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# In Silico Analysis of Bioactive Peptides in Invasive Sea Grass *Halophila stipulacea*

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Academic Editor: Ka-Chun Wong

Received: 4 April 2019; Accepted: 30 May 2019; Published: 7 June 2019



**Abstract:** *Halophila stipulacea* is a well-known invasive marine sea grass in the Mediterranean Sea. Having been introduced into the Mediterranean Sea via the Suez Channel, it is considered a Lessepsian migrant. Although, unlike other invasive marine seaweeds, it has not demonstrated serious negative impacts on indigenous species, it does have remarkable invasive properties. The present in-silico study reveals the biotechnological features of *H. stipulacea* by showing bioactive peptides from its rubisc/o protein. These are features such as antioxidant and hypolipideamic activities, dipeptidyl peptidase-IV and angiotensin converting enzyme inhibitions. The reported data open up new applications for such bioactive peptides in the field of pharmacy, medicine and also the food industry.

**Keywords:** bioactive peptides; *Halophila stipulacea*; in silico analysis; DPP-IV; angiotensin converting enzyme inhibitors

## 1. Introduction

Invasive species are becoming an important problem in the Mediterranean Sea where many vectors exist, including inflowing water from the Suez Channel and ships' ballast waters. One of the best-known invasive sea grasses is *Halophila stipulacea* (Forsskål) Ascherson [1], which was first reported in the Mediterranean Sea by Friscth [2]. This sea grass is abundant in the eastern Mediterranean Basin and very common along the Turkish coastline [3]. No validated eradication method for this invasive species is described in the scientific literature. A few reports mention the negative impacts of *H. stipulacea* on indigenous species [4], although Willette and Ambrose [5] mention that little is known of *H. stipulacea*'s effects in its recently discovered Caribbean locations, while Van Tussenbroek et al. [6] report that *H. stipulacea* may be harmful for native species. Although most efforts so far have been devoted to finding alternative ways of evaluating non-indigenous species [7,8], invasive species secrete interesting secondary metabolites that can be exploited economically under the title of *blue biotechnology*. Indeed, recent years have seen growing interest in blue biotechnology-based products [9]. For example, bioactive peptides are one of the candidate targets that can be isolated from invasive species. However, to the best of our knowledge, the bioactive peptides from invasive *H. stipulacea* have not been assessed, and the aim of the present contribution is to fill in this gap in the literature.

Bioactive peptides (hereafter BPs) comprise 3–20 free amino acid food protein fragments [10] composed of covalently bonded (amide/peptide bonds) amino acids [11]. According to the BP database

called BIOPEP [12], there are more than 3500 different BPs. The sources of natural BPs can be land-, marine- or food-derived, and include seaweeds [13,14] tropical amphibians [15], cyanobacterium [16], fermented soybean meal [17], sea cucumber [18], cereal crops [19] and milk [20]. BPs are of great importance because of their positive impact on human health. Daliry et al. [21] classified food-derived BPs as anti-cancer, antidiabetic, antihypertensive, antimicrobial, cholesterol-lowering peptides, and multifunctional peptides [21]. Since they help prevent the oxidation and microbial degradation of foods, BPs could be defined as a “new generation” of bioactive regulators [11]. Although BPs are generally coded in the parent protein structure, some BPs are found free in natural sources [11]. Due to their biological and pharmaceutical properties, the production of BPs is of great importance, whether it be by enzymatic hydrolysis [22], chemical synthesis [23] or microbial fermentation [24]. Other production processes include separation and purification techniques such as gel filtration, ultrafiltration [25,26], reverse phase ultra-flow liquid chromatography (RP-UFLC) [25], and reverse phase high performance liquid chromatography (RP-HPLC) and characterization methods such as ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) [27]. Since these production, isolation, purification and characterization protocols are time- and solvent-consuming, bioinformatics tools are increasingly used [13,16]. The role of database-aided bioinformatics tools is to quantitatively predict the structure–activity relationship. Many tools have been developed such as BIOPEP [12], Antimicrobial Peptide Database (APD) [28] and PepBank [29]. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO, E.C. 4.1.1.39) is an important photosynthetic enzyme and the most abundant protein in the world [14,30]. RubisCO is a bifunctional multimeric enzyme and it plays a role in photorespiration and carbon fixation in the Calvin cycle [19,30]. Thirty to fifty percent of RubisCO is soluble and contains eight large (56 kDa) and eight small (14 kDa) subunits [31]. The small subunits of RubisCO contain high amounts of cationic and hydrophobic amino acids [31], while a bioactive dipeptide (Phe-Cys), which suppresses oxidative stress, has been obtained from the large subunit of RubisCO by in-silico thermolysin hydrolysis [32]. Some RubisCO derived peptides have revealed opioid activity, and some are G-protein coupled receptor ligands which constitute the most important class of drug targets [30]. Although there have been attempts at the chemical analysis of *H. stipulacea* [33], its detailed chemical composition is still not known in full.

This contribution presents an alternative and sustainable method for evaluating the invasive sea grass *H. stipulacea* by using in silico analysis of BPs in the large chain of RubisCO. Bioactive peptides are of great importance for the preparation of functional foods because of their excellent health-related effects. Many bioactivities, including antioxidant, antihypertensive and enzyme inhibitory properties have been associated with bioactive peptides from RubisCO of plants [13,19].

## 2. Materials and Methods

### 2.1. Sequence of *H. stipulacea* Rubisco

Ribulose bisphosphate carboxylase large chain of *H. stipulacea* (H6TQS9) was retrieved from the UniProtKB/Swiss-Prot database at the ExPASy Bioinformatics Resource Portal [34]. According to the portal, the sequence provided is a fragment and consists of 200 amino acids.

### 2.2. In silico BIOPEP Parameters

All in-silico calculations were performed using the codes implemented in the BIOPEP webserver [12]. One of the main theoretical parameters ( $A$ ) is defined as the frequency of occurrence of bioactive fragments in the protein chain  $A$  [12,35,36], which can be calculated by using Equation (1):

$$A = a/N \quad (1)$$

where  $a$  is the number of fragments with given activity in a protein sequence and  $N$  is the number of amino acid residues in the protein chain [12,35,36].

The frequency with which fragments with given activity were released by enzymes ( $A_E$ ) and the relative frequency of release of fragments with given activity by enzymes ( $W$ ) were calculated based on Equations (2) and (3), respectively.

$$A_E = d/N \quad (2)$$

where  $d$  is the number of fragments with given activity in the protein sequence that could be released by enzymes, and  $N$  is the number of amino acid residues in the protein chain. The relative frequency of release of fragments with given activity by selected enzymes ( $W$ ) is given by:

$$W = A_E/A \quad (3)$$

The values of  $A_E$  and  $A$  are defined according to Equations (1) and (2), respectively.

Potential biological activity of protein ( $B$ ) [ $\mu\text{M}^{-1}$ ]:

$$B = \frac{\sum_{i=1}^k \frac{a_i}{EC_{50i}}}{N} \quad (4)$$

In Equation (4),  $a_i$  is the number of repetitions of the  $i$ -th bioactive fragment in protein sequence,  $EC_{50i}$  is the concentration of the  $i$ -th bioactive peptide corresponding to its half-maximal activity [ $\mu\text{M}$ ],  $k$  is the number of different fragments with given activity and  $N$  is the number of amino acid residues [35].

The theoretical degree of hydrolysis ( $DH_t$ ) was also calculated using the following Equation (5):

$$DH_t = \frac{d}{D} \times 100\% \quad (5)$$

In Equation (5),  $d$  is number of hydrolyzed peptide bonds and  $D$  is total number of peptide bonds in a protein chain.

The relative activity of fragments with given activity released by selected enzymes ( $V$ ) is:

$$V = \frac{B_E}{B} \quad (6)$$

In Equation (6),  $B_E$  is the activity of fragments potentially released by proteolytic enzyme (enzymes) and  $B$  is the potential biological activity of the protein.

The amino acid composition of protein was determined based on protein sequences, using the ProtParam program [37] available at [38].

### 3. Results

*H. stipulacea* large chain RubisCO was retrieved from expasy.org. After in silico proteolytic fragmentation of the RubisCO by BIOPEP tools, bioactive peptides were obtained. The raw data can be found in Appendix A (Tables A1–A56). BIOPEP parameters ( $A$ ,  $A_E$ ,  $W$ ,  $BH_t$  and  $V$ ) were extracted from the raw data and the results are presented in the Tables 1–10.

The angiotensin-converting enzyme (ACE) inhibitor properties of bioactive peptides from RubisCO of *H. stipulacea* were given in Table 1. The values were not calculated for prolyl endopeptidase, clostripain, thrombin, glutamyl endopeptidase II, Xaa-dipeptidase, chymosin, ginger protease (zingipain). From this table, maximum and minimum  $A$  values were found to be 0.5833 and 0.5808, respectively. The highest values were observed in V-protease, endopeptidase, V-8 protease (Glutamyl endopeptidase), and the minimum values were found in trypsin, plasmin and oligopeptidase B. The maximum  $A_E$  value was found to be 0.0874 (calpain 2) and the minimum  $A_E$  values were found to be 0.0097 (plasmin, oligopeptidase B, tripsin). The maximum and minimum relative frequency of release of fragments ( $W$ ) were found if RubisCO is cleaved by calpain 2 (0.1500) and V-protease, glycylic endopeptidase, V8-protease (glutamyl endopeptidase) (0.0084).

**Table 1.** The values of the parameters describing the predicted efficiency of bioactive Angiotensin Converting Enzyme (ACE) inhibitor fragment release from *Halophila stipulacea*-RubisCO by proteases.

| Proteases                                     | A      | A <sub>E</sub> | W      | B      | V      |
|---|--------|----------------|--------|--------|--------|
| Chymotrypsin A                                | 0.5822 | 0.0485         | 0.0833 | 0.0017 | 0.0645 |
| Trypsin                                       | 0.5808 | 0.0097         | 0.0167 | 0.0000 | 0.0004 |
| Pepsin  | 0.5826 | 0.0194         | 0.0333 | 0.0000 | 0.0009 |
| Proteinase K                                  | 0.5825 | 0.0777         | 0.1334 | 0.0033 | 0.1240 |
| Pancreatic elastase                           | 0.5827 | 0.0437         | 0.0750 | 0.0007 | 0.0256 |
| V-protease                                    | 0.5833 | 0.0049         | 0.0084 | 0.0000 | 0.0003 |
| Thermolysin                                   | 0.5825 | 0.0777         | 0.1334 | 0.0052 | 0.1932 |
| Chymotrypsin C                                | 0.5826 | 0.0631         | 0.1083 | 0.0018 | 0.0671 |
| Plasmin                                       | 0.5808 | 0.0097         | 0.0167 | 0.0000 | 0.0004 |
| Cathepsin G                                   | 0.5826 | 0.0388         | 0.0666 | 0.0008 | 0.0308 |
| Chymase                                       | 0.5826 | 0.0388         | 0.0666 | 0.0008 | 0.0308 |
| Papain  | 0.5826 | 0.0631         | 0.1083 | 0.0019 | 0.0718 |
| Ficin   | 0.5827 | 0.0680         | 0.1167 | 0.0016 | 0.0592 |
| Leukocyte elastase                            | 0.5822 | 0.0485         | 0.0833 | 0.0025 | 0.0916 |
| Metridin                                      | 0.5826 | 0.0388         | 0.0666 | 0.0008 | 0.0308 |
| Pancreatic elastase II                        | 0.5826 | 0.0194         | 0.0333 | 0.0000 | 0.0009 |
| Bromelain                                     | 0.5822 | 0.0340         | 0.0584 | 0.0007 | 0.0276 |
| Oligopeptidase B                              | 0.5808 | 0.0097         | 0.0167 | 0.0000 | 0.0004 |
| Calpain 2                                     | 0.5827 | 0.0874         | 0.1500 | 0.0034 | 0.1285 |
| Glycyl endopeptidase                          | 0.5833 | 0.0049         | 0.0084 | 0.0000 | 0.0002 |
| Oligopeptidase F                              | 0.5826 | 0.0194         | 0.0333 | 0.0000 | 0.0009 |
| Proteinase P1 (lactocepin)                    | 0.5827 | 0.0243         | 0.0417 | 0.0005 | 0.0198 |
| Pepsin (pH > 2)                               | 0.5825 | 0.0777         | 0.1334 | 0.0022 | 0.0828 |
| Coccolysin                                    | 0.5827 | 0.0680         | 0.1167 | 0.0021 | 0.0800 |
| Subtilisin                                    | 0.5823 | 0.0534         | 0.0917 | 0.0055 | 0.2057 |
| V-8 protease (Glutamyl endopeptidase, pH:7.8) | 0.5833 | 0.0049         | 0.0084 | 0.0000 | 0.0003 |

**Table 2.** The values of parameters describing the predicted efficiency of release of the bioactive antioxidative fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases                  | A      | A <sub>E</sub> | W      | B | V |
|----------------------------|--------|----------------|--------|---|---|
| Chymotrypsin A             | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Proteinase K               | 0.0728 | 0.0243         | 0.3338 | 0 | 0 |
| Chymotrypsin C             | 0.0728 | 0.0097         | 0.1332 | 0 | 0 |
| Cathepsin                  | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Chymase                    | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Papain                     | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Ficin                      | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Leukocyte elastase         | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Metridin                   | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Bromelain                  | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Calpain 2                  | 0.0728 | 0.0097         | 0.1332 | 0 | 0 |
| Proteinase P1 (lactocepin) | 0.0728 | 0.0097         | 0.1332 | 0 | 0 |
| Pepsin (pH > 2)            | 0.0728 | 0.0049         | 0.0673 | 0 | 0 |
| Pubtilisin                 | 0.0728 | 0.0146         | 0.2005 | 0 | 0 |

**Table 3.** The values of parameters describing the predicted efficiency of release of bioactive dipeptidyl peptidase IV inhibitor fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases                             | A      | A <sub>E</sub> | W      | B      | V      |
|---------------------------------------|--------|----------------|--------|--------|--------|
| Chymotrypsin A                        | 0.6501 | 0.0340         | 0.0523 | 0.0000 | 0.0086 |
| Trypsin                               | 0.6518 | 0.0146         | 0.0224 | 0.0000 | 0.0000 |
| Pepsin                                | 0.6518 | 0.0146         | 0.0224 | 0.0000 | 0.0086 |
| Proteinase K                          | 0.6507 | 0.0922         | 0.1417 | 0.0000 | 0.0345 |
| Pancreatic elastase                   | 0.6508 | 0.0777         | 0.1194 | 0.0000 | 0.0969 |
| Prolyl oligopeptidase                 | 0.6533 | 0.0049         | 0.0075 | 0.0000 | 0.0000 |
| V-protease                            | 0.6533 | 0.0049         | 0.0075 | 0.0000 | 0.0000 |
| Thermolysin                           | 0.6504 | 0.0534         | 0.0821 | 0.0000 | 0.0071 |
| Chymotrypsin C                        | 0.6501 | 0.0485         | 0.0746 | 0.0000 | 0.0242 |
| Plasmin                               | 0.6518 | 0.0146         | 0.0224 | 0.0000 | 0.0000 |
| Cathepsin                             | 0.6497 | 0.0243         | 0.0374 | 0.0000 | 0.0086 |
| Clostripain                           | 0.6533 | 0.0049         | 0.0075 | 0.0000 | 0.0000 |
| Chymase                               | 0.6497 | 0.0243         | 0.0374 | 0.0000 | 0.0086 |
| Papain                                | 0.6506 | 0.0825         | 0.1268 | 0.0000 | 0.1066 |
| Ficin                                 | 0.6503 | 0.0874         | 0.1344 | 0.0000 | 0.0510 |
| Leukocyte elastase                    | 0.6506 | 0.0728         | 0.1119 | 0.0000 | 0.1056 |
| Metridin                              | 0.6497 | 0.0243         | 0.0374 | 0.0000 | 0.0086 |
| Pancreatic elastase II                | 0.6518 | 0.0146         | 0.0224 | 0.0000 | 0.0086 |
| Bromelain                             | 0.6507 | 0.0680         | 0.1045 | 0.0000 | 0.1005 |
| Oligopeptidase B                      | 0.6518 | 0.0146         | 0.0224 | 0.0000 | 0.0000 |
| Calpain 2                             | 0.6506 | 0.1214         | 0.1866 | 0.0000 | 0.1066 |
| Glycyl endopeptidase                  | 0.6533 | 0.0049         | 0.0075 | 0.0000 | 0.0000 |
| Oligopeptidase F                      | 0.6518 | 0.0146         | 0.0224 | 0.0000 | 0.0086 |
| Proteinase P1 (lactocepin)            | 0.6501 | 0.0340         | 0.0523 | 0.0000 | 0.0000 |
| Pepsin (pH > 2)                       | 0.6505 | 0.1165         | 0.1791 | 0.0001 | 0.4569 |
| Cocolysin                             | 0.6503 | 0.0437         | 0.0672 | 0.0000 | 0.0071 |
| Subtilisin                            | 0.6507 | 0.0583         | 0.0896 | 0.0000 | 0.0086 |
| V-8 protease (Glutamyl endopeptidase) | 0.6510 | 0.0097         | 0.0149 | 0.0000 | 0.0000 |

**Table 4.** The values of parameters describing the predicted efficiency of release of bioactive activating ubiquitin-mediated proteolysis fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases                  | A      | A <sub>E</sub> | W      | B | V |
|----------------------------|--------|----------------|--------|---|---|
| Pancreatic elastase        | 0.0146 | 0.0097         | 0.6644 | 0 | - |
| Leukocyte elastase         | 0.0146 | 0.0049         | 0.3356 | 0 | - |
| Proteinase P1 (lactocepin) | 0.0146 | 0.0049         | 0.3356 | 0 | - |
| Pepsin (pH > 2)            | 0.0146 | 0.0097         | 0.6644 | 0 | - |

**Table 5.** The values of parameters describing the predicted efficiency of the release of bioactive regulating fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases       | A      | A <sub>E</sub> | W      | B | V |
|-----------------|--------|----------------|--------|---|---|
| Chymotrypsin C  | 0.0146 | 0.0049         | 0.3356 | 0 | - |
| Ficin           | 0.0146 | 0.0049         | 0.3356 | 0 | - |
| Calpain 2       | 0.0146 | 0.0049         | 0.3356 | 0 | - |
| Pepsin (pH > 2) | 0.0146 | 0.0049         | 0.3356 | 0 | - |

**Table 6.** The values of parameters describing the predicted efficiency of release of bioactive antithrombotic fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases       | A      | A <sub>E</sub> | W      | B | V |
|-----------------|--------|----------------|--------|---|---|
| Chymotrypsin C  | 0.0097 | 0.0049         | 0.5052 | 0 | - |
| Calpain 2       | 0.0097 | 0.0049         | 0.5052 | 0 | - |
| Pepsin (pH > 2) | 0.0097 | 0.0049         | 0.5052 | 0 | - |

**Table 7.** The values of parameters describing the predicted efficiency of release of bioactive anti-inflammatory fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases       | A      | A <sub>E</sub> | W      | B | V |
|-----------------|--------|----------------|--------|---|---|
| Chymotrypsin C  | 0.0097 | 0.0049         | 0.5052 | 0 | - |
| Calpain 2       | 0.0097 | 0.0049         | 0.5052 | 0 | - |
| Pepsin (pH > 2) | 0.0097 | 0.0049         | 0.5052 | 0 | - |

**Table 8.** The values of parameters describing the predicted efficiency of release of bioactive stimulating fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases       | A      | A <sub>E</sub> | W      | B | V |
|-----------------|--------|----------------|--------|---|---|
| Papain          | 0.0340 | 0.0049         | 0.1441 | 0 | - |
| Pepsin (pH > 2) | 0.0340 | 0.0049         | 0.1441 | 0 | - |

**Table 9.** The values of parameters describing the predicted efficiency of release of bioactive immunomodulating fragment from *Halophila stipulacea*-RubisCO by proteases.

| Proteases | A      | A <sub>E</sub> | W      | B | V |
|-----------|--------|----------------|--------|---|---|
| Calpain 2 | 0.0097 | 0.0049         | 0.5052 | 0 | 0 |

**Table 10.** Theoretical degree of hydrolysis ( $DH_t$ ) values for the following enzymes.

| Proteases                             | $DH_t$  |
|---------------------------------------|---------|
| Chymotrypsin A                        | 23.4146 |
| Trypsin                               | 10.7317 |
| Pepsin                                | 12.1951 |
| Proteinase K                          | 37.0732 |
| Pancreatic elastase                   | 54.6341 |
| Prolyl oligopeptidase                 | 6.8293  |
| V-protease                            | 7.3171  |
| Thermolysin                           | 36.5854 |
| Chymotrypsin C                        | 35.6098 |
| Plasmin                               | 10.7317 |
| Cathepsin                             | 20.4878 |
| Clostripain                           | 5.3659  |
| Chymase                               | 19.5122 |
| Papain                                | 43.4146 |
| Ficin                                 | 44.3902 |
| Leukocyte elastase                    | 38.5366 |
| Metridin                              | 19.5122 |
| Thrombin                              | 0       |
| Pancreatic elastase II                | 13.1707 |
| Bromelain                             | 54.1463 |
| Endopeptidase II                      | 0       |
| Oligopeptidase B                      | 10.7317 |
| Calpain 2                             | 47.3171 |
| Glycyl endopeptidase                  | 9.7561  |
| Oligopeptidase F                      | 12.1951 |
| Proteinase P1 (lactocepine)           | 41.4634 |
| Xaa-Pro dipeptidase                   | 0       |
| Pepsin (pH>2)                         | 70.7317 |
| Cocolysin                             | 30.2439 |
| Subtilisin                            | 29.2683 |
| Chymosin                              | 0       |
| Ginger protease (zingipain)           | 0       |
| V-8 protease (Glutamyl endopeptidase) | 11.7073 |

The highest  $B$  value was found to be 0.0055 (subtilisin) and the lowest  $B$  values were found to be 0 for trypsin, pepsin, plasmin, pancreatic elastase II, oligopeptidase B, glycyI endopeptidase, oligopeptidase F and V-8 protease (Glutamyl endopeptidase). The maximum and minimum  $V$  values were found to be 0.2057 and 0.0002, respectively. The maximum value was observed in subtilisin and the minimum value was found in glycyI endopeptidase. ACE is known as dipeptidyl carboxypeptidase and one of its major roles is controlling blood pressure [39]. In the literature, Agirbasli and Cavas [13] evaluated the frequency of occurrence ( $A$ ) values of ACE inhibitor peptides in *Caulerpa* RubisCO and found that *C. racemosa* var. *lamourouxii*, *C. taxifolia* and *C. racemosa* f. *occidentalis* had the highest  $A$  values (0.4330, 0.4330 and 0.3993, respectively) and *C. racemosa* var. *turbinata* exhibited the lowest (0.3822). Another study revealed that *C. microphysa* had potential ACE inhibitory activity as a result of pepsin cleavage [40]. However, the number of bioactive peptides in the BIOPEP database has increased, and so the frequency values might have been altered.

The antioxidative properties of BPs from RubisCO of *H. stipulacea* are listed in Table 2. Results reveal that maximum and minimum  $A$  values were 0.07282 and 0.07280, respectively. The minimum  $A$  value was obtained when proteinase K was used as protease. Minimum  $A$  values were found in calpain 2 and proteinase P1 (lactocepin). The maximum and minimum  $A_E$  values were obtained as 0.0243 (proteinase K) and 0.0049 (chymotrypsin, cathepsin, chymase, papain, ficin, leukocyte elastase, metridin, bromelain and pepsin), respectively. Proteinase K had the maximum  $W$  value (0.3338) and chymase, papain, ficin, leukocyte elastase, metridin, bromelain and pepsin had the lowest  $W$  values (0.0673). When thrombin, endopeptidase II, Xaa-Pro dipeptidase, chymosin and ginger protease (zingipain) were used for cleaving, no antioxidative fragment from RubisCO of *H. stipulacea* was found. The inhibition of lipid peroxidation, scavenging of radicals and metal chelation are among the antioxidative properties of BPs [41]. In the literature, the antioxidative activity of BPs have been evaluated in-silico. According to a recent study, the highest  $A$  value for the antioxidative properties of RubisCO was found in *Caulerpa taxifolia* (0.0785) samples and *C. cylindracea* (0.0759) species [13]. Also, Udenigwe et al. [19,30] found the maximum and minimum  $A$  value of antioxidative properties of RubisCO of cereal crops to be 0.0568 and 0.0464, respectively [19].

The inhibition effects of bioactive peptides from RubisCO of *H. stipulacea* on dipeptidyl peptidase IV (DPP-IV) (E.C. 3.4.14.5) are given in Table 3. According to the results, prolyl oligopeptidase, V-protease, clostripain and glycyI endopeptidase have the maximum (0.6533) and cathepsin, chymase and metridin have the minimum (0.6497)  $A$  value. The maximum  $A_E$  value was 0.1214 when calpain 2 was used as a protease in DPP-IV inhibitor activity. The minimum  $A_E$  value (0.0049) was found in prolyl oligopeptidase, V-protease, clostripain and glycyI endopeptidase. Calpain 2 had the maximum (0.1866) and prolyl oligopeptidase, V-protease and clostripain had the minimum (0.0075)  $W$  value. We found very low  $B$  values for all the enzymes studied. The highest  $V$  value was found in pepsin ( $\text{pH} > 2$ ) (0.4569), while trypsin, prolyl oligopeptidase, V8-protease, plasmin, clostripain, oligopeptidase B, glycyI endopeptidase and proteinase P1 had the lowest  $V$  value (0.0000). DPP-IV is crucial in glucose metabolism and it degrades the incretins [42]. Thus, DPP-IV inhibitors play a major role in type-2 diabetes mellitus in which insulin secretion and blood glucose level stability are of great importance. [42]. Agirbasli and Cavas found the  $A$  value of DPP-IV to be between 0.0550 and 0.0714 in all of *Caulerpa* species [13]. They also mentioned that caulerpenyne is found in *Caulerpa* species and its alpha-amylase inhibition activity may play an important role in reducing starch degradation. In another in silico study carried out by Udenigwe et al. [19,30], rice and oat showed the highest  $A$  value (0.0758).

Table 4 shows the ubiquitin-mediated proteolysis (UbMP) activating properties of bioactive peptides from RubisCO of *H. stipulacea*. The  $A$  value was 0.0146 in pancreatic elastase, leukocyte elastase, proteinase P1 (lactocepin) and pepsin ( $\text{pH} > 2$ ). The maximum  $A_E$  value was 0.0097 (pancreatic elastase) and the minimum  $A_E$  values were 0.0049 for leukocyte elastase and proteinase P1. Also, when thrombin, endopeptidase II and Xaa-Pro dipeptidase, chymosin and ginger protease were used, a ubiquitin-mediated proteolysis fragment from *H. stipulacea* was not found. Pancreatic elastase and



pepsin have the highest  $W$  value (0.6644) and leukocyte elastase and proteinase P1 have the lowest  $W$  value (0.3356). No  $B$  or  $W$  value was found for UbMP properties of bioactive peptides from RubisCO of *H. stipulacea*. UbMP is crucial for brain development [43] and its absence causes neurodegenerative diseases such as Parkinson's and Alzheimer's [44]. In the literature, Minkiewicz et al. [36] carried out an in-silico evaluation of bovine meat proteins and found that the highest  $A$  value of activating UbMP was 0.028 in tropomyosin  $\alpha$ -1 chain [36].

Results for bioactive regulating fragments from *H. stipulacea* by proteases are shown in Table 5. The results reveal that the only  $A$  value (0.0146) was found in chymotrypsin, ficin, calpain 2 and pepsin. The maximum  $A_E$  value (0.0049) was obtained in chymotrypsin, ficin, calpain 2 and pepsin. Chymotrypsin, ficin, calpain 2 and pepsin had the same  $W$  values (0.3356).  $B$  and  $V$  values were not found for the bioactive regulating activity of *H. stipulacea* by proteases.

Table 6 shows the antithrombotic activity of BPs from *H. stipulacea*. According to the results, maximum  $A$ ,  $A_E$  and  $W$  values (0.0097, 0.0049 and 0.5052, respectively) were obtained for chymotrypsin, calpain 2 and pepsin. The  $B$  and  $V$  values of the antithrombotic properties of *H. stipulacea* by proteases were not found. Antithrombotic activity is essential for the reduction of thrombin. In the study of Agirbasli and Cavas [13], the  $A$  values of antithrombotic activity of BPs from *Caulerpa* genus were found within the range of 0.0010 to 0.0100 [13].

The anti-amnesic activity values of the bioactive peptides are given in Table 7. The only  $A$  value found (0.0097) was found in chymotrypsin, calpain 2 and pepsin. Also, the maximum  $A_E$  and  $W$  values (0.0049 and 0.5052, respectively) were obtained by chymotrypsin, calpain 2 and pepsin. Agirbasli and Cavas [13] found the  $A$  values of anti-amnesic activity of BPs from *Caulerpa* genus to be within the range of 0.0010 to 0.0100, the same as for antithrombotic activity, perhaps because they act in the similar pathway way [13].

The results of stimulating fragments of *H. stipulacea* are given in Table 8. The highest  $A$ ,  $A_E$  and  $W$  values (0.0340, 0.0049 and 0.1441) were obtained by papain and pepsin ( $\text{pH} > 2$ ).

Table 9 provides the immunomodulating activity results of BPs from *H. stipulacea*. The results reveal that calpain 2 has the highest  $A$ ,  $A_E$  and  $W$  values (0.0097, 0.0049 and 0.5052, respectively). It is interesting to note  $A$ ,  $A_E$ ,  $W$ ,  $B$  or  $V$  values could not be calculated for other enzymes in this study.

In Table 10, the theoretical degree of hydrolysis ( $DH_t$ ) are given for the following proteases: Chymotrypsin A, trypsin, pepsin, proteinase K, pancreatic elastase, prolyl oligopeptidase, V8-protease, thermolysin, chymotrypsin C, plasmin, cathepsin, clostripain, chymase, papain, ficin, leukocyte elastase, metridin, thrombin, pancreatic elastase II, bromelain, endopeptidase II, oligopeptidase B, calpain 2, glycy endopeptidase, oligopeptidase F, proteinase P1 (lactocepain), Xaa-Pro dipeptidase, pepsin ( $\text{pH} > 2$ ), cocolysin, subtilisin, chymosin, ginger protease (zingipain) and V-8 protease (Glutamyl endopeptidase). According to the results, the highest and the lowest  $DH_t$  values were found in pepsin  $\text{pH} > 2$  (70.7317) and thrombin, endopeptidase II, Xaa-Pro dipeptidase, chymocine, ginger protease (0), respectively.

In-silico analysis is regarded as an important tool by food scientists since in-silico results may reflect in-vitro and in-vivo results [10,45–48]. Lafarga et al. [48] defined new bioactive peptides that show ACE and DPP IV inhibition. They confirmed their biological activity by synthetic tripeptides. Sayd et al. [49] also used a similar strategy, grouping the bioactive meat proteins into three categories based on their digestion dynamic. In recent years, there has been a growth in meat consumption as a result of an increasing population. This demand may increase the use of growth hormones, which, in some countries, are banned, but in others allowed [50]. Therefore, an alternative protein source to meat would be of great interest. In this respect, marine seaweeds and seagrasses can be exploited on an industrial scale since there is no hormone ingredient.



#### 4. Discussion

Blue biotechnology and blue growth have become two of the hottest topics in recent years. The evaluation of invasive species may open up a new door in the search for novel agents such as vaccines, secondary metabolites and medicines. The present paper reveals that *H.stipulacea* contains bioactive peptides. These peptides can be harvested and evaluated in the countries affected. However, any possible industrial collection of *H.stipulacea* would have to be approved by local authorities. Since *H.stipulacea* forms a mixed vegetation with local Mediterranean macrophytes and seaweeds, its uncontrolled collection might damage the local species. In conclusion, invasive species in the Mediterranean Sea contain very important secondary metabolites and bioactive peptides. Instead of applying blunt eradication methods, biotechnological evaluation is needed.

**Author Contributions:** Conceptualization, L.C. and H.P.-S.; methodology, L.C.; software, C.K.-C., N.M.-O.; validation, C.K.-C., N.M.-O.; formal analysis, L.C. and H.P.-S.; investigation, L.C. and H.P.-S.; resources, L.C. and H.P.-S.; data curation, C.K.-C., N.M.-O.; writing—original draft preparation, L.C. and C.K.-C., N.M.O.; writing—review and editing, L.C., C.K.-C. and H.P.-S.; visualization, L.C., C.K.-C.; supervision, L.C., and H.P.-S.; project administration, L.C. and H.P.-S.; funding acquisition, L.C. and H.P.-S.

**Funding:** This project was financially supported by Turkish Higher Education Council (YÖK) under Mevlana-Project Based Academic Exchange Programme (MEV-2017-230) and by grants from the Spanish Ministry of Economy and Competitiveness (CTQ2017-87974-R) and by the Fundación Séneca del Centro de Coordinación de la Investigación de la Región de Murcia under Projects 20988/PI/18 and 20524/PDC/18.

**Acknowledgments:** This project was financially supported by Turkish Higher Education Council (YÖK) under Mevlana-Project Based Academic Exchange Programme (MEV-2017-230) and by grants from the Spanish Ministry of Economy and Competitiveness (CTQ2017-87974-R) and by the Fundación Séneca del Centro de Coordinación de la Investigación de la Región de Murcia under Projects 20988/PI/18 and 20524/PDC/18.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A (Raw Data)

Table A1. Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using chymotrypsine.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 3384       | VF       | (128–129) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 264.1360          | 264.3100      |
| 3   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 4   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 5   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 6   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 7   | 7691       | KY       | (168–169) | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 309.1570          | 309.3440      |
| 8   | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 259.1780          | 259.3340      |
| 9   | 8219       | TY       | (23–24)   | antioxidative peptide                                |  | antioxidative                     | 282.1100          | 282.2750      |
| 10  | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 11  | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 12  | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 13  | 8819       | KY       | (168–169) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |
| 14  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 15  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |

Table A1. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 16  | 8917       | VF       | (128–129) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 264.1360          | 264.3100      |
| 17  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 365.1840          | 365.4090      |
| 18  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

Table A2.  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage carried out by using chymotrypsin.

| $DH_t$ (%) |                                   |        |        |                    |                    |
|------------|-----------------------------------|--------|--------|--------------------|--------------------|
| 23.4146    |                                   |        |        |                    |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                |
| 1          | ACE inhibitor                     | 0.0485 | 0.0833 | 0.0017261314434853 | 0.06450702174869   |
| 2          | antioxidative                     | 0.0049 | 0.0673 | 0                  | 0                  |
| 3          | dipeptidyl peptidase IV inhibitor | 0.0340 | 0.0523 | 1.8563339357632E-6 | 0.0086054864513139 |

Table A3. Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using trypsin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 7603       | GR       | (84–85)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 231.1220          | 231.2400      |
| 2   | 7697       | YK       | (82–83)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 309.1570          | 309.3440      |
| 3   | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 4   | 8858       | PK       | (180–181) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 5   | 8939       | YK       | (82–83)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |

**Table A4.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage carried out by using trypsin.

| $DH_t$ (%)<br>10.7317 |                                   |        |        |                    |                     |
|-----------------------|-----------------------------------|--------|--------|--------------------|---------------------|
| No.                   | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                 |
| 1                     | ACE inhibitor                     | 0.0097 | 0.0167 | 9.4749721470635E-6 | 0.00035408788633428 |
| 2                     | dipeptidyl peptidase IV inhibitor | 0.0146 | 0.0224 | 0                  | 0                   |

**Table A5.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using pepsin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 3   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 4   | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 5   | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 6   | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 7   | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A6.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage carried out by using pepsin.

| $DH_t$ (%)<br>12.1951 |                                   |        |        |                    |                     |
|-----------------------|-----------------------------------|--------|--------|--------------------|---------------------|
| No.                   | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                 |
| 1                     | ACE inhibitor                     | 0.0194 | 0.0333 | 2.5006640432763E-5 | 0.00093451973448837 |
| 2                     | dipeptidyl peptidase IV inhibitor | 0.0146 | 0.0224 | 1.8563339357632E-6 | 0.0086054864513139  |

**Table A7.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using proteinase K.

| No. | Peptide ID | Sequence | Location  | Name            | Function   | Activity      | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|-----------------|--|---------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 287.1850          | 287.3480      |
| 2   | 3378       | GRP      | (170–172) | ACE inhibitor   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 328.1740          | 328.3570      |
| 3   | 3563       | AY       | (101–102) | ACE inhibitor   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 252.1000          | 252.2490      |
| 4   | 3563       | AY       | (104–105) | ACE inhibitor   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 252.1000          | 252.2490      |
| 5   | 3563       | AY       | (147–148) | ACE inhibitor   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 252.1000          | 252.2490      |
| 6   | 7591       | GF       | (12–13)   | ACE inhibitor   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 222.0890          | 222.2290      |
| 7   | 7591       | GF       | (130–131) | ACE inhibitor   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 222.0890          | 222.2290      |
| 8   | 7599       | GL       | (183–184) | ACE inhibitor   | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor | 188.1050          | 188.2120      |

Table A7. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function  | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|---|-----------------------------------|-------------------|---------------|
| 9   | 7608       | GV       | (47–48)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                     | 174.0890          | 174.1850      |
| 10  | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |   | ACE inhibitor                     | 259.1780          | 259.3340      |
| 11  | 7693       | KL       | (181–182) | ACE inhibitor from wakame                            |   | ACE inhibitor                     | 259.1780          | 259.3340      |
| 12  | 7752       | EY       | (28–29)   | ACE inhibitor from shark meat hydrolysate            | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                     | 310.1050          | 310.2860      |
| 13  | 7810       | KP       | (179–180) | ACE inhibitor from anchovy and bonito                | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                     | 243.1460          | 243.2910      |
| 14  | 7866       | AY       | (101–102) | peptide from Okara protein                           | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | antioxidative                     | 252.1000          | 252.2490      |
| 15  | 7866       | AY       | (104–105) | peptide from Okara protein                           | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | antioxidative                     | 252.1000          | 252.2490      |
| 16  | 7866       | AY       | (147–148) | peptide from Okara protein                           | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | antioxidative                     | 252.1000          | 252.2490      |
| 17  | 8218       | KP       | (179–180) | Antioxidative peptide                                | Free radical scavenger  | antioxidative                     | 243.1460          | 243.2910      |
| 18  | 8219       | TY       | (23–24)   | antioxidative peptide                                |   | antioxidative                     | 282.1100          | 282.2750      |
| 19  | 8503       | TP       | (26–27)   | Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 216.0990          | 216.2220      |
| 20  | 8505       | SP       | (2–3)     | Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 202.0840          | 202.1970      |
| 21  | 8505       | SP       | (43–44)   | Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 202.0840          | 202.1970      |
| 22  | 8519       | KP       | (179–180) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 23  | 8529       | EP       | (90–91)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 244.0940          | 244.2330      |
| 24  | 8532       | QP       | (45–46)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 243.1100          | 243.2480      |
| 25  | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |

Table A7. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 26  | 8765       | AY       | (101–102) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 252.1000          | 252.2490      |
| 27  | 8765       | AY       | (104–105) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 252.1000          | 252.2490      |
| 28  | 8765       | AY       | (147–148) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 252.1000          | 252.2490      |
| 29  | 8777       | EY       | (28–29)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 310.1050          | 310.2860      |
| 30  | 8782       | GF       | (12–13)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 31  | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 32  | 8786       | GV       | (47–48)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 33  | 8793       | HI       | (88–89)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 268.1420          | 268.3020      |
| 34  | 8879       | QV       | (160–161) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1260          | 245.2640      |
| 35  | 8884       | RI       | (143–144) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 36  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 37  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 38  | 9073       | TP       | (26–27)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 216.0990          | 216.2220      |
| 39  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |
| 40  | 9146       | QGP      | (153–155) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 300.1310          | 300.3000      |



**Table A8.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by proteinase K.

| $DH_t$ (%)<br>37.0732 |                                   |        |        |                    |                   |
|-----------------------|-----------------------------------|--------|--------|--------------------|-------------------|
| No.                   | Activity                          | $A_E$  | $W$    | $B_E$              | $V$               |
| 1                     | ACE inhibitor                     | 0.0777 | 0.1334 | 0.0033188734698932 | 0.12402916586194  |
| 2                     | antioxidative                     | 0.0243 | 0.3338 | 0                  | 0                 |
| 3                     | dipeptidyl peptidase IV inhibitor | 0.0922 | 0.1417 | 7.4392944998736E-6 | 0.034486655009987 |

**Table A9.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using pancreatic elastase.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                                  | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|---|-------------------|---------------|
| 1   | 3174       | KA       | (8–9)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 217.1310          | 217.2530      |
| 2   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 287.1850          | 287.3480      |
| 3   | 4005       | RA       | (135–136) |  | Activation of ubiquitin-dependent proteolysis  | activating ubiquitin-mediated proteolysis | 245.1380          | 245.2670      |
| 4   | 4005       | RA       | (193–194) |  | Activation of ubiquitin-dependent proteolysis  | activating ubiquitin-mediated proteolysis | 245.1380          | 245.2670      |
| 5   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                             | 228.1360          | 228.2770      |
| 6   | 7588       | RA       | (135–136) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 245.1380          | 245.2670      |
| 7   | 7588       | RA       | (193–194) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 245.1380          | 245.2670      |
| 8   | 7604       | KG       | (83–84)   | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1; MEROPS ID: XM02-001)        | ACE inhibitor                             | 203.1150          | 203.2260      |
| 9   | 7605       | FG       | (129–130) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 222.0890          | 222.2290      |

Table A9. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 10  | 7681       | DG       | (74–75)   | ACE inhibitor from soy                               | Inhibitor of Angiotensin-converting enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 190.0479          | 190.1410      |
| 11  | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 259.1780          | 259.3340      |
| 12  | 7743       | KA       | (8–9)     | ACE inhibitor  | Inhibitor of angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 217.1310          | 217.2530      |
| 13  | 8526       | RA       | (135–136) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1380          | 245.2670      |
| 14  | 8526       | RA       | (193–194) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1380          | 245.2670      |
| 15  | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 16  | 8685       | WT       | (68–69)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 17  | 8685       | WT       | (72–73)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 18  | 8774       | ET       | (6–7)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 19  | 8774       | ET       | (30–31)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 20  | 8793       | HI       | (88–89)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 268.1420          | 268.3020      |
| 21  | 8810       | KG       | (83–84)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 203.1150          | 203.2260      |
| 22  | 8816       | KT       | (150–151) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 247.1410          | 247.2790      |
| 23  | 8851       | NV       | (127–128) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 231.1109          | 231.2370      |
| 24  | 8879       | QV       | (160–161) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1260          | 245.2640      |
| 25  | 8882       | RG       | (200–201) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 231.1220          | 231.2400      |
| 26  | 8884       | RI       | (143–144) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 27  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |

**Table A10.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by pancreatic elastase.

| $DH_t$ (%) |   |        |        |                    |                   |
|------------|---|--------|--------|--------------------|-------------------|
| 54.6341    |   |        |        |                    |                   |
| No.        | Activity                                  | $A_E$  | $W$    | $B_E$              | $V$               |
| 1          | dipeptidyl peptidase IV inhibitor         | 0.0777 | 0.1194 | 2.0912653098933E-5 | 0.096945678488557 |
| 2          | ACE inhibitor                             | 0.0437 | 0.0750 | 0.0006857879028624 | 0.025628485786464 |
| 3          | activating ubiquitin-mediated proteolysis | 0.0097 | 0.6644 | 0                  |                   |

**Table A11.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using prolyl oligopeptidase.

| No. | Peptide ID | Sequence | Location | Name   | Function  | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|----------|--|---|-----------------------------------|-------------------|---------------|
| 1   | 8532       | QP       | (45–46)  | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003) | dipeptidyl peptidase IV inhibitor | 243.1100          | 243.2480      |

**Table A12.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by prolyl oligopeptidase.

| $DH_t$ (%) |                                   |        |        |       |     |
|------------|-----------------------------------|--------|--------|-------|-----|
| 6.8293     |                                   |        |        |       |     |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$ | $V$ |
| 1          | dipeptidyl peptidase IV inhibitor | 0.0049 | 0.0075 | 0     | 0   |

**Table A13.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using V-protease.

| No. | Peptide ID | Sequence | Location | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 8934       | YE       | (29–30)  | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 310.1050          | 310.2860      |
| 2   | 9078       | YE       | (29–30)  | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 310.1050          | 310.2860      |

**Table A14.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by V-protease.

| $DH_t$ (%) |                                   |        |        |                    |                    |
|------------|-----------------------------------|--------|--------|--------------------|--------------------|
| 7.3171     |                                   |        |        |                    |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                |
| 1          | dipeptidyl peptidase IV inhibitor | 0.0049 | 0.0075 | 0                  | 0                  |
| 2          | ACE inhibitor                     | 0.0049 | 0.0084 | 7.6943555746376E-6 | 0.0002875447082947 |

**Table A15.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using thermolysin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3522       | IPP      | (144–146) | ACE inhibitor (from bovine b-CN)                     |  | ACE inhibitor                     | 325.1880          | 325.3940      |
| 2   | 3666       | YP       | (105–106) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 278.1150          | 278.2870      |
| 3   | 7592       | FR       | (40–41)   | ACE inhibitor  |  | ACE inhibitor                     | 321.1690          | 321.3650      |
| 4   | 7594       | VG       | (11–12)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 174.0890          | 174.1850      |
| 5   | 7600       | AG       | (9–10)    | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 6   | 7600       | AG       | (15–16)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 7   | 7600       | AG       | (53–54)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 8   | 7605       | FG       | (129–130) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 9   | 7619       | LG       | (182–183) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 10  | 7697       | YK       | (20–21)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 309.1570          | 309.3440      |
| 11  | 7859       | IEP      | (89–91)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 357.1780          | 357.3930      |
| 12  | 8521       | YP       | (105–106) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 278.1150          | 278.2870      |

Table A15. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 13  | 8760       | AG       | (9–10)    | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 14  | 8760       | AG       | (15–16)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 15  | 8760       | AG       | (53–54)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 16  | 8779       | FQ       | (13–14)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 293.1260          | 293.3080      |
| 17  | 8780       | FR       | (40–41)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 321.1690          | 321.3650      |
| 18  | 8805       | IQ       | (159–160) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 259.1420          | 259.2910      |
| 19  | 8824       | LT       | (22–23)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 232.1310          | 232.2650      |
| 20  | 8918       | VG       | (11–12)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 21  | 8937       | YH       | (87–88)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 318.1210          | 318.3120      |
| 22  | 8939       | YK       | (20–21)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |
| 23  | 9076       | FQ       | (13–14)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 293.1260          | 293.3080      |
| 24  | 9087       | YH       | (87–88)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 318.1210          | 318.3120      |
| 25  | 9213       | LR       | (134–135) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 287.1850          | 287.3480      |
| 26  | 9213       | LR       | (137–138) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 287.1850          | 287.3480      |
| 27  | 9213       | LR       | (142–143) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 287.1850          | 287.3480      |

**Table A16.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by thermolysin.

| $DH_t$ (%) |                                   |        |        |                    |                    |
|------------|-----------------------------------|--------|--------|--------------------|--------------------|
| 36.5854    |                                   |        |        |                    |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                |
| 1          | ACE inhibitor                     | 0.0777 | 0.1334 | 0.0051700207807555 | 0.19320813846713   |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0534 | 0.0821 | 1.5313466662583E-6 | 0.0070989290961449 |

**Table A17.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using Chymotrypsin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 2753       | GP       | (154–155) | peptide regulating the stomach mucosal membrane activity | regulating the stomach mucosal membrane activity                                     | regulating                        | 172.0730          | 172.1690      |
| 2   | 3169       | GP       | (154–155) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)     | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 172.0730          | 172.1690      |
| 3   | 3257       | RL       | (138–139) | beta-lactokinin  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 4   | 3283       | GP       | (154–155) |  | antithrombotic   | antithrombotic                    | 172.0730          | 172.1690      |
| 5   | 3378       | GRP      | (170–172) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 328.1740          | 328.3570      |
| 6   | 3461       | GP       | (154–155) | Prolyl endopeptidase inhibitor                           | Inhibitor of Prolyl Endopeptidase (PEP) (EC 3.4.21.26) (MEROPS ID: S09.001)          | antiamnestic                      | 172.0730          | 172.1690      |
| 7   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 8   | 3563       | AY       | (147–148) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 252.1000          | 252.2490      |
| 9   | 7512       | GP       | (154–155) | ACE inhibitor from Alaskan pollack skin                  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 172.0730          | 172.1690      |
| 10  | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |

Table A17. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function  | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|---|-----------------------------------|-------------------|---------------|
| 11  | 7691       | KY       | (168–169) | ACE inhibitor from wakame                            |   | ACE inhibitor                     | 309.1570          | 309.3440      |
| 12  | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |   | ACE inhibitor                     | 259.1780          | 259.3340      |
| 13  | 7693       | KL       | (181–182) | ACE inhibitor from wakame                            |   | ACE inhibitor                     | 259.1780          | 259.3340      |
| 14  | 7829       | VE       | (161–162) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                     | 246.1100          | 246.2490      |
| 15  | 7830       | TE       | (5–6)     | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)              | ACE inhibitor                     | 248.0890          | 248.2210      |
| 16  | 7866       | AY       | (147–148) | peptide from Okara protein                           | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | antioxidative                     | 252.1000          | 252.2490      |
| 17  | 8219       | TY       | (23–24)   | antioxidative peptide                                |   | antioxidative                     | 282.1100          | 282.2750      |
| 18  | 8503       | TP       | (26–27)   | Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 216.0990          | 216.2220      |
| 19  | 8505       | SP       | (2–3)     | Dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 202.0840          | 202.1970      |
| 20  | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 21  | 8765       | AY       | (147–148) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 252.1000          | 252.2490      |
| 22  | 8819       | KY       | (168–169) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |
| 23  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 24  | 8899       | TE       | (5–6)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 25  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 26  | 8916       | VE       | (161–162) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor | 246.1100          | 246.2490      |
| 27  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)         | ACE inhibitor                     | 365.1840          | 365.4090      |
| 28  | 9073       | TP       | (26–27)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)         | ACE inhibitor                     | 216.0990          | 216.2220      |



**Table A18.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by Chymotrypsin.

| $DH_t$ (%) |                                   |        |        |                    |                   |
|------------|-----------------------------------|--------|--------|--------------------|-------------------|
| 35.6098    |                                   |        |        |                    |                   |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$               |
| 1          | regulating                        | 0.0049 | 0.3356 | 0                  |                   |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0485 | 0.0746 | 5.217325115104E-6  | 0.024186176702991 |
| 3          | ACE inhibitor                     | 0.0631 | 0.1083 | 0.0017951962898486 | 0.067088034662415 |
| 4          | antithrombotic                    | 0.0049 | 0.5052 | 0                  |                   |
| 5          | antiamnestic                      | 0.0049 | 0.5052 | 0                  |                   |
| 6          | antioxidative                     | 0.0097 | 0.1332 | 0                  | 0                 |

**Table A19.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using plasmin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 7603       | GR       | (84–85)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 231.1220          | 231.2400      |
| 2   | 7697       | YK       | (82–83)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 309.1570          | 309.3440      |
| 3   | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 4   | 8858       | PK       | (180–181) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 5   | 8939       | YK       | (82–83)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |

**Table A20.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by plasmin.

| $DH_t$ (%) |                                   |        |        |                    |                     |
|------------|-----------------------------------|--------|--------|--------------------|---------------------|
| 10.7317    |                                   |        |        |                    |                     |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                 |
| 1          | ACE inhibitor                     | 0.0097 | 0.0167 | 9.4749721470635E-6 | 0.00035408788633428 |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0146 | 0.0224 | 0                  | 0                   |

**Table A21.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using cathepsin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 3   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 4   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 5   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 6   | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 259.1780          | 259.3340      |
| 7   | 8219       | TY       | (23–24)   | antioxidative peptide                                |  | antioxidative                     | 282.1100          | 282.2750      |
| 8   | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 9   | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 10  | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 11  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 12  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 13  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 365.1840          | 365.4090      |
| 14  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A22.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by cathepsin.

| $DH_t$ (%) |                                   |        |        |                     |                    |
|------------|-----------------------------------|--------|--------|---------------------|--------------------|
| 20.4878    |                                   |        |        |                     |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$               | $V$                |
| 1          | ACE inhibitor                     | 0.0388 | 0.0666 | 0.00082506965175237 | 0.030833564947006  |
| 2          | antioxidative                     | 0.0049 | 0.0673 | 0                   | 0                  |
| 3          | dipeptidyl peptidase IV inhibitor | 0.0243 | 0.0374 | 1.8563339357632E-6  | 0.0086054864513139 |

**Table A23.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using clostripain.

| No. | Peptide ID | Sequence | Location  | Name   | Function  | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|---|-----------------------------------|-------------------|---------------|
| 1   | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003) | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |

**Table A24.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by clostripain.

| $DH_t$ (%) |                                   |        |        |       |     |
|------------|-----------------------------------|--------|--------|-------|-----|
| 5.3659     |                                   |        |        |       |     |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$ | $V$ |
| 1          | dipeptidyl peptidase IV inhibitor | 0.0049 | 0.0075 | 0     | 0   |

**Table A25.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using chymase.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 3   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 4   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 5   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 6   | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 259.1780          | 259.3340      |
| 7   | 8219       | TY       | (23–24)   | antioxidative peptide                                |  | antioxidative                     | 282.1100          | 282.2750      |
| 8   | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 9   | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 10  | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 11  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 12  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 13  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 365.1840          | 365.4090      |
| 14  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A26.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by chymase.

| $DH_t$ (%) |                                   |        |        |                     |                    |  |
|------------|-----------------------------------|--------|--------|---------------------|--------------------|--|
| 19.5122    |                                   |        |        |                     |                    |  |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$               | $V$                |  |
| 1          | ACE inhibitor                     | 0.0388 | 0.0666 | 0.00082506965175237 | 0.030833564947006  |  |
| 2          | antioxidative                     | 0.0049 | 0.0673 | 0                   | 0                  |  |
| 3          | dipeptidyl peptidase IV inhibitor | 0.0243 | 0.0374 | 1.8563339357632E-6  | 0.0086054864513139 |  |

**Table A27.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using papain.

| No. | Peptide ID | Sequence | Location  | Name                                     | Function   | Activity      | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|---------------|-------------------|---------------|
| 1   | 3351       | EEE      | (95–97)   | Stimulating vasoactive substance release |  | stimulating   | 405.1260          | 405.3480      |
| 2   | 3522       | IPP      | (144–146) | ACE inhibitor (from bovine b-CN)         |  | ACE inhibitor | 325.1880          | 325.3940      |
| 3   | 3538       | VSP      | (42–44)   | ACE inhibitor                            | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 301.1520          | 301.3300      |
| 4   | 7513       | PL       | (172–173) | ACE inhibitor from Alaskan pollack skin  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor | 228.1360          | 228.2770      |
| 5   | 7583       | AF       | (39–40)   | ACE inhibitor                            | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 236.1050          | 236.2560      |
| 6   | 7594       | VG       | (11–12)   | ACE inhibitor                            | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 174.0890          | 174.1850      |
| 7   | 7600       | AG       | (9–10)    | ACE inhibitor                            | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 146.0580          | 146.1310      |
| 8   | 7600       | AG       | (15–16)   | ACE inhibitor                            | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor | 146.0580          | 146.1310      |

Table A27. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 9   | 7600       | AG       | (53–54)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 10  | 7600       | AG       | (93–94)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 11  | 7617       | QG       | (153–154) | ACE inhibitor  |  | ACE inhibitor                     | 203.0790          | 203.1830      |
| 12  | 7681       | DG       | (74–75)   | ACE inhibitor from soy                               | Inhibitor of Angiotensin-converting enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 190.0479          | 190.1410      |
| 13  | 7951       | YYT      | (24–26)   | synthetic peptide                                    |  | antioxidative                     | 445.1730          | 445.4450      |
| 14  | 8559       | AL       | (133–134) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 202.1210          | 202.2390      |
| 15  | 8559       | AL       | (136–137) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 202.1210          | 202.2390      |
| 16  | 8560       | SL       | (78–79)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 218.1160          | 218.2400      |
| 17  | 8638       | PL       | (172–173) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 18  | 8685       | WT       | (68–69)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 19  | 8759       | AF       | (39–40)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 236.1050          | 236.2560      |
| 20  | 8760       | AG       | (9–10)    | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 21  | 8760       | AG       | (15–16)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 22  | 8760       | AG       | (53–54)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 23  | 8760       | AG       | (93–94)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 24  | 8764       | AV       | (56–57)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 25  | 8769       | DR       | (80–81)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |

Table A27. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 26  | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 27  | 8774       | ET       | (6–7)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 28  | 8871       | QG       | (153–154) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 203.0790          | 203.1830      |
| 29  | 8878       | QT       | (4–5)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 247.1050          | 247.2360      |
| 30  | 8918       | VG       | (11–12)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 31  | 8951       | AV       | (56–57)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 188.1050          | 188.2120      |
| 32  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

Table A28.  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using papain.

| $DH_t$ (%) |                                   |        |        |                    |                   |
|------------|-----------------------------------|--------|--------|--------------------|-------------------|
| 43.4146    |                                   |        |        |                    |                   |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$               |
| 1          | stimulating                       | 0.0049 | 0.1441 | 0                  |                   |
| 2          | ACE inhibitor                     | 0.0631 | 0.1083 | 0.0019221967090735 | 0.071834149934202 |
| 3          | antioxidative                     | 0.0049 | 0.0673 | 0                  | 0                 |
| 4          | dipeptidyl peptidase IV inhibitor | 0.0825 | 0.1268 | 2.3003804384162E-5 | 0.10663971774841  |



**Table A29.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using ficin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 2749       | DY       | (19–20)   | ion flow regulating peptide                          | ion flow regulating peptide  | regulating                        | 296.0899          | 296.2590      |
| 2   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 3   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 4   | 7513       | PL       | (172–173) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 5   | 7558       | VK       | (17–18)   | ACE inhibitor from buckwheat                         |  | ACE inhibitor                     | 245.1620          | 245.3070      |
| 6   | 7594       | VG       | (11–12)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 174.0890          | 174.1850      |
| 7   | 7600       | AG       | (9–10)    | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 8   | 7617       | QG       | (153–154) | ACE inhibitor  |  | ACE inhibitor                     | 203.0790          | 203.1830      |
| 9   | 7621       | TG       | (65–66)   | ACE inhibitor  |  | ACE inhibitor                     | 176.0680          | 176.1570      |
| 10  | 7682       | NY       | (190–191) | ACE inhibitor from garlic                            |  | ACE inhibitor                     | 295.1059          | 295.2740      |
| 11  | 7698       | NK       | (167–168) | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 260.1369          | 260.2780      |
| 12  | 8185       | TF       | (151–152) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 266.1150          | 266.2820      |
| 13  | 8219       | TY       | (23–24)   | antioxidative peptide                                |  | antioxidative                     | 282.1100          | 282.2750      |
| 14  | 8559       | AL       | (133–134) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 202.1210          | 202.2390      |
| 15  | 8559       | AL       | (136–137) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 202.1210          | 202.2390      |
| 16  | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 17  | 8638       | PL       | (172–173) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 18  | 8760       | AG       | (9–10)    | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 19  | 8769       | DR       | (80–81)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |

Table A29. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 20  | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 21  | 8853       | NY       | (190–191) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 295.1059          | 295.2740      |
| 22  | 8858       | PK       | (180–181) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 23  | 8871       | QG       | (153–154) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 203.0790          | 203.1830      |
| 24  | 8900       | TF       | (151–152) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 266.1150          | 266.2820      |
| 25  | 8901       | TG       | (65–66)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 176.0680          | 176.1570      |
| 26  | 8910       | TS       | (77–78)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 206.0790          | 206.1850      |
| 27  | 8910       | TS       | (120–121) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 206.0790          | 206.1850      |
| 28  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 29  | 8918       | VG       | (11–12)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 30  | 8921       | VK       | (17–18)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1620          | 245.3070      |
| 31  | 8926       | VS       | (42–43)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 204.1000          | 204.2130      |
| 32  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 365.1840          | 365.4090      |
| 33  | 9072       | DY       | (19–20)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 296.0899          | 296.2590      |
| 34  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A30.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using ficin.

| $DH_t$ (%) |                                   |        |        |                    |                   |
|------------|-----------------------------------|--------|--------|--------------------|-------------------|
| 44.3902    |                                   |        |        |                    |                   |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$               |
| 1          | regulating                        | 0.0049 | 0.3356 | 0                  |                   |
| 2          | ACE inhibitor                     | 0.0680 | 0.1167 | 0.001585167723556  | 0.059239085878814 |
| 3          | antioxidative                     | 0.0049 | 0.0673 | 0                  | 0                 |
| 4          | dipeptidyl peptidase IV inhibitor | 0.0874 | 0.1344 | 1.1006017099609E-5 | 0.051021063187465 |

**Table A31.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using leukocyte elastase.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                                  | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|---|-------------------|---------------|
| 1   | 3174       | KA       | (8–9)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 217.1310          | 217.2530      |
| 2   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 287.1850          | 287.3480      |
| 3   | 4005       | RA       | (135–136) |  | Activation of ubiquitin-dependent proteolysis  | activating ubiquitin-mediated proteolysis | 245.1380          | 245.2670      |
| 4   | 7588       | RA       | (135–136) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 245.1380          | 245.2670      |
| 5   | 7598       | GA       | (54–55)   | ACE inhibitor  |  | ACE inhibitor                             | 146.0580          | 146.1310      |
| 6   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                             | 188.1050          | 188.2120      |
| 7   | 7608       | GV       | (10–11)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 174.0890          | 174.1850      |
| 8   | 7608       | GV       | (16–17)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 174.0890          | 174.1850      |
| 9   | 7612       | GT       | (66–67)   | ACE inhibitor  |  | ACE inhibitor                             | 176.0680          | 176.1570      |
| 10  | 7743       | KA       | (8–9)     | ACE inhibitor  | Inhibitor of angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                             | 217.1310          | 217.2530      |

Table A31. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 11  | 7951       | YYT      | (24–26)   | synthetic peptide                                    |  | antioxidative                     | 445.1730          | 445.4450      |
| 12  | 8524       | GA       | (54–55)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 13  | 8526       | RA       | (135–136) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1380          | 245.2670      |
| 14  | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 15  | 8685       | WT       | (68–69)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 16  | 8685       | WT       | (72–73)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 17  | 8774       | ET       | (6–7)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 18  | 8786       | GV       | (10–11)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 19  | 8786       | GV       | (16–17)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 20  | 8816       | KT       | (150–151) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 247.1410          | 247.2790      |
| 21  | 8879       | QV       | (160–161) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1260          | 245.2640      |
| 22  | 8884       | RI       | (143–144) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 23  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 24  | 8945       | YS       | (148–149) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 268.0950          | 268.2500      |
| 25  | 8946       | YV       | (102–103) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 280.1310          | 280.3030      |
| 26  | 9056       | DGL      | (74–76)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 303.1319          | 303.3010      |
| 27  | 9077       | YV       | (102–103) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.1310          | 280.3030      |

**Table A32.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using leukocyte elastase.

| $DH_t$ (%) |   |        |        |                    |                   |
|------------|---|--------|--------|--------------------|-------------------|
| 38.5366    |   |        |        |                    |                   |
| No.        | Activity                                  | $A_E$  | $W$    | $B_E$              | $V$               |
| 1          | dipeptidyl peptidase IV inhibitor         | 0.0728 | 0.1119 | 2.2768987034696E-5 | 0.10555116493987  |
| 2          | ACE inhibitor                             | 0.0485 | 0.0833 | 0.0024508146173148 | 0.091589057379035 |
| 3          | activating ubiquitin-mediated proteolysis | 0.0049 | 0.3356 | 0                  |                   |
| 4          | antioxidative                             | 0.0049 | 0.0673 | 0                  | 0                 |

**Table A33.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using metridin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 3   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 4   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 5   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 6   | 7693       | KL       | (21–22)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 259.1780          | 259.3340      |
| 7   | 8219       | TY       | (23–24)   | antioxidative peptide                                |  | antioxidative                     | 282.1100          | 282.2750      |
| 8   | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 9   | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 10  | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 11  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 12  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 13  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 365.1840          | 365.4090      |
| 14  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A34.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using metridin.

| $DH_t$ (%)<br>19.5122 |                                   |        |        |                     |                    |
|-----------------------|-----------------------------------|--------|--------|---------------------|--------------------|
| No.                   | Activity                          | $A_E$  | $W$    | $B_E$               | $V$                |
| 1                     | ACE inhibitor                     | 0.0388 | 0.0666 | 0.00082506965175237 | 0.030833564947006  |
| 2                     | antioxidative                     | 0.0049 | 0.0673 | 0                   | 0                  |
| 3                     | dipeptidyl peptidase IV inhibitor | 0.0243 | 0.0374 | 1.8563339357632E-6  | 0.0086054864513139 |

**Table A35.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using pancreatic elastase II.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 3   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 4   | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 5   | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 6   | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 7   | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A36.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using pancreatic elastase II.

| $DH_t$ (%) |                                   |        |        |                    |                     |
|------------|-----------------------------------|--------|--------|--------------------|---------------------|
| 13.1707    |                                   |        |        |                    |                     |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                 |
| 1          | ACE inhibitor                     | 0.0194 | 0.0333 | 2.5006640432763E-5 | 0.00093451973448837 |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0146 | 0.0224 | 1.8563339357632E-6 | 0.0086054864513139  |

**Table A37.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using stem bromelain.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3174       | KA       | (8–9)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 217.1310          | 217.2530      |
| 2   | 3174       | KA       | (132–133) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 217.1310          | 217.2530      |
| 3   | 7513       | PL       | (172–173) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 4   | 7617       | QG       | (153–154) | ACE inhibitor  |  | ACE inhibitor                     | 203.0790          | 203.1830      |
| 5   | 7681       | DG       | (74–75)   | ACE inhibitor from soy                               | Inhibitor of Angiotensin-converting enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 190.0479          | 190.1410      |
| 6   | 7743       | KA       | (8–9)     | ACE inhibitor  | Inhibitor of angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 217.1310          | 217.2530      |
| 7   | 7743       | KA       | (132–133) | ACE inhibitor  | Inhibitor of angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 217.1310          | 217.2530      |
| 8   | 7951       | YYT      | (24–26)   | synthetic peptide                                    |  | antioxidative                     | 445.1730          | 445.4450      |
| 9   | 8638       | PL       | (172–173) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 10  | 8685       | WT       | (68–69)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 11  | 8685       | WT       | (72–73)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 12  | 8769       | DR       | (80–81)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |

Table A37. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 13  | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 14  | 8774       | ET       | (6–7)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 15  | 8816       | KT       | (150–151) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 247.1410          | 247.2790      |
| 16  | 8851       | NV       | (127–128) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 231.1109          | 231.2370      |
| 17  | 8867       | QA       | (14–15)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 217.0950          | 217.2100      |
| 18  | 8871       | QG       | (153–154) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 203.0790          | 203.1830      |
| 19  | 8945       | YS       | (148–149) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 268.0950          | 268.2500      |
| 20  | 8946       | YV       | (102–103) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 280.1310          | 280.3030      |
| 21  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |
| 22  | 9077       | YV       | (102–103) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.1310          | 280.3030      |

Table A38.  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using stem bromelain.

| $DH_t$ (%) |                                   |        |        |                     |                   |
|------------|-----------------------------------|--------|--------|---------------------|-------------------|
| 54.1463    |                                   |        |        |                     |                   |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$               | $V$               |
| 1          | dipeptidyl peptidase IV inhibitor | 0.0680 | 0.1045 | 2.1686874619195E-5  | 0.10053477023254  |
| 2          | ACE inhibitor                     | 0.0340 | 0.0584 | 0.00073973077367093 | 0.027644377422971 |
| 3          | antioxidative                     | 0.0049 | 0.0673 | 0                   | 0                 |



**Table A39.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using oligopeptidase B.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 7603       | GR       | (84–85)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 231.1220          | 231.2400      |
| 2   | 7697       | YK       | (82–83)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 309.1570          | 309.3440      |
| 3   | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 4   | 8858       | PK       | (180–181) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 5   | 8939       | YK       | (82–83)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |

**Table A40.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using oligopeptidase B.

| $DH_t$ (%) |                                   |        |        |                    |                     |
|------------|-----------------------------------|--------|--------|--------------------|---------------------|
| 10.7317    |                                   |        |        |                    |                     |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                 |
| 1          | ACE inhibitor                     | 0.0097 | 0.0167 | 9.4749721470635E-6 | 0.00035408788633428 |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0146 | 0.0224 | 0                  | 0                   |

**Table A41.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using calpain 2.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity         | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|------------------|-------------------|---------------|
| 1   | 2754       | PG       | (46–47)   | peptide regulating the stomach mucosal membrane activity | regulating the stomach mucosal membrane activity                                     | regulating       | 172.0730          | 172.1690      |
| 2   | 2882       | YG       | (169–170) | Immunostimulating peptide                                | Enhancing protein biosynthesis in lymphocytes  | immunomodulating | 238.0840          | 238.2220      |
| 3   | 3285       | PG       | (46–47)   | Antithrombotic peptide                                   | Antithrombotic   | antithrombotic   | 172.0730          | 172.1690      |
| 4   | 3460       | PG       | (46–47)   | Prolyl endopeptidase inhibitor                           | Inhibitor of Prolyl Endopeptidase (PEP) (EC 3.4.21.26) (MEROPS ID: S09.001)          | antiamnesic      | 172.0730          | 172.1690      |
| 5   | 3522       | IPP      | (144–146) | ACE inhibitor (from bovine b-CN)                         |  | ACE inhibitor    | 325.1880          | 325.3940      |
| 6   | 3553       | YG       | (169–170) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 238.0840          | 238.2220      |
| 7   | 3563       | AY       | (147–148) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 252.1000          | 252.2490      |
| 8   | 7513       | PL       | (172–173) | ACE inhibitor from Alaskan pollack skin                  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor    | 228.1360          | 228.2770      |
| 9   | 7558       | VK       | (17–18)   | ACE inhibitor from buckwheat                             |  | ACE inhibitor    | 245.1620          | 245.3070      |
| 10  | 7594       | VG       | (11–12)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 174.0890          | 174.1850      |
| 11  | 7600       | AG       | (9–10)    | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 146.0580          | 146.1310      |
| 12  | 7600       | AG       | (15–16)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 146.0580          | 146.1310      |
| 13  | 7600       | AG       | (53–54)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 146.0580          | 146.1310      |
| 14  | 7600       | AG       | (93–94)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor    | 146.0580          | 146.1310      |

Table A41. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function  | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|---|-----------------------------------|-------------------|---------------|
| 15  | 7625       | PG       | (46–47)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)         | ACE inhibitor                     | 172.0730          | 172.1690      |
| 16  | 7681       | DG       | (74–75)   | ACE inhibitor from soy                               | Inhibitor of Angiotensin-converting enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                     | 190.0479          | 190.1410      |
| 17  | 7697       | YK       | (82–83)   | ACE inhibitor from wakame                            | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | ACE inhibitor                     | 309.1570          | 309.3440      |
| 18  | 7698       | NK       | (167–168) | ACE inhibitor from wakame                            |   | ACE inhibitor                     | 260.1369          | 260.2780      |
| 19  | 7866       | AY       | (147–148) | peptide from Okara protein                           |   | antioxidative                     | 252.1000          | 252.2490      |
| 20  | 7951       | YYT      | (24–26)   | synthetic peptide                                    | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | antioxidative                     | 445.1730          | 445.4450      |
| 21  | 8559       | AL       | (133–134) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 202.1210          | 202.2390      |
| 22  | 8559       | AL       | (136–137) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 202.1210          | 202.2390      |
| 23  | 8560       | SL       | (78–79)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 218.1160          | 218.2400      |
| 24  | 8638       | PL       | (172–173) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 25  | 8685       | WT       | (68–69)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 305.1260          | 305.3180      |
| 26  | 8760       | AG       | (9–10)    | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 27  | 8760       | AG       | (15–16)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 28  | 8760       | AG       | (53–54)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 29  | 8760       | AG       | (93–94)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 30  | 8764       | AV       | (56–57)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 31  | 8765       | AY       | (147–148) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 252.1000          | 252.2490      |
| 32  | 8769       | DR       | (80–81)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) |   | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |

Table A41. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 33  | 8769       | DR       | (164–165) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 289.1279          | 289.2770      |
| 34  | 8774       | ET       | (6–7)     | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 248.0890          | 248.2210      |
| 35  | 8779       | FQ       | (13–14)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 293.1260          | 293.3080      |
| 36  | 8779       | FQ       | (152–153) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 293.1260          | 293.3080      |
| 37  | 8805       | IQ       | (159–160) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 259.1420          | 259.2910      |
| 38  | 8855       | PG       | (46–47)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 172.0730          | 172.1690      |
| 39  | 8858       | PK       | (180–181) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 40  | 8894       | SK       | (149–150) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 233.1260          | 233.2540      |
| 41  | 8918       | VG       | (11–12)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 42  | 8921       | VK       | (17–18)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1620          | 245.3070      |
| 43  | 8936       | YG       | (169–170) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 238.0840          | 238.2220      |
| 44  | 8938       | YI       | (99–100)  | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 294.1470          | 294.3300      |
| 45  | 8939       | YK       | (82–83)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |
| 46  | 8951       | AV       | (56–57)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 188.1050          | 188.2120      |
| 47  | 9076       | FQ       | (13–14)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 293.1260          | 293.3080      |
| 48  | 9076       | FQ       | (152–153) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 293.1260          | 293.3080      |
| 49  | 9184       | ST       | (64–65)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 206.0790          | 206.1850      |

**Table A42.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using calpain 2.

| $DH_t$ (%) |                                   |        |        |                    |                  |
|------------|-----------------------------------|--------|--------|--------------------|------------------|
| 47.3171    |                                   |        |        |                    |                  |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$              |
| 1          | regulating                        | 0.0049 | 0.3356 | 0                  |                  |
| 2          | immunomodulating                  | 0.0049 | 0.5052 | 0                  |                  |
| 3          | antithrombotic                    | 0.0049 | 0.5052 | 0                  |                  |
| 4          | antiamnestic                      | 0.0049 | 0.5052 | 0                  |                  |
| 5          | ACE inhibitor                     | 0.0874 | 0.1500 | 0.0034373644220009 | 0.12845727500362 |
| 6          | antioxidative                     | 0.0097 | 0.1332 | 0                  | 0                |
| 7          | dipeptidyl peptidase IV inhibitor | 0.1214 | 0.1866 | 2.3003804384162E-5 | 0.10663971774841 |

**Table A43.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using glycy endopeptidase.

| No. | Peptide ID | Sequence | Location | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 7594       | VG       | (11–12)  | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 174.0890          | 174.1850      |
| 2   | 8918       | VG       | (11–12)  | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |

**Table A44.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using glycy endopeptidase.

| $DH_t$ (%) |                                   |        |        |                    |                     |
|------------|-----------------------------------|--------|--------|--------------------|---------------------|
| 9.7561     |                                   |        |        |                    |                     |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                 |
| 1          | ACE inhibitor                     | 0.0049 | 0.0084 | 4.4130626654898E-6 | 0.00016491996042102 |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0049 | 0.0075 | 0                  | 0                   |

**Table A45.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using oligopeptidase F.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin                                      | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 3   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 4   | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 5   | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |
| 6   | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 7   | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

**Table A46.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using oligopeptidase F.

| $DH_t$ (%) |                                   | 12.1951 |        |                    |                     |
|------------|-----------------------------------|---------|--------|--------------------|---------------------|
| No.        | Activity                          | $A_E$   | $W$    | $B_E$              | $V$                 |
| 1          | ACE inhibitor                     | 0.0194  | 0.0333 | 2.5006640432763E-5 | 0.00093451973448837 |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0146  | 0.0224 | 1.8563339357632E-6 | 0.0086054864513139  |

**Table A47.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using proteinase P1 (lactocepin).

| No. | Peptide ID | Sequence | Location  | Name   | Function  | Activity                                  | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|---|---|-------------------|---------------|
| 1   | 3563       | AY       | (104–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                             | 252.1000          | 252.2490      |
| 2   | 3563       | AY       | (147–148) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                             | 252.1000          | 252.2490      |
| 3   | 4005       | RA       | (135–136) |  | Activation of ubiquitin-dependent proteolysis   | activating ubiquitin-mediated proteolysis | 245.1380          | 245.2670      |
| 4   | 7588       | RA       | (135–136) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                             | 245.1380          | 245.2670      |
| 5   | 7591       | GF       | (12–13)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                             | 222.0890          | 222.2290      |
| 6   | 7608       | GV       | (47–48)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001)        | ACE inhibitor                             | 174.0890          | 174.1850      |
| 7   | 7866       | AY       | (104–105) | peptide from Okara protein                           | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | antioxidative                             | 252.1000          | 252.2490      |
| 8   | 7866       | AY       | (147–148) | peptide from Okara protein                           | Peptide obtained by hydrolysis of Okara protein by use of enzymatic preparation Protease N. | antioxidative                             | 252.1000          | 252.2490      |
| 9   | 8526       | RA       | (135–136) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 245.1380          | 245.2670      |
| 10  | 8765       | AY       | (104–105) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 252.1000          | 252.2490      |
| 11  | 8765       | AY       | (147–148) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 252.1000          | 252.2490      |
| 12  | 8782       | GF       | (12–13)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 222.0890          | 222.2290      |
| 13  | 8786       | GV       | (47–48)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 174.0890          | 174.1850      |
| 14  | 8884       | RI       | (143–144) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 287.1850          | 287.3480      |
| 15  | 8895       | SV       | (114–115) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)                     | dipeptidyl peptidase IV inhibitor         | 204.1000          | 204.2130      |

**Table A48.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage carried out by using proteinase P1 (lactocepin).

| $DH_t$ (%) |   |        |        |                     |                   |
|------------|---|--------|--------|---------------------|-------------------|
| 41.4634    |   |        |        |                     |                   |
| No.        | Activity                                  | $A_E$  | $W$    | $B_E$               | $V$               |
| 1          | ACE inhibitor                             | 0.0243 | 0.0417 | 0.00053029982441682 | 0.019817762103858 |
| 2          | activating ubiquitin-mediated proteolysis | 0.0049 | 0.3356 | 0                   |                   |
| 3          | antioxidative                             | 0.0097 | 0.1332 | 0                   | 0                 |
| 4          | dipeptidyl peptidase IV inhibitor         | 0.0340 | 0.0523 | 0                   | 0                 |

**Table A49.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using pepsin (pH >2).

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                                  | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|---|-------------------|---------------|
| 1   | 2754       | PG       | (46–47)   | peptide regulating the stomach mucosal membrane activity | regulating the stomach mucosal membrane activity                                     | regulating                                | 172.0730          | 172.1690      |
| 2   | 3172       | VA       | (57–58)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)     | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 188.1050          | 188.2120      |
| 3   | 3172       | VA       | (92–93)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)     | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 188.1050          | 188.2120      |
| 4   | 3172       | VA       | (103–104) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)     | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 188.1050          | 188.2120      |
| 5   | 3257       | RL       | (138–139) | beta-lactokinin  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 287.1850          | 287.3480      |
| 6   | 3257       | RL       | (165–166) | beta-lactokinin  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 287.1850          | 287.3480      |
| 7   | 3285       | PG       | (46–47)   | Antithrombotic peptide                                   | Antithrombotic   | antithrombotic                            | 172.0730          | 172.1690      |
| 8   | 3380       | RY       | (81–82)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 337.1640          | 337.3580      |
| 9   | 3384       | VF       | (128–129) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 264.1360          | 264.3100      |
| 10  | 3460       | PG       | (46–47)   | Prolyl endopeptidase inhibitor                           | Inhibitor of Prolyl Endopeptidase (PEP) (EC 3.4.21.26) (MEROPS ID: S09.001)          | antiamnestic                              | 172.0730          | 172.1690      |
| 11  | 3492       | VY       | (195–196) | ACE inhibitor from sake                                  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 280.1310          | 280.3030      |
| 12  | 4005       | RA       | (135–136) |  | Activation of ubiquitin-dependent proteolysis  | activating ubiquitin-mediated proteolysis | 245.1380          | 245.2670      |



Table A49. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                                  | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|---|-------------------|---------------|
| 13  | 4005       | RA       | (193–194) |  | Activation of ubiquitin-dependent proteolysis  | activating ubiquitin-mediated proteolysis | 245.1380          | 245.2670      |
| 14  | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin              | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                             | 228.1360          | 228.2770      |
| 15  | 7558       | VK       | (17–18)   | ACE inhibitor from buckwheat                         |  | ACE inhibitor                             | 245.1620          | 245.3070      |
| 16  | 7562       | IA       | (100–101) | ACE inhibitor from soy hydrolysate                   |  | ACE inhibitor                             | 202.1210          | 202.2390      |
| 17  | 7588       | RA       | (135–136) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 245.1380          | 245.2670      |
| 18  | 7588       | RA       | (193–194) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 245.1380          | 245.2670      |
| 19  | 7594       | VG       | (11–12)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 174.0890          | 174.1850      |
| 20  | 7594       | VG       | (125–126) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 174.0890          | 174.1850      |
| 21  | 7625       | PG       | (46–47)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                             | 172.0730          | 172.1690      |
| 22  | 7827       | IE       | (89–90)   | ACE inhibitor  |  | ACE inhibitor                             | 260.1260          | 260.2760      |
| 23  | 7829       | VE       | (161–162) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                             | 246.1100          | 246.2490      |
| 24  | 8224       | VY       | (195–196) | antioxidative peptide                                | free radical scavenging  | antioxidative                             | 280.1310          | 280.3030      |
| 25  | 8323       | IL       | (36–37)   | Glucose uptake stimulating peptide                   | Glucose uptake stimulating   | stimulating                               | 244.1680          | 244.3200      |
| 26  | 8525       | IA       | (100–101) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 202.1210          | 202.2390      |
| 27  | 8526       | RA       | (135–136) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 245.1380          | 245.2670      |
| 28  | 8526       | RA       | (193–194) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 245.1380          | 245.2670      |
| 29  | 8560       | SL       | (78–79)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 218.1160          | 218.2400      |
| 30  | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 228.1360          | 228.2770      |
| 31  | 8685       | WT       | (68–69)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 305.1260          | 305.3180      |
| 32  | 8802       | IL       | (36–37)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor         | 244.1680          | 244.3200      |

Table A49. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 33  | 8805       | IQ       | (159–160) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 259.1420          | 259.2910      |
| 34  | 8855       | PG       | (46–47)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 172.0730          | 172.1690      |
| 35  | 8858       | PK       | (180–181) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 243.1460          | 243.2910      |
| 36  | 8882       | RG       | (200–201) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 231.1220          | 231.2400      |
| 37  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 38  | 8886       | RL       | (165–166) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 39  | 8894       | SK       | (149–150) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 233.1260          | 233.2540      |
| 40  | 8916       | VE       | (161–162) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 246.1100          | 246.2490      |
| 41  | 8917       | VF       | (128–129) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 264.1360          | 264.3100      |
| 42  | 8918       | VG       | (11–12)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 43  | 8918       | VG       | (125–126) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 174.0890          | 174.1850      |
| 44  | 8921       | VK       | (17–18)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 245.1620          | 245.3070      |
| 45  | 8927       | VT       | (115–116) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 218.1150          | 218.2380      |
| 46  | 8929       | VY       | (195–196) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 280.1310          | 280.3030      |
| 47  | 9079       | IL       | (36–37)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 244.1680          | 244.3200      |

**Table A50.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage carried out by using pepsin (pH >2).

| $DH_t$ (%)<br>70.7317 |   |        |        |                    |                   |
|-----------------------|---|--------|--------|--------------------|-------------------|
| No.                   | Activity                                  | $A_E$  | $W$    | $B_E$              | $V$               |
| 1                     | regulating                                | 0.0049 | 0.3356 | 0                  |                   |
| 2                     | dipeptidyl peptidase IV inhibitor         | 0.1165 | 0.1791 | 9.8559287499226E-5 | 0.45689549540936  |
| 3                     | ACE inhibitor                             | 0.0777 | 0.1334 | 0.002215755283035  | 0.082804687193409 |
| 4                     | antithrombotic                            | 0.0049 | 0.5052 | 0                  |                   |
| 5                     | antiemetic                                | 0.0049 | 0.5052 | 0                  |                   |
| 6                     | activating ubiquitin-mediated proteolysis | 0.0097 | 0.6644 | 0                  |                   |
| 7                     | antioxidative                             | 0.0049 | 0.0673 | 0                  | 0                 |
| 8                     | stimulating                               | 0.0049 | 0.1441 | 0                  |                   |

**Table A51.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using cocolysin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3522       | IPP      | (144–146) | ACE inhibitor (from bovine b-CN)                     |  | ACE inhibitor                     | 325.1880          | 325.3940      |
| 2   | 3666       | YP       | (105–106) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 278.1150          | 278.2870      |
| 3   | 7600       | AG       | (53–54)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 146.0580          | 146.1310      |
| 4   | 7605       | FG       | (129–130) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 5   | 7619       | LG       | (182–183) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 6   | 7697       | YK       | (20–21)   | ACE inhibitor from wakame                            |  | ACE inhibitor                     | 309.1570          | 309.3440      |
| 7   | 8521       | YP       | (105–106) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 278.1150          | 278.2870      |
| 8   | 8760       | AG       | (53–54)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 146.0580          | 146.1310      |
| 9   | 8764       | AV       | (56–57)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 10  | 8764       | AV       | (194–195) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 11  | 8779       | FQ       | (13–14)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 293.1260          | 293.3080      |

Table A51. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 12  | 8824       | LT       | (22–23)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 232.1310          | 232.2650      |
| 13  | 8937       | YH       | (87–88)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 318.1210          | 318.3120      |
| 14  | 8939       | YK       | (20–21)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 309.1570          | 309.3440      |
| 15  | 8946       | YV       | (102–103) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 280.1310          | 280.3030      |
| 16  | 8951       | AV       | (56–57)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 188.1050          | 188.2120      |
| 17  | 8951       | AV       | (194–195) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 188.1050          | 188.2120      |
| 18  | 9076       | FQ       | (13–14)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 293.1260          | 293.3080      |
| 19  | 9077       | YV       | (102–103) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.1310          | 280.3030      |
| 20  | 9087       | YH       | (87–88)   | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 318.1210          | 318.3120      |
| 21  | 9213       | LR       | (134–135) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 287.1850          | 287.3480      |
| 22  | 9213       | LR       | (137–138) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 287.1850          | 287.3480      |
| 23  | 9213       | LR       | (142–143) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 287.1850          | 287.3480      |

Table A52.  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using cocolysin.

| $DH_t$ (%) |                                   |        |        |                    |                    |
|------------|-----------------------------------|--------|--------|--------------------|--------------------|
| 30.2439    |                                   |        |        |                    |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                |
| 1          | ACE inhibitor                     | 0.0680 | 0.1167 | 0.0021410562742961 | 0.080013120769247  |
| 2          | dipeptidyl peptidase IV inhibitor | 0.0437 | 0.0672 | 1.5313466662583E-6 | 0.0070989290961449 |

**Table A53.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using subtilisin.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 3257       | RL       | (138–139) | beta-lactokinin  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 287.1850          | 287.3480      |
| 2   | 3384       | VF       | (128–129) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 264.1360          | 264.3100      |
| 3   | 3486       | VW       | (71–72)   | ACE inhibitor from sake lees   | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 303.1470          | 303.3460      |
| 4   | 3492       | VY       | (195–196) | ACE inhibitor from sake  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.1310          | 280.3030      |
| 5   | 3546       | VAY      | (103–105) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 351.1680          | 351.3820      |
| 6   | 7513       | PL       | (106–107) | ACE inhibitor from Alaskan pollack skin                                | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 228.1360          | 228.2770      |
| 7   | 7591       | GF       | (130–131) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 222.0890          | 222.2290      |
| 8   | 7599       | GL       | (183–184) | ACE inhibitor  | Inhibitor of Angiotensin-converting enzyme (EC 3.4.15.1) (MEROPS ID: XM02-001)       | ACE inhibitor                     | 188.1050          | 188.2120      |
| 9   | 7693       | KL       | (21–22)   | ACE inhibitor from wakame  |  | ACE inhibitor                     | 259.1780          | 259.3340      |
| 10  | 8219       | TY       | (23–24)   | antioxidative peptide  |  | antioxidative                     | 282.1100          | 282.2750      |
| 11  | 8224       | VY       | (195–196) | antioxidative peptide  | free radical scavenging  | antioxidative                     | 280.1310          | 280.3030      |
| 12  | 8461       | VW       | (71–72)   | Antioxidant peptide from marine bivalve ( <i>Mactra veneriformis</i> ) | Antioxidant  | antioxidative                     | 303.1470          | 303.3460      |
| 13  | 8561       | GL       | (183–184) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)                   | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 188.1050          | 188.2120      |
| 14  | 8638       | PL       | (106–107) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)                   | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 228.1360          | 228.2770      |
| 15  | 8782       | GF       | (130–131) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor)                   | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 222.0890          | 222.2290      |

Table A53. Cont.

| No. | Peptide ID | Sequence | Location  | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|-----------|--|--|-----------------------------------|-------------------|---------------|
| 16  | 8886       | RL       | (138–139) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 287.1850          | 287.3480      |
| 17  | 8910       | TS       | (77–78)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 206.0790          | 206.1850      |
| 18  | 8910       | TS       | (120–121) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 206.0790          | 206.1850      |
| 19  | 8911       | TT       | (69–70)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 220.0940          | 220.2100      |
| 20  | 8914       | TY       | (23–24)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 282.1100          | 282.2750      |
| 21  | 8917       | VF       | (128–129) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 264.1360          | 264.3100      |
| 22  | 8926       | VS       | (42–43)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 204.1000          | 204.2130      |
| 23  | 8928       | VW       | (71–72)   | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 303.1470          | 303.3460      |
| 24  | 8929       | VY       | (195–196) | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 280.1310          | 280.3030      |
| 25  | 9071       | IAY      | (100–102) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: M02-001)  | ACE inhibitor                     | 365.1840          | 365.4090      |
| 26  | 9074       | DF       | (204–205) | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 280.0949          | 280.2660      |

Table A54.  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using subtilisin.

| $DH_t$ (%) |                                   |        |        |                    |                    |
|------------|-----------------------------------|--------|--------|--------------------|--------------------|
| 29.2683    |                                   |        |        |                    |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                |
| 1          | ACE inhibitor                     | 0.0534 | 0.0917 | 0.0055038387628308 | 0.20568320455268   |
| 2          | antioxidative                     | 0.0146 | 0.2005 | 0                  | 0                  |
| 3          | dipeptidyl peptidase IV inhibitor | 0.0583 | 0.0896 | 1.8563339357632E-6 | 0.0086054864513139 |

**Table A55.** Bioactive peptides from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using V-8 protease (Glutamyl endopeptidase).

| No. | Peptide ID | Sequence | Location | Name   | Function   | Activity                          | Monoisotopic Mass | Chemical Mass |
|-----|------------|----------|----------|--|--|-----------------------------------|-------------------|---------------|
| 1   | 8898       | TD       | (34–35)  | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 234.0739          | 234.1940      |
| 2   | 8934       | YE       | (29–30)  | dipeptidyl peptidase IV inhibitor (DPP IV inhibitor) | Inhibitor of Dipeptidyl Peptidase IV (EC 3.4.14.5) (MEROPS ID: S09.003)              | dipeptidyl peptidase IV inhibitor | 310.1050          | 310.2860      |
| 3   | 9078       | YE       | (29–30)  | ACE inhibitor  | Inhibitor of Angiotensin-Converting Enzyme (ACE) (EC 3.4.15.1) (MEROPS ID: XM02-001) | ACE inhibitor                     | 310.1050          | 310.2860      |

**Table A56.**  $A_E$ ,  $DH_t$ ,  $W$ ,  $B_E$  and  $V$  values from RubisCO of *Halophila stipulacea*. In silico enzymatic cleavage was carried out by using V-8 protease (Glutamyl endopeptidase).

| $DH_t$ (%) |                                   |        |        |                    |                    |
|------------|-----------------------------------|--------|--------|--------------------|--------------------|
| 11.7073    |                                   |        |        |                    |                    |
| No.        | Activity                          | $A_E$  | $W$    | $B_E$              | $V$                |
| 1          | dipeptidyl peptidase IV inhibitor | 0.0097 | 0.0149 | 0                  | 0                  |
| 2          | ACE inhibitor                     | 0.0049 | 0.0084 | 7.6943555746376E-6 | 0.0002875447082947 |

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