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## Bioactivities from Novel Toxins of *Pterois volitans*: A Bioinformatics Approach

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Keywords	Abstract
Angiotensin Converting Enzyme Bioactive Peptides Dipeptidyl Peptidase-IV <i>Pterois volitans</i>	<i>Pterois volitans</i> (Linnaeus, 1758), native of the Indo-Pacific Ocean, is an invasive fish species in the Western-Atlantic. Due to the very long venomous spines, they increase their populations in newly invaded habitats. No validated eradication method has been existed for this species yet. Therefore, there is an urgent need for alternative utilization methods for this fish species. Bioactive peptides are of great importance for human health since they exhibit excellent inhibitory properties for some medicinally important enzymes in human metabolisms such as angiotensin converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV). In this study, the bioactive peptides in protein-based venom of <i>P. volitans</i> , Pvtoxin-a and Pvtoxin-b, were investigated by using in silico tools. Total number of negatively charged residues (Asp + Glu) for Pvtoxin-a and Pvtoxin-b were found as 21 and 18, respectively. Total number of positively charged residues (Arg + Lys) for Pvtoxin-a and Pvtoxin-b were observed to be 22 and 24, respectively. According to BIOPEP results, A <sub>E</sub> values for ACE and DPP-IV were found to be 0.0305 and 0.0666 for Pvtoxin-a and Pvtoxin-b as 0.0333 and 0.0569, respectively. It is very interesting to note that A <sub>E</sub> values related to antioxidant property were observed to be 0.0083 for both Pvtoxin-a and Pvtoxin-b. Although A <sub>E</sub> value for alpha-glucosidase inhibitor was found to be 0.0014 for Pvtoxin-a, no A <sub>E</sub> value was obtained for Pvtoxin-b. In conclusion, the BIOPEP results clearly show that after a possible eradication study, protein-based venoms from <i>P. volitans</i> can be evaluated in the production of bioactive peptides. A possible economical value may create a pressure on its increasing population in newly invaded areas.

### Cite

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## 1. INTRODUCTION

The entry of invasive species has been increasing and becoming a major problem in the Mediterranean Sea (Carballo-Cárdenas, 2015; Bédry et al., 2021). The name “lionfish” is given for the members of the genera *Pterois*, *Parapterois* and *Dendrochirus*. Among this large family, *Pterois volitans* (Linnaeus, 1758) and *P. miles* (Bennett, 1828) (Actinoptery: Scorpaeniformes: Scorpaenidae) are of great importance due to their invasive properties. The lionfish invasion is one of the important concerns for marine ecosystems since they have an ability to alter native fish populations and damage to the native biodiversity (Sutherland et al., 2010; Andradi-Brown, 2019). Although *P. volitans* are native to the western parts of the Indo-Pacific Ocean, they have so far been reported from western Atlantic (Schofield, 2009; 2010), USA (Meister et al., 2005) and southeast Brazil (Ferreira et al., 2015). The invasion of lionfish has still been confused for the Mediterranean Sea due to some published reports in the scientific journals. As an example, although some authors (Gürlek et al., 2016; Turan et al., 2017; 2020, Ayas et al., 2018) previously identified the lionfish in the Mediterranean Sea coastline of Turkey as *P. volitans*, according to the recent papers by Ulman et al. (2020) and Cinar et al. (2021), the lionfish off the Turkish coastline is defined as *P. miles*. The reason of the confusion is based on the misinterpretation of the samples due to i) morphological properties cannot be enough to distinguish *P. volitans*

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and *P. miles*, ii) correct bio-sequences should be selected to identify correct taxon in the genetic studies (Ulman et al., 2020; Cinar et al., 2021, Kovačić et al., 2021 ). Lionfish invasion along Turkish coastline is in increasing trend. It is very important to note that the lionfish is also mentioned in the recent novel of Zülfü Livaneli, one of the famous writers in Turkey, titled "*Balıkçı ve Oğlu*" (Livaneli, 2021). The lionfish has venomous dorsal spiky rays and pectoral fins (Dağhan & Demirhan, 2020). Therefore, hunting of lionfish through spear fishing is dangerous and also risky because of its venomous spines. On the other hand, spear fishing tournaments specific to lionfish organized by various local organizations to reduce their numbers on the shores (Antalya Büyükşehir Belediyesi, 2021). However, this kind of organizations must be carried out by experts. Yuzvik et al. (2018) developed a submarine robot which has properties of hunting lionfish to control of *P. volitans* population in the Caribbean. Therefore, it could be stated that lionfish may harm the ecosystem due to their huge appetites and preferences for native species, however they may also present economic benefits through development of commercial fisheries for them (Kleitou et al., 2019). It has maroon or brown and white stripes (Davis, 2016) also it has 13 back, 3 anal spines and one on each pelvic fin (Galloway & Porter, 2019). *P. volitans* uses its back spines to present itself larger and stronger (Galloway & Porter, 2019). Its spines contain non-protein based ichthyotoxin with low molecular mass. This venom can be isolated from only alive fishes (Nair et al., 1985). Toxins can cause to symptoms that last for a few days as a result of contact with humans. Burning symptom is observed within the first 15-20 minutes after the spines come into contact. Also, tachycardia, hypertension, and numbness can be observed. After 3 hours, the venom spreading into the body can cause limb paralysis (Vetrano et al., 2002). Therefore, it is dangerous if the spines penetrate humans, but once their spines are removed, they are safe to consume and are considered a very delicious fish. The lionfish is at the top of the underwater food chain and is a major threat to native fish. *P. volitans* is observed in the ecosystem where it skillfully hunts smaller fish and crustaceans. It damages native biodiversity. Especially, the Mediterranean Sea and the Caribbean are under the threat since lionfish has widely distributed areas in these marine ecosystems (Carballo-Cárdenas, 2015; Ulman et al., 2020).

Bioactive peptides are obtained from food proteins. They are component of the functional foods and nutraceuticals and therefore they play important roles in human health (Liu et al., 2020). Hyperlipidemia, inflammation, diabetes, cancer, immune disorder can be given as example disorders in which bioactive peptides can show effectiveness (Agyei & Danquah, 2012). They are related with biological activities such as antimicrobial, antioxidant, antihypertensive, immunomodulatory, antidiabetes, anti-cancer (Daroit & Brandelli, 2021). Bioactive peptides consist of 2-20 amino acid residues. Long peptides are inactive without hydrolysis. They are activated via hydrolysis methods such as fermentation (proteolytic enzymes, microbial or vegetable proteolytic enzymes) (Weaver, 2014). Compared to proteins, bioactive peptides are absorbed by the intestine completely due to their sizes (Zhou et al., 2020). Bioactive peptides are new to humans but safety studies have been limited yet for commercial use although they are derived from foods. There has so far been no information yet about if new bioactive peptides carry sequences with allergenic or even toxic (Liu et al., 2020). The origin of bioactive peptides can be plant or animal. Grains and legumes are peptide rich sources (Jang & Lee, 2005). According to Chakrabarti et al. (2018), there are three challenges on the utilization of food-derived bioactive peptides and they are production, oral consumption and regulation. Bioactive peptides may be used for the chronic diseases but further studies are required.

Kiriake & Shiomi (2011) discovered two proteinaceous toxins from two lionfish species (*Pterois antennata* and *Pterois volitans*). The authors investigated the toxin structures via cDNA cloning using primers designed from the highly conserved sequences of the stonefish toxins. Since these toxins homologous are most probably existed in Scorpaeniformes members (Kiriake & Shiomi, 2011), *P. miles* which has a second colonization in the Mediterranean Sea may have these toxins and may show similar symptoms when preys are exposed to these toxins. *P. miles* is also widely distributed within the Mediterranean Sea, we wanted to investigate bioactive peptides in Pvtoxin-a and Pvtoxin-b in *P. volitans* by using bioinformatics tools developed for identification of bioactive peptides within protein sequences.

## 2. MATERIALS AND METHODS

The proteins related to *P. volitans* were searched by using UniProt.org and 560 proteins were listed. The longest protein was Pvtoxin-a (F2ZAF0) with 699 amino acids and the shortest protein was ATP synthase protein 8 (A0A0U1WQN3) with 55 amino acids (Morgat et al., 2020). In this study, Pvtoxin-a (F2ZAF0) and

Pvtoxin-b (F2ZAF1) were selected as model proteins. FASTA formats of proteins were obtained from UniProt.org. In order to obtain protein parameters, Protparam tool was used (Gasteiger et al., 2005). Pairwise sequence alignment was carried out by using Clustal Omega (Sievers et al., 2011). BIOPEP-UWM (<http://www.uwm.edu.pl/biochemia/index.php/en/biopep>) was used to estimate bioactive peptides in Pvtoxin-a and Pvtoxin-b. Chymotrypsin, trypsin and pepsin (pH = 1.3) were used for in silico digestions. The reason for selection of these 3 proteolytic enzymes was their importance in human protein digestion system (Minkiewicz et al., 2019). The analytical parameters with respect to bioactive peptides were estimated from BIOPEP-UWM and then they were compared. The Basic Local Alignment Search Tool (BLAST) was used to find similar proteins in the protein database.

### 3. RESULTS

#### 3.1. Protparam Results

Protein parameters in Pvtoxin-a and -b were studied in this paper by using Protparam tool (Gasteiger et al., 2005). According to Table 1, most two abundant amino acids in Pvtoxin-a were leucine (% 8.8) and valine (% 7.7). Maximum percentages of amino acids in Pvtoxin-b were found as leucine (% 7.8) and glycine and lysine (% 7.3). From the results, it could be said that essential amino acid leucine is very high in both studied proteins. Theoretical pI value was found for Pvtoxin-a as 7.89 and for Pvtoxin-b as 9.14. Total number of negatively charged residues (Asp + Glu) for Pvtoxin-a and Pvtoxin-b were found as 21 and 18, respectively. Total number of positively charged residues (Arg + Lys) for Pvtoxin-a and Pvtoxin-b were observed to be 22 and 24, respectively. It is very interesting to note that the net charges of the proteins studied, Pvtoxin-a and Pvtoxin-b, were found as 1 and 6, respectively.

**Table 1.** Number, Percentages and Theoretical pI, Total Number of Positively & Negatively Charged Residues and Net Charge of Amino Acids of Pvtoxin-a and Pvtoxin-b Proteins of *Pterois volitans* (aa: amino acids)

	Pvtoxin-a		Pvtoxin-b	
	#aa	aa%	#aa	aa%
<b>Ala (A)</b>	6	3.1	10	5.2
<b>Arg (R)</b>	8	4.1	10	5.2
<b>Asn (N)</b>	14	7.2	8	4.1
<b>Asp (D)</b>	10	5.2	11	5.7
<b>Cys (C)</b>	3	1.5	4	2.1
<b>Gln (Q)</b>	5	2.6	10	5.2
<b>Glu (E)</b>	11	5.7	7	3.6
<b>Gly (G)</b>	14	7.2	14	7.3
<b>His (H)</b>	8	4.1	6	3.1
<b>Ile (I)</b>	9	4.6	10	5.2
<b>Leu (L)</b>	17	8.8	15	7.8
<b>Lys (K)</b>	14	7.2	14	7.3
<b>Met (M)</b>	3	1.5	1	0.5
<b>Phe (F)</b>	10	5.2	1	5.7
<b>Pro (P)</b>	10	5.2	10	5.2
<b>Ser (S)</b>	14	7.2	12	6.2
<b>Thr (T)</b>	10	5.2	12	6.2
<b>Trp (W)</b>	5	2.6	8	4.1
<b>Tyr (Y)</b>	8	4.1	8	4.1
<b>Val (V)</b>	15	7.7	12	6.2
<b>Theoretical pI</b>	7.89		9.14	
<b>Total number of negatively charged residues (Asp + Glu):</b>	21		18	
<b>Total number of positively charged residues (Arg + Lys):</b>	22		24	
<b>Net charge</b>	1		6	

### 3.2. Pairwise Sequence Alignment results

Clustal Omega tool was used to determine the similarity of Pvtoxin-a and -b (Siever et al., 2011). According to Figure 1, their both N-terminals are methionine (M) and their C-terminals are leucine (L), respectively. There is very high similarity in the amino acid sequences of the proteinous toxins, Pvtoxin-a and -b.



**Figure 1.** Clustal Omega Results of Pvtoxin-a and Pvtoxin-b. (\*' symbol is used to show conserved residues, ':' symbol used to show very similar amino acids in terms of physicochemical characters and '.' symbol is used to show similar amino acids in terms of physicochemical characters. Empty spaces are used for deleted or inserted amino acids)

### 3.3. BIOPEP-UWM Results

BIOPEP-UWM was used for in silico hydrolysis of bioactive peptides which are obtained from Pvtoxin-a and -b (Minkiewicz et al., 2019). When Table 2 is examined, DHt value of Pvtoxin-a was found as 40.1389. A<sub>E</sub> value corresponds to the frequency of release of fragments by selected enzymes. A<sub>E</sub> values related to dipeptidyl peptidase-III and -IV inhibitors were as 0.0042 and 0.066, respectively. A<sub>E</sub> values of Angiotensin converting Enzyme (ACE) inhibitor as 0.0305. The A<sub>E</sub> values related to other bioactivities such as stimulating, antioxidative, renin inhibitor, CaMPDE inhibitor and alpha-glucosidase inhibitor can also be found in Table 2. According to Table 3, DHt value of Pvtoxin-b was found as 39.7775. The A<sub>E</sub> values were found for dipeptidyl peptidase-III and -IV inhibitors to be 0.0069 and 0.0569, respectively. The A<sub>E</sub> values with respect to ACE inhibitor as 0.0333. The other bioactivities such as anti-amnestic, stimulating, antioxidative and renin inhibitor were depicted in Table 3.

**Table 2.** *In Silico Hydrolysis of Pvtoxin-a Protein of Pterois volitans Using BIOPEP-UWM Database*

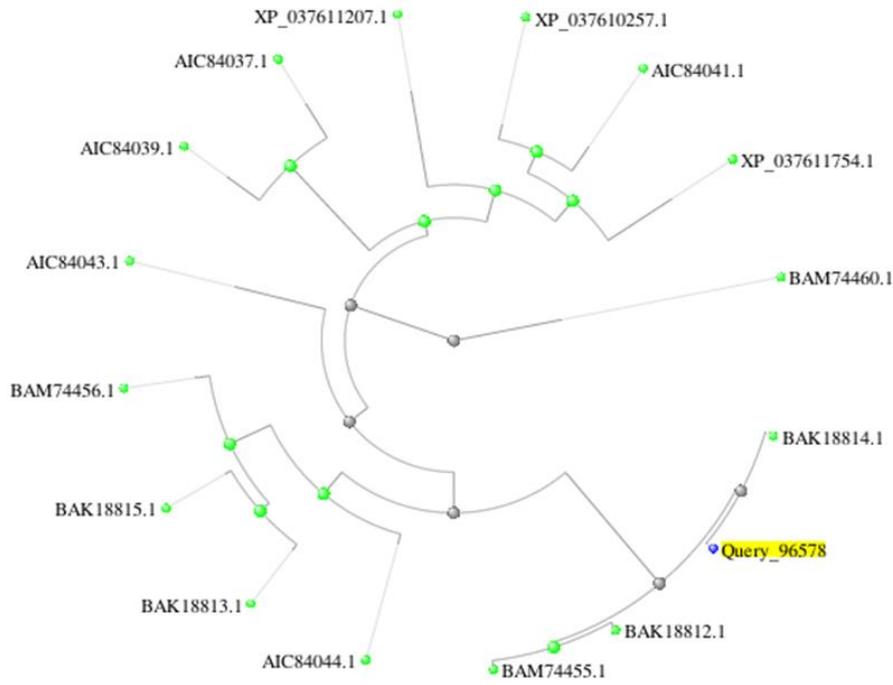
DHt [%] 40.1389					
No	Activity	A <sub>E</sub>	W	B <sub>E</sub>	V
1	ACE inhibitor	0.0305	0.0830	0.0023	0.1357
2	Stimulating	0.0055	0.1134	0	Nd
3	Antioxidative	0.0083	0.1273	0	0
4	renin inhibitor	0.0042	0.1317	0.0002	0.3332
5	CaMPDE inhibitor	0.0014	0.1687	0	nd
6	dipeptidyl peptidase IV inhibitor	0.0666	0.1109	0.0001	0.2552
7	alpha-glucosidase inhibitor	0.0014	0.0530	6.1370E-5	0.9683
8	dipeptidyl peptidase III inhibitor	0.0042	0.0445	0	nd

**Table 3.** *In Silico Hydrolysis of Pvtoxin-b Protein of Pterois volitans Using BIOPEP-UWM Database*

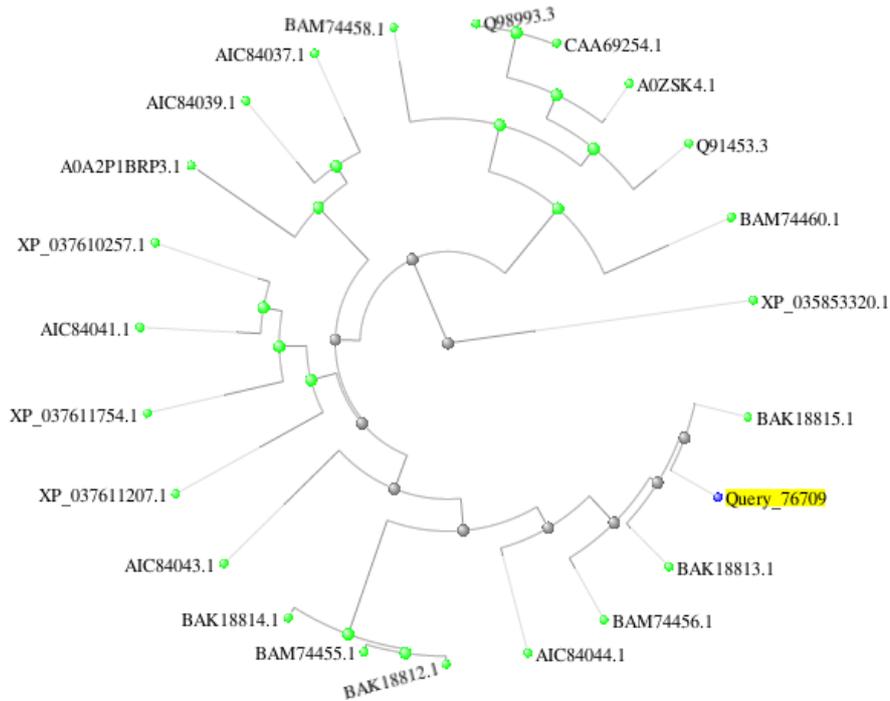
DHt [%] 39.7775					
No	Activity	A <sub>E</sub>	W	B <sub>E</sub>	V
1	antiamnestic	0.0014	0.2500	2.9551E-5	1
2	stimulating	0.0028	0.0695	0	
3	ACE inhibitor	0.0333	0.0885	0.0010	0.0905
4	antioxidative	0.0083	0.1390	0	0
5	dipeptidyl peptidase IV inhibitor	0.0569	0.0962	0.0001	0.2193
6	renin inhibitor	0.0042	0.1317	8.9606E-7	0.0058
7	dipeptidyl peptidase III inhibitor	0.0069	0.0776	0	

### 3.4. BLAST Results

In order to find similar proteins, BLAST-p analysis was carried out by using BLAST server (Altschul et al., 1990). Blast-p results of Pvtoxin-a and -b were given in Figure 2 and Figure 3, respectively. Pvtoxin-a like proteins were also existed in the member of same genus such as *P.lunulata* and *P.antennata*. However, similar proteins are also existed in different genus such as *Scorpaenopsis*, *Sebastapistes*, *Dendrochrius* and *Hypodytes*. The Pvtoxin-a is highlighted as yellow in Figure 2. The BLAST-p results for Pvtoxin-b were given Figure 3. Contrary to Pvtoxin-a, Tx gamma-subunit from *Dendrochrius zebra* was in the same clade with Pvtoxin-b. Very similar proteins with most probably same bioactivities are existed in the genus of *Inimicus*, *Sebastepiscus*, *Scorpaenopsis*, *Scorpaena*, *Sebastes*, *Sebastiscus*, *Dendrochrius* and *Hypodytes* (Figure 3). The Pvtoxin-b is highlighted as yellow in Figure 3. Table 4-5 show codes and names of the proteins in the phylogenetic tree. Since Pvtoxin-a and -b are searched, the original sequences were also found after BLAST search.



**Figure 2.** BLAST-p Analysis Results for Pvtoxin-a  
 (The Codes and Names of the Proteins are Explained in the Table 4)



**Figure 3.** BLAST-p Analysis Results for Pvtoxin-b  
 (The Codes and Names of the Proteins are Explained in the Table 5)

**Table 4.** A list of the codes and names of proteins obtained for Pvtoxin-a from BLAST-p

The Codes	The Name of The Proteins
Query_96578	pvtoxin-a [ <i>Pterois volitans</i> ]
BAK18814.1	pvtoxin-a [ <i>Pterois volitans</i> ]
BAM74455.1	pltoxin-a subunit [ <i>Pterois lunulata</i> ]
BAK18812.1	patoxin-a subunit [ <i>Pterois antennata</i> ]
BAM74456.1	pltoxin-b subunit [ <i>Pterois lunulata</i> ]
BAK18815.1	pvtoxin-b [ <i>Pterois volitans</i> ]
BAK18813.1	patoxin-b subunit [ <i>Pterois antennata</i> ]
XP_037611754.1	stonustoxin subunit beta-like [ <i>Sebastes umbrosus</i> ]
AIC84041.1	Tx beta-subunit [ <i>Sebastiscus marmoratus</i> ]
AIC84037.1	Tx beta-subunit [ <i>Sebastapistes strongia</i> ]
XP_037610257.1	stonustoxin subunit beta-like [ <i>Sebastes umbrosus</i> ]
XP_037611207.1	stonustoxin subunit beta-like [ <i>Sebastes umbrosus</i> ]
AIC84039.1	Tx beta-subunit [ <i>Scorpaenopsis oxycephala</i> ]
AIC84044.1	Tx gamma-subunit [ <i>Dendrochirus zebra</i> ]
BAM74460.1	hrtoxin-b subunit [ <i>Hypodytes rubripinnis</i> ]
AIC84043.1	Tx beta-subunit [ <i>Dendrochirus zebra</i> ]

**Table 5.** A list of the codes and names of proteins obtained for Pvtoxin-b from BLAST-p

The Codes	The Name of The Proteins
Query_76709	pvtoxin-b [ <i>Pterois volitans</i> ]
BAK18815.1	pvtoxin-b [ <i>Pterois volitans</i> ]
BAK18813.1	patoxin-b subunit [ <i>Pterois antennata</i> ]
BAM74456.1	pltoxin-b subunit [ <i>Pterois lunulata</i> ]
XP_037610257.1	stonustoxin subunit beta-like [ <i>Sebastes umbrosus</i> ]
AIC84041.1	Tx beta-subunit [ <i>Sebastiscus marmoratus</i> ]
AIC84037.1	Tx beta-subunit [ <i>Sebastapistes strongia</i> ]
BAK18814.1	pvtoxin-a [ <i>Pterois volitans</i> ]
AIC84044.1	Tx gamma-subunit [ <i>Dendrochirus zebra</i> ]
BAM74455.1	pltoxin-a subunit [ <i>Pterois lunulata</i> ]
XP_037611207.1	stonustoxin subunit beta-like [ <i>Sebastes umbrosus</i> ]
BAK18812.1	patoxin-a subunit [ <i>Pterois antennata</i> ]
AIC84039.1	Tx beta-subunit [ <i>Scorpaenopsis oxycephala</i> ]
XP_037611754.1	stonustoxin subunit beta-like [ <i>Sebastes umbrosus</i> ]
BAM74460.1	hrtoxin-b subunit [ <i>Hypodytes rubripinnis</i> ]
Q91453.3	RecName: Full=Stonustoxin subunit beta; Short=SNTX subunit beta; AltName: Full=DELTA-synanceitoxin-Sh1b; Short=DELTA-SYTX-Sh1b; AltName: Full=Trachynilysin subunit beta; Short=TLY subunit beta [ <i>Synanceia horrida</i> ]
A0ZSK4.1	RecName: Full=Neoverrucotoxin subunit beta; Short=NeoVTX subunit beta [ <i>Synanceia verrucosa</i> ]
XP_035853320.1	LOW QUALITY PROTEIN: stonustoxin subunit beta-like [ <i>Sander lucioperca</i> ]
BAM74458.1	ijtoxin-b subunit [ <i>Inimicus japonicus</i> ]
A0A2P1BRP3.1	RecName: Full=Cytolytic toxin-beta; Short=Sp-CTx-beta [ <i>Scorpaena plumieri</i> ]
AIC84043.1	Tx beta-subunit [ <i>Dendrochirus zebra</i> ]
CAA69254.1	verrucotoxin alpha [ <i>Synanceia verrucosa</i> ]
Q98993.3	RecName: Full=Verrucotoxin subunit beta; Short=VTX subunit beta [ <i>Synanceia verrucosa</i> ]

#### 4. DISCUSSION

Introduction of alien or non-indigenous species into a new habitat may not always harm to ecosystem. In order to define an alien species as an invasive species, alien species must negatively impact the biodiversity, economy or human health (Ulman et al., 2020). As an example, *P.miles* invasion in the Mediterranean Sea coastline of Turkey impressed not only scientists, but also writers. Lionfish invasion and its social effects from the eyes of a local fisherman in Turkey are described in the recent novel of Zülfü Livaneli (Livaneli, 2021). The current situation of *P.volitans* in Turkey is also complicated due to previously published and confused papers where the lionfish is defined as *P.volitans* (Gürlek et al., 2016; Turan et al., 2017; 2020, Ayas et al., 2018), on the other hand, recent papers underline that the lionfish in Turkish coastlines is *P.miles* (Ulman et al., 2020; Çinar et al., 2021). Bioactive peptides are obtained via wet lab studies. The bioinformatic tools are also used for discovering of bioactive peptides (Agirbasli & Cavas, 2017; Kandemir-Cavas et al., 2019). In this study, Pvtoxin-a and Pvtoxin-b from *P. volitans* were studied as model proteins. Amino acid sequences were compared by using well-known bioinformatics tools such as ProtParam and multiple sequence alignment. The novelty of the paper is to study BIOPEP parameters of Pvtoxin-a and Pvtoxin-b from *P. volitans*. There are some differences in protein parameters in Pvtoxin-a and Pvtoxin-b from *P. volitans* such as theoretical pI, net charge and total number of negatively and positively charged residues (Table 1). These differences are caused by the amino acid sequence variations in these proteins (Figure 1). These toxins are still classified as unreviewed proteins in the uniprot.org. However, based on the sequence homology, it is said that these proteins show their functions outside the cell membranes since they are toxin-based proteins. Since they are long protein, consisted of more than 698 amino acids, their short-bioactive peptides are worthwhile to study. Among bioactive peptides, ACE inhibitors are of great importance in terms of hypertension. Since the hypertension is very common not only in developing countries but also in developed countries, it is very important to propose the ACE inhibitors for the production of functional food-based inhibitors. From this perspectives, isolated *P. volitans* toxins can be an alternative source of ACE inhibitors. After partial purification of these toxins, ACE-inhibitors based bioactive peptide cocktails can be prepared. The stimulating is one of the parameters that BIOPEP provided. The stimulating in BIOPEP is defined as the peptides stimulating various biological processes (Minkiewicz et al., 2019). The information related to specific biological activities are also given for each individual protein. When two proteins are compared, it is clear from Table 2 and 3 that Pvtoxin-a has remarkable stimulating activities than Pvtoxin-b. Specific biological activities can be accessed from BIOPEP web page after enzymatic in silico digestion of these proteins. For example, after enzymatic digestion of Pvtoxin-a via trypsin, chymotrypsin and pepsin, VPL was found as a stimulating peptide. Its biological activities are matched with stimulating release of vasoactive substance with 327.41 chemical mass value. The antioxidative term in BIOPEP is defined as peptides inhibiting oxidation. When Pvtoxin-a and -b were compared, the differences in the W values were observed. On the other hand, their A<sub>E</sub> values were the same. According to Table 3, Pvtoxin-b has more antioxidative activities than Pvtoxin-a. After digestion of Pvtoxin-b, SDF was found as an antioxidative peptide. AH and EL can also be given as examples for this activity. W and A<sub>E</sub> values of Pvtoxin-a and -b for Renin inhibitor were found same. SF and TF were found as renin inhibitor peptides after digestion of Pvtoxin-b. DPP-IV is a serine protease which catalyzes amide bonds. It is reported that DPP-4 inhibitors have an effect on diabetes type-II. Diabetes is a very common health issue and it is very common among 35-40 aged people (Misumi & Ikehara, 2013). According to Table 2 and 3, when A<sub>E</sub> and W values were compared for the two proteins, Pvtoxin-a has more DPP-IV inhibitors than Pvtoxin-b. VP, PL, AF, AH, DN can be given as examples as DPP-IV inhibitor peptides. Dipeptidyl peptidase III (DPP-III) is a zinc-dependent hydrolase involved in degrading oligopeptides with 4–12 amino acid residues (Jha et al., 2020). When two proteins were compared, Pvtoxin-b had noticeably more DPP-III inhibitors than Pvtoxin-a. TF and PF can be given as examples for DPP-III inhibitor peptides. BLAST-p was used to find similar proteins. According to Figure 2, *Pterois lunulata*, *Pterois antennata*, *Sebastes umbrosus*, *Sebastiscus marmoratus*, *Sebastapistes strongia*, *Scorpaenopsis oxycephala*, *Dendrochirus zebra* and *Hypodytes rubripinnis* have Pvtoxin-a like protein. Their percent identities were found to be between 98.57 and 70.67%. The shortest sequence was found to be 583 in *D. zebra* and the longest sequence was 752 in *S. umbrosus*. According to Figure 3, Pvtoxin-b like protein was found in the genus *Inimicus*, *Sebastepiscus*, *Scorpaenopsis*, *Scorpaena*, *Sebastes*, *Sebastiscus*, *Dendrochirus* and *Hypodytes*. When two proteins were compared, Tx gamma-subunit from *D. zebra*, *Synanceia horrida*, *Synanceia verrucosa*, *Sander lucioperca* and *Inimicus japonicus* were observed for Pvtoxin-b. Most of these fishes belong to the Scorpaenidae family and they are distributed in tropical waters.

Harris et al. (2020) tested the collapsible trap (Gittings trap) for mitigation of *P. volitans*. The trap technology is very important since SCUBA divers may not always access the depth limits of *P. volitans*. The authors tested their trap in the northern Gulf of Mexico. It is reported that 327 lionfish and 28 native fish were trapped. On the other hand, removal efficacy was given between as 12-24%. According to the authors, even if this range is lower than that of spearfishing, more studies are recommended to develop the traps.

Sommeng et al. (2019) studied the antiviral activity of *P. volitans* phospholipase A2 (PLA2) from Indonesia for its effects on the human immunodeficiency virus. The authors reported that PLA2 from *P. volitans* showed strong inhibition on the SRV2-A549 cell lines, therefore, they suggested to evaluate their effects *in vivo*. Sommeng et al. (2020) also investigated the coagulant activity of *P. volitans* spine venom. The authors studied the coagulant activity via prothrombin time and activated partial thromboplastin time. They found out that the responsible procoagulant molecule in *P. volitans* venom is Nomega-nitro-L-arginine methyl ester. Becerra-Amezcuca et al. (2020) investigated the effects of the venom of *P. volitans* on the cholinergic and dopaminergic systems via nicotinic acetylcholine receptors and dopaminergic neurons. Zebra fish was used as a model organisms in their study and it was found by the authors that the venom from *P. volitans* significantly affected the dopaminergic neurons and retarded embryonic development due to the venom also affects growth hormone secretion. The authors also concluded that lionfish venom should be further studied for its possible pharmaceutical effects on the Alzheimers' disease.

Hoo Fung et al. (2013) investigated concentrations of essential, non-essential and toxic elements in the tissues of *P. volitans* collected from Jamaican waters. While the muscular tissues of the *P. volitans* (lionfish) were evaluated, researchers demonstrated that it might make a significant contribution as an alternative nutrition source since it did not reveal conceivably hazardous substances. This study appears partly to confirm the findings of our study. Cavas et al. (2020), in our previous study, proposed that the toxins of *Lagocephalus sceleratus* can be removed and the residues including toxins could be exploited in some industries such as pharmacy inasmuch as their analgesic and anti-cancer effects have been reported for these toxins. From the study of Cavas et al. (2020), it could be said that the removed toxins of *L. sceleratus* may also be evaluated in different industries such as pharmacy inasmuch as their analgesic and anti-cancer effects.

Galloway & Porter (2019) studied the mechanical properties of *P. volitans* spines by quantifying the impacts of shape and tapering on the mechanical properties of the spine. Well-known mechanical properties such as Young's modulus, elastic energy store and flexural stiffness were studied by Galloway & Porter (2019). Bending properties of the spines are also investigated in their study. The authors suggested that the mechanical properties of the spines are important in many functions such as defense, protection and intimidation.

## 5. CONCLUSION

From the published papers, it could be said that the systematic of the genera *Pterois* is very complex. Even if there has so far been a confusion in the taxonomic identification, the toxins mentioned in this paper most probably exist in *P. miles* which has colonized the Mediterranean Sea. After carefully removal of the venomous spines, the rest of the lionfish tissues can be consumed. From the results of the present study, it could be said that bioactive peptides exist in proteinaceous toxins of *P. volitans* and these bioactive peptides can be used as an alternative protein source. A possible economical values assigned to these invasive fishes may create extra stress on their populations and may help to control their populations. More studies on lionfish species, are strongly warranted to evaluate their values in the preparation of functional foods.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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