biological activity and favorable safety profiles before experimental validation. Similar computational approaches have been successfully employed for the discovery of bioactive peptides from other natural sources, including marine organisms, demonstrating the utility of in silico methodologies for accelerating peptide-based drug discovery [30].

An important observation was that approximately one-third of the peptides predicted as ACPs were subsequently classified as “Toxin” by ToxinPred 3.0. This finding emphasizes that anticancer activity prediction alone is insufficient for peptide prioritization and underscores the importance of incorporating toxicity screening into peptide discovery pipelines. Several peptides exhibiting relatively high AntiCP scores, such as KRQFAMC, KRQFTMC, and SCAPTF, were excluded after toxicity assessment, illustrating how safety-related predictions can substantially refine the pool of potential therapeutic candidates. Consequently, the integration of toxicity prediction represents an important computational filtering step for reducing the number of peptides with predicted toxicity and prioritizing candidates with more favorable predicted profiles for future experimental evaluation [30].

Both β - and γ -conglutins contributed substantially to the final pool of predicted “Non-toxin” ACP candidates, although a slightly higher number of unique peptides originated from β -conglutins. This observation suggests that both storage protein families represent valuable sources of encrypted bioactive sequences. Previous studies have similarly demonstrated that lupin proteins constitute an important source of peptides with diverse biological activities, including antioxidant, anti-inflammatory, antihypertensive, and enzyme-inhibitory properties [18,31]. Therefore, the identification of ACP candidates from both conglutin families further expands the spectrum of biological activities that may be associated with *L. albus* storage proteins.

To provide additional context, the final peptide candidates identified in the present study were compared with representative lupin-derived bioactive peptides previously reported in the literature. No exact sequence matches were observed between our final candidates and reported lupin-derived peptides such as IQDKEGIPDQQR, AKIQDKEGIPDQQR, GPETAFLR, FVPY, LTFPGSAED, LILPKHSDAD, AGGPQQR, PSELSGAH, or VVVVDE [16–18,31]. Thus, this comparison should be interpreted as a literature-based

contextual analysis rather than as evidence of shared bioactivity, sequence homology, anticancer activity, or multifunctionality.

Studies on other plant protein sources also support the relevance of seeds and legumes as reservoirs of peptides with anticancer potential. For example, peptides identified from *Salvia hispanica* seeds, including KLKKNL, MLKSKR, KKYRVF, FRTKKK, and SV-VAKAPVGKR, have been evaluated through combined in silico and in vitro approaches, showing interactions with cancer cell membrane models and reduced viability in different cancer cell lines [32]. Similarly, peptides derived from soybean β -conglycinin and cowpea β -vignin hydrolysates have been reported to inhibit breast and prostate cancer cell growth, with in silico analyses identifying candidate Bcl-2 homology 3 mimetic (BH3-mimetic) peptides such as VIPAAY [33]. In addition, recent reviews indicate that plant-derived anticancer peptides have been described from diverse sources, including legumes, cereals, oilseeds, and marine plants [34]. In this context, the peptide candidates identified in the present study are consistent with the broader use of plant storage proteins as sources of encrypted bioactive sequences. Accordingly, the similarities discussed here should be considered descriptive and hypothesis-generating, and future studies should include sequence-similarity analyses and experimental assays to determine whether these candidates share functional properties with plant-derived anticancer peptides reported elsewhere.

The physicochemical characterization of the selected peptides revealed several features commonly associated with ACPs. Most fully characterized candidates were short peptides ranging from 5 to 16 amino acid residues, which is consistent with previous reports indicating that ACPs frequently exhibit relatively short sequence lengths that may influence cellular interaction and membrane permeation [28,29]. Furthermore, several peptides displayed high theoretical isoelectric points, including NSQRF, SSQRF, RVRFPTL, and LHWGNILK, suggesting the presence of basic amino acid residues in these sequences. However, because net charge at physiological pH, amphipathicity, and hydrophobic moment were not calculated in the present study, the theoretical pI values alone should not be interpreted as direct evidence of membrane-interaction potential. Previous studies have shown that cationic residues, hydrophobicity, amphipathicity, amino acid composition, and secondary structure can influence ACP–membrane interactions and anticancer peptide prediction [28–31,35]. Therefore, the physicochemical properties reported here should be interpreted as preliminary descriptors for candidate prioritization rather than as evidence of a specific membrane-associated mechanism.

Hydrophobicity also appears to play an important role in the biological potential of the identified peptides. Previous studies have demonstrated that an appropriate balance between positive charge and hydrophobicity is essential for the anticancer activity of cationic peptides, influencing their ability to interact with cellular membranes and modulate intracellular targets. In the present study, peptides such as AQIFPF, VLQYLFSCAPSFLAQK, and LGPMVR exhibited relatively high GRAVY values, suggesting increased hydrophobic character compared with the other selected candidates. However, GRAVY values alone do not demonstrate membrane interaction, membrane disruption, or cellular uptake. Therefore, these hydrophobicity-related results should be interpreted only as preliminary physicochemical descriptors, and additional structural and membrane-interaction analyses would be required to evaluate their possible role in ACP mechanisms [36].

Interestingly, several of the identified peptides contained arginine residues at terminal positions, including NSQRF. Similar arginine-containing motifs have previously been reported in bioactive peptides derived from *Lupinus albus*, particularly in studies investigating arginase-inhibitory peptides generated through simulated gastrointestinal digestion. However, arginase inhibition and anticancer peptide prediction represent different biological endpoints. Therefore, this comparison should be interpreted only as a motif-level

observation and not as evidence of anticancer activity. The occurrence of comparable structural features supports the broader concept that lupin proteins may contain encrypted bioactive peptide sequences, but the anticancer relevance of the peptides identified in the present study requires experimental validation [35].

The predicted stability of certain peptides may also support their future evaluation. Among the candidates analyzed, NSKAIF, RVRFPTL, LGPMVR, and NPYHFNSQR were classified as stable according to the instability index. Predicted peptide stability is an important consideration during early-stage candidate selection because degradation susceptibility represents a major challenge in peptide-based therapeutics. Therefore, these candidates may warrant further investigation in experimental models to determine whether their predicted stability translates into favorable biological performance [30,36].

Despite the promising results obtained, several limitations should be acknowledged. The present study relied exclusively on computational predictions, which provide valuable preliminary information but cannot fully reproduce the complexity of biological systems. Previous investigations involving lupin-derived peptides have shown that predicted biological activities do not always correlate perfectly with experimental outcomes, emphasizing the need for validation through in vitro and in vivo studies [35]. Furthermore, parameters such as cellular uptake, metabolic stability, bioavailability, immunogenicity, allergenicity, hemolytic potential, solubility, digestive stability, and selectivity toward cancer cells were not evaluated in the present study and should be addressed in future research. Although some of these aspects can be partially explored using additional computational tools, they were not included in the current workflow because the study was designed as an initial screening strategy focused on enzymatic peptide release, general bioactivity prediction, anticancer peptide classification, toxicity prediction, and basic physicochemical characterization. Similarly, antioxidant and anti-inflammatory activities were not evaluated in the present study, although these bioactivities may be relevant for assessing the potential multifunctionality of the prioritized peptides in future studies. Consequently, the peptides identified in this work should be considered candidates for further computational refinement and experimental investigation rather than confirmed anticancer agents.

5. Conclusions

This study showed that *Lupinus albus* β - and γ -conglutins may represent sources of encrypted peptide sequences with predicted anticancer potential. Through a stepwise in silico workflow combining independent enzyme-specific hydrolysis, bioactivity prediction, anticancer peptide screening, toxicity prediction, and physicochemical characterization, 18 unique predicted non-toxic peptide candidates were prioritized from an initial set of 1610 hydrolysis-derived fragments. The progressive reduction in candidates throughout the screening process highlights the usefulness of integrating multiple computational tools to prioritize peptides with predicted anticancer potential and favorable predicted toxicity profiles.

The selected peptides exhibited physicochemical characteristics commonly associated with anticancer peptides, including short sequence length, the presence of basic residues, and variable degrees of hydrophobicity. Furthermore, both β - and γ -conglutins contributed to the generation of candidate sequences, supporting the value of lupin storage proteins as reservoirs of encrypted bioactive peptide candidates.

However, the findings of the present study are based exclusively on computational predictions and do not validate anticancer activity, cancer-cell selectivity, toxicity, stability, bioavailability, or mechanisms of action. Therefore, the identified peptides should be regarded only as candidates for further computational refinement and experimental testing.

Future studies should evaluate their anticancer activity, toxicity, selectivity, stability, and mechanisms of action in relevant in vitro and/or in vivo models.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biomedinformatics6040048/s1>, Supplementary Data S1: Input protein sequences; Supplementary Data S2: Complete hydrolysis-derived peptide fragments; Supplementary Data S3: PeptideRanker results for peptides within the 4–50 amino acid range; Supplementary Data S4: AntiCP 2.0 prediction results; Supplementary Data S5: ToxinPred 3.0 prediction results.

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Abbreviations

The following abbreviations are used in this manuscript:

AA	Amino Acid(s)
ACE	Angiotensin-Converting Enzyme
ACP	Anticancer Peptide
BH3-mimetic	Bcl-2 homology 3 mimetic
Da	Dalton
DPP-IV	Dipeptidyl Peptidase IV
GRAVY	Grand Average of Hydropathy
MERCI	Motif-Emerging and with Classes-Identification
ML	Machine Learning
MMP-9	Matrix Metalloproteinase-9
MW	Molecular Weight
pI	Isoelectric Point
SVM	Support Vector Machine

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