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Investigation of bioactive peptides from *Scolymus hispanicus* by using *in silico* methods

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Abstract: Due to increases in the soil pollution related to agricultural areas, the interests on the wild edible greens have been increasing nowadays. *Scolymus hispanicus* (=Golden thistle) is a wild edible plant which is widely and naturally spread in Türkiye. An *in silico*-based bioinformatics approach has been proposed for the evaluation of bioactive peptides from this species. *In silico* digestion and also bioactive peptides of RubisCO from *S.hispanicus* were studied by using BIOPEP-UWM. Protparam and Clustal Omega were also used to determine physicochemical parameters and sequence similarity, respectively. The A_E values related to angiotensin converting enzyme and dipeptidyl peptidase-IV were 0.0847 and 0.1059 after *in silico* pepsin digestion (pH>2), respectively. While the antioxidant property obtained after pepsin (pH>2) digestion was found to be 0.0127, the value of 0.042 was obtained for ficin on this parameter. BIOPEP-UWM also exhibit important properties related to the bioactivities of the peptides such as antioxidant, dipeptidyl peptidase-IV and angiotensin converting enzyme inhibitions. From the results, it could be said that *S. hispanicus* has very important bioactive peptides which could be evaluated in the production of functional foods. Moreover, isolated bioactive peptides and also secondary metabolites can also be utilized in pharmaceutical industry. Further *in vitro* and *in vivo* studies are strongly recommended on *S. hispanicus*.

Keywords: antioxidant; bioactive peptides; bioinformatics; *Scolymus hispanicus*; Golden thistle; functional foods

1 Introduction

Bioinformatics tools provide important contributions to understanding of biological functions in living organisms in various levels. Due to the big data related to protein and DNA sequences in various database, fast, robust and unbiased computational tools are necessary to explain structures in life sciences (Baxevanis et al. 2020). It is frequently observed in the literature that protein and DNA sequences evaluated in terms of bioinformatics are converted into numerical values and evaluated through computer-based algorithms (Kandemir-Cavas, 2023; Negi et al. 2023; Iqbal and Kumar, 2023).

Biopeptides can be determined as short chains of amino acids with diverse biological functions, including roles as signaling molecules, hormones, and antimicrobial agents. Bioinformatics also plays a crucial role in the analysis of biopeptides, providing valuable tools and methods to find out the complex information embedded in peptide sequences. It is of great importance to obtain biopeptides in their structures in order to evaluate agricultural products (Du et al. 2023). There are bioinformatics tools used to identify, characterize, detail and produce the bioactive mechanisms of bioactive peptides belonging to proteins that are very popular in the food and cosmetic industries today (Nuñez et al. 2020).

Wild food plants form an important part of many people's diets in a variety of local dishes (Guarrera and Savo, 2016). Golden thistle (*Scolymus hispanicus* L.), belongs to the Asteraceae family (Sergio, 2023). *S. hispanicus* is a thorny perennial herbaceous plant. This wild plant can be existed in wasteland, along the road, weedy areas and roadside ditches (Sergio, 2023, Polo et al. 2009). It is used as food in the Mediterranean countries such as Turkey, Spain, Portugal, Morocco and Greece (Polo et al. 2009). Southern European nations are particularly interested in it due to its nutritional value and health benefits (Paschoalinotto, 2023). Young leaves, midribs, and root bark are all regarded as vegetables. Golden thistle is consumed with olive oil and meat, as well as in the form of tea (Figure 1).

Fig. 1 *Scolymus hispanicus* in Dokuz Eylül University Tınaztepe Campus, İzmir, Türkiye.

The essential oil of *S. hispanicus* included 15 constituents. Heneicosan (19.4%), acetone with hexahydrofarnel (17.0%), and phytol (17.0%) are the major chemicals. The most common were saturated n-alkane differentiation (35.2%), oxygenated sesquiterpenes (25.6%), and diterpenes (17%) (Servi, 2019). In its chemical analysis, high α-tocopherol content (2.79 ± 0.07 mg/100 g) in flowers, high gallic acid content (187.01 ± 10.19 mg/kg) in leaves and 3 flavonoids and 13 phenolic acids were identified (Marmouzi et al. 2017). This wild herb has hypoglycemic, antioxidant and anti-inflammatory properties (Berdja et al. 2021). Wild plants have been used for medical purposes since ancient times. Today, usage of wild edible plants has been gaining popularity as a healthful resource (Altiner and Sahan, 2016). In addition to its food properties, golden thistle also shows medicinal properties. The effect of the medicine called "Lityazol Cemil" on the kidney and bladder has been proven in clinical studies (Karik, 2019). Coşkun et al. (2021) investigated the effect of the extract obtained from the root of this plant in healing kidney stones, also known as calcium oxalate nephrolithiasis. Experimental results revealed the healing effect of the extract. Berdja et al. (2021) showed that studies showed that inclusion of *S. hispanicus* in a diet program or use as a nutritional supplement prevents metabolic damage by creating a hypoglycemic and hypolipidemic impact. It decreases oxidative stress, inflammation, and lipid buildup to improve glucose tolerance, hyperlipidemia, and hepatic steatosis (Berdja et al. 2021). Marmouzi et al. 2017 showed that the antioxidant and antidiabetic activities of the plant are through the inhibition of α-glucosidase and α-amylase. Components isolated from *S. hispanicus* inhibited NF-κB p65 expression in PHA-stimulated human PBMCs and subsequently showed anti-inflammatory properties in vitro as a result of reducing inflammatory cytokines (Kandil et al. 2020). *S. hispanicus* extract has a cytotoxic effect on Caco-2 cell growth, suggesting that it may have protective effects against colon cancer (Ahmad, 2017). Proteins in foods support health with their physiochemical roles as well as serving as nutrients (Daliri et al. 2017). Bioactive peptides are obtained from animal or plant-based sources (Cavas and Bilgin, 2021). Food-derived peptides display a number of biological functions in addition to their nutritional benefits, with effects such as antioxidative, hypoglycemic, hypocholesterolemic and antimicrobial (Nasri, 2017). Typically, bioactive peptides have 2 - 20 amino acid residues (Fan et al. 2014). Inhibition of the enzymes such as angiotensin converting enzyme (ACE) (EC 3.4.15.1) and dipeptidyl peptidase IV (DPP-IV) (EC 3.4.14.5) via bioactive peptides provides information about the function of biopeptides (Iwaniak et al. 2020). Bioactive peptides from foods are safe, effective, cost-effective, and bioavailable, leading to their investigation (Duffuler et al. 2022). This study reports, for the first time, the results from *in silico* tools related to the ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) bioactive peptides of the golden thistle plant. Additionally, the aim of the study was to estimate the functional food potential of the bioactive peptides from *S.hispanicus.*

2 Materials and Method

The proteins were searched on Uniprot.org using the term "*Scolymus hispanicus*" (The UniProt Consortium, 2019) and then the sequence of RubisCO from *S.hispanicus* was retrieved. The uniprot ID of RubisCO from *S.hispanicus* is G0WZG7. The amino acid sequence of *S. hispanicus* protein was retrieved from Uniprot.org in FASTA format (Morgat et al. 2019).

The physical and chemical characteristics of the protein from *S. hispanicus* were identified using the ProtParam tool (Gasteiger et al. 2005). This tool was used to calculate molecular weight, number, net charge, amino acid percentage and theoretical pI value and instability index.

Amino acid or nucleotide sequence multiple sequence alignments are quickly and accurately provided using Clustal Omega tool (Sievers and Higgins, 2014). The sequences of RubisCO proteins of the plants were compared using the Clustal Omega tool.

Bioactive peptides reported in *S. hispanicus* were searched using the BIOPEP-UWM tool (Minkiewicz et al. 2019). To analyze the values of the quantitative parameters of BIOPEP-UWM for bioactive peptides, the following procedures were performed. The enzymes used in the research as follow: chymotrypsine (EC3.4.21.1), trypsin (EC3.4.21.4), pepsin (pH 1.3) (EC3.4.23.1), proteinase K (EC3.4.21.64), pancreatic elastase (EC3.4.21.36), prolyl oligopeptidase (EC3.4.21.26), V-8 protease (Glutamyl endopeptidase; pH=4 (EC3.4.21.19), thermolysin (EC3.4.24.27), chymotrypsin C

(EC3.4.21.2), plasmin (EC3.4.21.7), cathepsin (EC3.4.21.20), clostripain (EC3.4.22.8), chymase (EC3.4.21.39), papain (EC3.4.22.2), ficin (EC3.4.22.3), leukocyte elastase (EC3.4.21.37), metridin (EC3.4.21.3), pancreatic elastase II (EC3.4.21.71), stem bromelain (EC3.4.22.32), glutamyl endopeptidase II (EC3.4.21.82), oligopeptidase B (EC3.4.21.83), calpain 2 (EC3.4.22.53), glycyl endopeptidase (EC3.4.22.25), oligopeptidase F, proteinase P1 (lactocepin) (EC3.4.21.96), Xaa-Pro dipeptidase (EC3.4.13.9), pepsin (pH>2) (EC3.4.23.1), coccolysin (EC3.4.24.30), subtilisin (EC3.4.21.62), chymosin (EC3.4.23.4), ginger protease (zingipain) (EC3.4.22.67) and V-8 protease (glutamyl endopeptidase); pH=7.8 (EC3.4.21.19).

3 Results and Discussion

RubisCO of *S.hispanicus* peptides was obtained by *in silico* proteolytic digestion with BIOPEP tools. The results were also compared with the common plants such as *Anethum graveolens* and *Eruca sativa*. The results from BIOPEP parameters (B_E, A_E, W, DH_t and V) were shown in Tables 1-5.

The theoretical degree of hydrolysis (DHt), indicates the enzyme's efficiency in producing peptides from *S. hispanicus*. The highest DHt values were obtained to be 71.9149, 52.3404 and 42.9787 by using pepsin (pH>2), pancreatic elastase and ficin enzymes, respectively. Although the highest values were observed in pepsin, pancreatic elastase and ficin, the minimum values were found when chymosin, ginger protease (zingipain) and thrombin were used. W is a measure of how frequently an enzyme releases fragments with a specific level of activity. B_E refers to the activity of potentially released fragments by the proteolytic enzymes. According to Gasteiger et al. (2005), V denotes the relative activity of fragments with particular activity produced by the selected enzymes. The frequency with which the chosen enzyme releases fragments with a particular activity is known as the parameter A_E (Minkiewicz et al. 2019). Using the BIOPEP-UWM tool, the functions of biologically active peptides such as antioxidant peptides, ACE inhibitor, dipeptidyl peptidase III, DPP-IV inhibitor can be determined *in silico* tools (Iwaniak et al. 2015).

Biological activities of *S. hispanicus*, *E. sativa* and *A. graveolens* biopeptides are shown in Tables 1-5. As a result of *in silico* analysis, regulating, activating ubiquitin-mediated proteolysis and stimulating activity were observed in biopeptides obtained from these foods. Anticoagulant peptides have different efficacy in preventing thrombosis. The incidence of cardiovascular disease can be prevented and decreased with the help of antithrombotic-rich diets (Cheng et al. 2019). The antiamnestic effect of some bioactive peptides may play an important role in the treatment and prevention of mental disorders (Garmidolova et al. 2022). DPP-III is involved in a number of physiological and pathological processes, including defense from oxidative stress, apoptosis, as inflammation, besides to its role in the protein cycle's final stages (Tomi et al. 2023). Alpha glucosidase inhibitors delay the absorption of glucose by reducing the digestion of carbohydrates and lower blood glucose levels without insulin being secreted (Abbasi et al. 2022).

Table 1 BIOPEP Bioactivity parameters related to enzymatic digestion (ficin (EC 3.4.22.3)) of *Scolymus hispanicus*. (NAN: not a number)

DH_t [% 42.9787]					
No	**Activity**	**A_E**	**W**	**B_E**	**V**
1	regulating	0.0042	0.1654	0	NAN
2	ACE inhibitor	0.0678	0.1111	0.0018	0.0511
3	antioxidative	0.0042	0.0367	0	0
4	dipeptidyl peptidase IV inhibitor	0.0763	0.1233	0.0001	0.4036
5	HMG-CoA reductase inhibitor	0.0042	1.0000	0	NAN
6	renin inhibitor	0.0042	0.1239	1.37E-6	1
7	dipeptidyl peptidase III inhibitor	0.0085	0.0669	0	NAN

Table 2 BIOPEP Bioactivity parameters related to enzymatic digestion (pancreatic elastase (EC 3.4.21.36)) of *Scolymus hispanicus*. (NAN: not a number)

DH_t [%52.3404]					
No	**Activity**	**A_E**	**W**	**B_E**	**V**
1	Regulating	0.0042	0.1654	0	NAN
2	dipeptidyl peptidase IV inhibitor	0.0975	0.1576	1.83E-5	0.0605
3	ACE inhibitor	0.0678	0.1127	0.0008	0.022
4	Antithrombotic	0.0042	0.3307	0	NAN
5	Antiamnestic	0.0042	0.3307	0	NAN
6	activating ubiquitin-mediated proteolysis	0.0085	0.6693	0	NAN
7	dipeptidyl peptidase III inhibitor	0.0042	0.0330	0	NAN
8	alpha-glucosidase inhibitor	0.0042	0.0708	2.49E-7	0.0013
9	anti inflammatory	0.0042	0.4941	0	NAN

The peptides that inhibit DPP-IV are therapeutic for diabetes type 2. Anti-diabetic peptide controls the levels of glucose in the blood and stops incretins from degrading by inhibiting DPP-IV (Lu et al. 2022). DPP-IV inhibition related bioactive peptides obtained from *S.hispanicus* RubisCO by cleavage with pancreatic elastase is 0.0975 (Table 1), after digestion with ficin it was found as 0.0763 (Table 2). As a result of *in silico* digestion by pepsin, DPP-IV value was found to be 0.1059 (Table 3). When Pepsin (pH>2) (EC3.4.23.1) was used, the DPP-IV based A_E values of the biopeptides obtained from *E. sativa* and *A. graveolens* RubisCOs were found to be 0.0593 and 0.0750, respectively.

Table 3 BIOPEP Bioactivity parameters related to enzymatic digestion (pepsin (pH>2) (EC3.4.23.1)) of *Scolymus hispanicus*. (NAN: not a number)

DH_t [% 71.9149]					
No	**Activity**	**A_E**	**W**	**B_E**	**V**
1	regulating	0.0042	0.1654	0	NAN
2	dipeptidyl peptidase IV inhibitor	0.1059	0.1712	5.92E-5	0.1962
3	ACE inhibitor	0.0847	0.1388	0.0045	0.1286
4	antithrombotic	0.0042	0.3307	0	NAN
5	antiamnestic	0.0042	0.3307	0	NAN
6	activating ubiquitin-mediated proteolysis	0.0085	0.6693	0	NAN
7	antioxidative	0.0127	0.1110	0	0
8	stimulating	0.0042	0.1654	0	NAN
9	dipeptidyl peptidase III inhibitor	0.0169	0.1330	0	NAN
10	alpha-glucosidase inhibitor	0.0085	0.1433	3.60E-7	0.0019
11	anti inflammatory	0.0042	0.4941	0	NAN

Iram et al. 2022 used an *in silico* method to analyze bioactive peptides for the breakdown of sheep protein from milk via digestive enzymes. DPP-IV inhibitory peptides observed in BIOPEP-UWM, including casein, s1 casein, s2 casein, lactalbumin and lactoglobulin had A_E values of 0.4504, 0.4158, 0.4304, 0.4929, and 0.4611, respectively. The bioactive peptide capacity of tomato grain, an advantageous industrial waste, was researched by Kartal et al. in 2020. The greatest A values for possible DPP-IV inhibitory effects came from 11S globulin, profilin, and SNF4 and were 0.6819, 0.6794, and 0.6676, respectively. Portuguese oyster (*Crassostrea angulata*) peptides were studied by Gomez et al. 2019, to examine their potential bioactivities *in vitro* as well as *in silico*. Among the 9 enzymes used, theoretical DPP-IV activity was found to be high in pepsin (pH > 2), papain and root bromelain. The bioactive peptide numbers of these three enzymes were found to be 333, 290 and 280, respectively.

Table 4 BIOPEP Bioactivity parameters related to enzymatic digestion (pepsin (pH>2) (EC3.4.23.1)) of *Eruca sativa.* (NAN: not a number)

DH_t [%47.0270]					
No	**Activity**	**A_E**	**W**	**B_E**	**V**
1	regulating	0.0054	0.4000	0	NAN
2	dipeptidyl peptidase IV inhibitor	0.0593	0.1583	3.87E-5	0.2014
3	ACE inhibitor	0.0404	0.1135	0.0021	0.0872
4	antithrombotic	0.0027	0.3333	0	NAN
5	antiamnestic	0.0027	0.3333	0	NAN
6	activating ubiquitin-mediated proteolysis	0.0027	0.3333	0	NAN
7	antioxidative	0.0054	0.0801	0	0
8	stimulating	0.0027	0.1667	0	NAN
9	dipeptidyl peptidase III inhibitor	0.0108	0.1484	0	NAN
10	alpha-glucosidase inhibitor	0.0054	0.1337	2.29E-7	0.0018

The A_E value of the ACE inhibitor of bioactive peptides obtained from RubisCO of *S. hispanicus* was 0.0678 when pancreatic elastase and ficin were used, while it was found as 0.0847 when pepsin was used. The A_E value of the ACE inhibitor of bioactive peptides obtained from RubisCO of *E. sativa* shown in Table 4 was 0.0404 for pepsin (pH>2) (EC3.4.23.1), while it was 0.0500 for *A. graveolens* in Table 5. It is used to reduce blood pressure in people with hypertension because it eliminates the vasodilator bradykinin and catalyzes the transformation of the angiotensin I-converting enzyme (ACE) into the strong vasoconstrictor angiotensin II (Iwaniak et al. 2015; Karami et al. 2019).

Panjaitan et al. (2018) demonstrated that protein bioactivities from giant grouper eggs using the BIOPEP-UWM database. The values obtained using BIOPEP-UWM for amino acid sequence, vitellogenin ACE inhibitor, apolipoprotein A-1 precursor, and *Epinephelus coioides* activity were 0.408, 0.388, and 0.334, respectively.. Utilizing the BIOPEP-UWM database, Arámburo-Gálvez et al. (2022) assessed legume and provicillin protein sequences for forecasting of ACE-I inhibitory peptides. ACE-I inhibitory peptides of legume, provicilin and papain have been identified to have A_E values of 0.0605-0.0442 and 0.0323-0.0287, respectively. Buffalo milk proteins' ability to release both familiar and new bioactive peptides was studied by Gu et al. in 2023. The ACE inhibitory values of α lactalbumin, β lactoglobulin, α s1 casein, α s2 casein, β casein and K casein were 0.394, 0.528, 0.556, 0.405, 0.679 and 0.447, respectively.

Table 5 BIOPEP Bioactivity parameters related to enzymatic digestion (pepsin (pH>2) (EC3.4.23.1)) of *Anethum graveolens.* (NAN: not a number)

DH_t [%48.7465]					
No	**Activity**	**A_E**	**W**	**B_E**	**V**
1	regulating	0.0056	0.4029	0	NAN
2	dipeptidyl peptidase IV inhibitor	0.0750	0.1929	7.96E-5	0.3502
3	ACE inhibitor	0.0500	0.1333	0.0024	0.1026
4	antithrombotic	0.0028	0.5000	0	NAN
5	antiamnestic	0.0028	0.5000	0	NAN
6	activating ubiquitin-mediated proteolysis	0.0056	0.6747	0	NAN
7	antioxidative	0.0056	0.0776	0	0
8	stimulating	0.0028	0.1677	0	NAN
9	dipeptidyl peptidase III inhibitor	0.0111	0.1377	0	NAN
10	alpha-glucosidase inhibitor	0.0056	0.1440	2.36E-7	0.0019

Antioxidant-active peptidesregulate oxidative processes within the body of humans. Numerous degenerative diseases can start or advance because of free radicals. Illnesses and oxidative stress are closely related. To lessen oxidative stress, many antioxidants have been obtained and produced from natural sources (Sarmadi and Ismail, 2010). Antioxidative properties of biopeptides from RubisCO of *S. hispanicus* when pepsin and ficin used were also studied in the present study. The A_E values were 0.0127 and 0.0042, respectively. As opposed to that, no antioxidative property was observed when pancreatic elastase was used. When the antioxidative properties of bioactive peptides obtained from RubisCO of *E. sativa* showed in Table 4 were examined, the A_E value was 0.0054 for pepsin (pH>2) (EC3.4.23.1), while it was 0.0056 for *A. graveolens* (Table 5). Antioxidant peptides are also of great importance in foods, as they can limit oxidative damage and affect nutritive and beneficial. This may lessen food oxidative deterioration. Biopeptides, when taken in the diet as antioxidants, protect body cells from oxidation (Leo et al. 2016). Panjaitan et al. 2022 investigated the antioxidant and Angiotensin-I converting enzyme (ACE-I) inhibitory effect of steam-cooked mackerel (*Scomber australasicus*) juice utilizing both *in silico* and *in vitro* analysis methods. The antioxidant activity was hydrolyzed utilizing proteases papain, pepsin, proteinase k, alkalase, bromelain and thermolysin 36, 23, 29, 20, 27, 41, 25, 16 and 22, respectively. Millan et al. 2022 studied antioxidant and antihypertensive activities as a result of hydrolysis with protease were investigated in cold *Lysobacter sp.* The A_E values were found to be 0.0169, 0.0103 and 0.0033, respectively, when β-lactoglobulin, lactoferrin, and serum albumin were studied. Cavas et al. 2020 studied the bioactive content of *Lagocephalus sceleratus*, known as a poisonous fish due to its toxins, were evaluated using an *in silico*-based biotechnological approach. The A_E value of the antioxidant activity of L. sceleratus protein cytochrome c oxidase subunit 1 was found to be 0.0108 using pepsin (pH = 1.3) enzymes.

The functions of the peptides depend on their specific amino acid composition and sequence, as well as some hydrophobic amino acids that bring about the antioxidant function of the biopeptides (Wen et al. 2020). The percentage of the essential amino acids was determined to be 40.6% in the *S. hispanicus* RuBisCO (Table 6). It was found that glycine (9.6%), alanine (8.7%), tyrosine (8.3%), and leucine (8.3%) made up the majority of RuBisCO. The content of proline amino acid was determined to be 6.1%. Table 7 lists the net charges of the proteins from *S. hispanicus, E. sativa,* and *A. graveolens* RubisCOs were found with the values of -2, -4, and -3, respectively. The stability index for the proteins, which were found to be 28.76, 33.34, and 28.41, respectively, indicated that the proteins were stable.

Using *in vitro* and *in silico* techniques, the ability of Gouda cheese with a modified casein content to inhibit ACE- and DPP-IV was investigated by Iwaniak et al (2021). The commonest amino acids found encompass all genetic variants of S1 according to the ProtParam study were 0.6-12.1% Glutamic acid, 7.5-10.3% Leucine, 7.9-9.1% Proline, 7.5-8.6% Serine, and 7.0-7.5% Lysine. Ağırbaşlı and Cavas (2017) studied bioactive peptides of 28 *Caulerpa spp.* using *in silico* methods. It was discovered that the range of total essential amino acids in *Caulerpa spp*'s RuBisCO findings was between 45 and 47%. It was found that 10.2-11.5% glycine, 8.6-10.9% alanine and 8.9-9.8% leucine made up the majority of RuBisCO. Cavas and Abeska 2020 examined the allergenicity of the endochitinase class 1 (Pers a 1) protein found in avocados using bioinformatics techniques in their study. According to Prot param results, the most numerous amino acids included in endochitinase of the allergen protein *Persea americana* plant are glycine (4.3%), alanine (11%) and serine (7.1%).

Figure 2 displays the level of similarity between *S. hispanicus, E. sativa,* and *A. graveolens* identified through the Clustal Omega database. An asterisk (*), a colon (:), and a period (.) are used in the cluster omega results to denote conserved residues, comparable characteristics, and barely similar traits, respectively. The study contrasts the RubisCo bioactive peptide contents of *S. hispanicus* with those of *A. graveolens*, and *E. sativa*. One may say that RubisCO of these species are remarkably similar to one another. As a result of different enzymatic hydrolysis, the highest DHt value was found in *S. hispanicus*. DHt (%) values obtained as a result of hydrolysis of *E. sativa* and *A. graveolens* with pepsin were found to be 47.0270 and 48.7465, respectively. The AE value of *S. hispanicus* due to ACE inhibition is significantly higher than the other plants examined. Since ACE, antioxidant and DDT-IV values of *E. sativa*, and *A. graveolens* are very close to each other, *S. hispanicus* can be used as an alternative source.

Table 6 Number and percentage of amino acids found in *Scolymus hispanicus.*

Amino acid composition:	#	%
Ala (A)	20	8.70%
Arg (R)	13	5.70%
Asn (N)	8	3.50%
Asp (D)	12	5.20%
Cys (C)	4	1.70%
Gln (Q)	6	2.60%
Glu (E)	17	7.40%
Gly (G)	22	9.60%
His (H)	2	0.90%
Ile (I)	9	3.90%
Leu (L)	19	8.30%
Lys (K)	14	6.10%
Met (M)	2	0.90%
Phe (F)	11	4.80%
Pro (P)	14	6.10%
Ser (S)	7	3.00%
Thr (T)	19	8.30%
Trp (W)	3	1.30%
Tyr (Y)	14	6.10%
Val (V)	14	6.10%
Pyl (O)	0	0.00%
Sec (U)	0	0.00%
(B)	0	0.00%
(Z)	0	0.00%
(X)	0	0.00%

Table 7 Protein parameters of *Scolymus hispanicus*, *Eruca sativa*, *Anethum graveolens* found in RubisCO.

Species	#aa	MW (Kda)	Theoretical pI	Negatively Charged Residues (Asp+Glu)	Positively Charged Residues (Arg+Lys)	Net Charge	Instability Index
Scolymus hispanicus	230	25635.02	5.84	29	27	-2	28.76
Eruca sativa	215	24105.25	5.36	28	24	-4	33.34
Anethum graveolens	215	24083.25	5.38	28	25	-3	28.41

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tr|A0A1B0YZN8|A0A1B0YZN8_ERUVS     ---EYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLT     57
tr|G0WZG7|G0WZG7_9ASTR             GVKDYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLT     60
tr|A0A1B0YW80|A0A1B0YW80_ANEGR     ---DYKLTYYTPEYETKDTDILAAFRVTPQPGVPPEEAGAAVAAESSTGTWTTVWTDGLT     57
                                      :********************************************************

tr|A0A1B0YZN8|A0A1B0YZN8_ERUVS     SLDRYKGRCYHIEPVPGEETQFIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALAALRL     117
tr|G0WZG7|G0WZG7_9ASTR             SLDRYKGRCYGIEPVPGEENQFIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRL     120
tr|A0A1B0YW80|A0A1B0YW80_ANEGR     SLDRYKGRCYGIEPVAGEENQYIAYVAYPLDLFEEGSVTNMFTSIVGNVFGFKALRALRL     117
                                   ********** **** ***.*:********************************* ****

tr|A0A1B0YZN8|A0A1B0YZN8_ERUVS     EDLRIPPAYTKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECLRG     177
tr|G0WZG7|G0WZG7_9ASTR             EDLRIPTAYVKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECLRG     180
tr|A0A1B0YW80|A0A1B0YW80_ANEGR     EDLRIPVAYVKTFQGPPHGIQVERDKLNKYGRPLLGCTIKPKLGLSAKNYGRAVYECLRG     177
                                   ****** **.**************************************************

tr|A0A1B0YZN8|A0A1B0YZN8_ERUVS     GLDFTKDDENVNSQPFMRWRDRFLFCAEAIYKSQAETG------------  215
tr|G0WZG7|G0WZG7_9ASTR             GLDFTKDDENVNSQPFMRWRDRFLFCAEAIYKAQAETGEIKGHYLNATAG  230
tr|A0A1B0YW80|A0A1B0YW80_ANEGR     GLDFTKDDENVNSQPFMRWRDRFLFCAEAIYKAQAETG------------  215
                                   ********************************:*****
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Fig. 2 Multiple sequence analysis of RubisCOs from *Scolymus hispanicus*, *Eruca sativa* and *Anethum graveolens*.

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5 Conclusion

Functional nutrition is of great importance in preventing and controlling autoimmune diseases that develop due to nutrition. From this point, it could be said that bioactive peptides from various plant sources are of great importance for development of functional foods (Tu el al. 2018; Peredo-Lovillo et al. 2022).

Determining the biopeptides in the structure of proteins in experimental environments is both time consuming and costly. Nowadays, accurate, precise results can be obtained in a short time by using bioinformatics tools for biopeptide analysis (Dang et al. 2022; Agyei et al. 2019).

Due to soil contamination and also interest on wild edible plants, importance of *S. hispanicus* is being increased in Türkiye. The price of *S.hispanicus* is about 5 Euro/kg in local bazaars. In order to assess the bioactive peptides of the wild *S. hispanicus* plant, which grows in wastelands, and untamed areas, an *in silico*-based biotechnological technique has been suggested in this study. The present article reveals that *S. hispanicus* contains important bioactive peptides. In the nations where the plant is produced, these peptides should be isolated and utilized. In the pharmaceutical industry, isolated bioactive peptides and secondary metabolites can also be employed. The results revealed from the study clearly show that the bioactive potential of *S. hispanicus* peptides should be effectively utilised. The results of this study also support the plant's suitability as a functional food. Golden thistle bioactive peptides thus offer promise for the development of nutraceutical products. However, to see the full potential of the plant, further *in vitro* and *in vivo* experiments are recommended to validate *in silico* results.

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The study's inception, design, data collecting, writing, original draft preparation, writing, review, and editing were all contributed by LC, SD and CKC.

Conflict of interest disclosure:

The authors declare no conflict of interest.

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