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MICROBIOLOGY

Algae as a source of peptides inhibitors of the angiotensin-converting enzyme: a systematic review

ANDREZA P. DE AMORIM, GABRIELLY H. DA SILVA, ROMERO M. P. BRANDÃO, ANA LÚCIA F. PORTO & RAQUEL P. BEZERRA

Abstract: Hypertension is a factor that contributes to the risk of chronic diseases. The inhibition of angiotensin-I converting enzyme (ACE) is a useful therapeutic approach to the hypertension treatment. The algae have been an alternative for the production of ACE inhibitory (ACEi) peptides from enzymatic hydrolysis due to their protein-rich biomass. The aim of this study was to systematically review the literature regarding the production, composition and activity of ACEi peptides derived from algae proteins. Systematic database searches identified 648 related articles. Among these, only 14 were selected according to the eligibility criteria to this review. Macroalgae are more studied than microalgae as sources of ACEi peptides. Furthermore, hydrolysates by thermolysin or bromelain exhibited the highest ACEi activity compared to other enzymes. The main features of the peptides with high ACE inhibition are low molecular weight, short amino acids sequence and non-competitive inhibition pattern. *In vivo* studies using hydrolysates and peptides derived from algae proteins showed antihypertensive activity in spontaneously hypertensive rats (SHR). Thus, it is suggested that ACEi peptides derived from algae can be considered as potential antihypertensive.

Key words: Algae protein hydrolysate, anti-hypertension, bioactive peptides, hypertension, inhibitory peptide.

INTRODUCTION

The American College of Cardiology (ACC) and American Heart Association (AHA) define systemic arterial hypertension (SAH) as a disease characterized by persistence of high blood pressure levels (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg), which is associated to a risk factor for chronic diseases, including cardiovascular disorders, stroke, renal diseases and diabetes (Cappuccio & Miller 2016, Whelton et al. 2018). Worldwide, it is estimated that high blood pressure affects 1 billion people and will increase to more than 1.6 billion by 2025 (Ferreira-Santos et al. 2017, Hippauf et al. 2016, World Health Organization 2013).

Hypertension can be prevented by decreasing the intake of salt, saturated and trans fats, and by lifestyle (Brook et al. 2013). Currently, hypertension control can be done by use of antihypertensive drugs, such as diuretics, calcium channel blockers, beta blockers, angiotensin-II receptor blockers and angiotensin-I conversion enzyme (ACE; EC 3.4.15.1) inhibitor (McManus et al. 2012).

One of the main mechanisms of blood pressure control is the renin-angiotensin-aldosterone system (RAAS) (Chen et al. 2018). When the system is activated, the renin is secreted

from the justaglomerular apparatus of the kidney and cleaves the circulating glycoprotein angiotensinogen to form angiotensin-I. The ACE, a zinc-containing dipeptide carboxypeptidase, promotes the conversion of angiotensin-I to the potent angiotensin-II vasoconstrictor (Putnam et al. 2012) and aldosterone activator. The aldosterone, which acts on the adrenal gland, promotes sodium reabsorption, water retention and loss of potassium and magnesium, thereby modulating the extracellular space volume and the blood pressure (Spät & Hunyady 2004). The ACE is also responsible for inactivates bradykinin, a vasodilator in the kinin–kallikrein system (KKS) (Cheng et al. 2009, Eriksson et al. 2002).

ACE inhibition is considered an useful therapeutic approach in the hypertension treatment (Lahogue et al. 2010) and it has become an important activity in the development of drugs to control the high blood pressure. Many chemically synthesized ACE inhibitory (ACEi), such Captopril©, Enalapril©, Alcacepril© and Lisinopril©, are currently used in the hypertension control (García-Mora et al. 2017). However, it can cause several side effects, as dry cough, taste disturbances, skin rash, renal insufficiency (Cooper et al. 2006). Recently, ACEi peptides derived from natural compounds have increased due to less side effects when compared by synthetic antihypertensive drugs (Udenigwe & Aluko 2012). ACEi peptides have been obtained from several natural origin sources, such as hazelnut (Liu et al. 2018), kefir milk (Amorim et al. 2019), chicken foot (Mas-Capdevila et al. 2018) and algae (Xie et al. 2018).

Algae are multicellular or single cell living organisms rich in compounds with several biotechnological applications (Kiuru et al. 2014, Loureiro et al. 2018, Fitzgerald et al. 2011). For example, lipid fraction can be used as renewable and sustainable biodiesel production (Frumento et al. 2013, Aratboni et al. 2019, Falkowski 2004); whole biomass can be applied on wastewater bioremediation (Salama et al. 2019) and aquaculture (Allen et al. 2019); bioactive compounds as peptides, protein, fatty acids, secondary metabolites has beneficial health properties (DeRose et al. 2019, Priyadarshani & Rath 2012) with action anti-inflammatory (Fernando et al. 2016), anti-cancer (Zhang et al. 2019), antimicrobial (Pane et al. 2015) and ACEi (Deng et al. 2018). Thus, the purpose of this study was to systematically review the literature regarding the production, composition and activity of ACEi peptides derived from algae proteins.

MATERIALS AND METHODS

Search strategy

A literature search was conducted using electronic databases, PubMed, Springerlink, ScienceDirect and Molecular Diversity Preservation International (MDPI), from 2012 to 2019. Keywords and search terms (individually or combined) were “ACE inhibitory peptides”, “inhibitory peptides”, “angiotensin-I-converting enzyme”, “algae”.

Eligibility criteria

The studies considered eligible were those written in English, describing ACEi activity of peptides isolated from algae. Review articles, short communication, letters, comments, abstracts congress and studies with insufficient information were excluded.

Assessment of risk of bias and quality criteria

The MetaAnalysis of Statistics Assessment and Review Instrument (MASTARI) (The Joanna Briggs Institute Reviewers’ Manual 2014), with some modifications, was used for verifying the risk of bias of each study. Seven quality criteria were evaluated by using the following questions:

(1) "Does the article reports the procedure to obtain bioactive compounds?", (2) "Does the article reports the description of the process of carrying out biological activity *in vitro*?", (3) "Does the article reports the molecular weight of peptides?", (4) "Does the article characterizes the ACEi peptides?", (5) "Does the article describes the inhibition mode of ACEi peptides?", (6) "Does the article uses different sequence of methods to identify the peptides?" and (7) "Does the article determines the IC_{50} of peptide?". Each item was evaluated as "yes", "no", "unclear" or "not applicable" and the risk of bias was ranked according to the sum of "yes" marks received as follows: 0 to 49% (high risk of bias), 50 to 70% (medium risk of bias), 71 to 100% (low risk of bias). In addition, the frequency of "yes" for each criterion was used to verify it quality and classified as good (>70%), moderate (70 ≤ criteria quality ≤ 50%) and bad (<50%).

RESULTS

Figure 1 shows the flow diagram for study selection. Through the literature search, we retrieved 295 articles from PubMed, 253 articles from ScienceDirect, 55 articles from SpringerLink

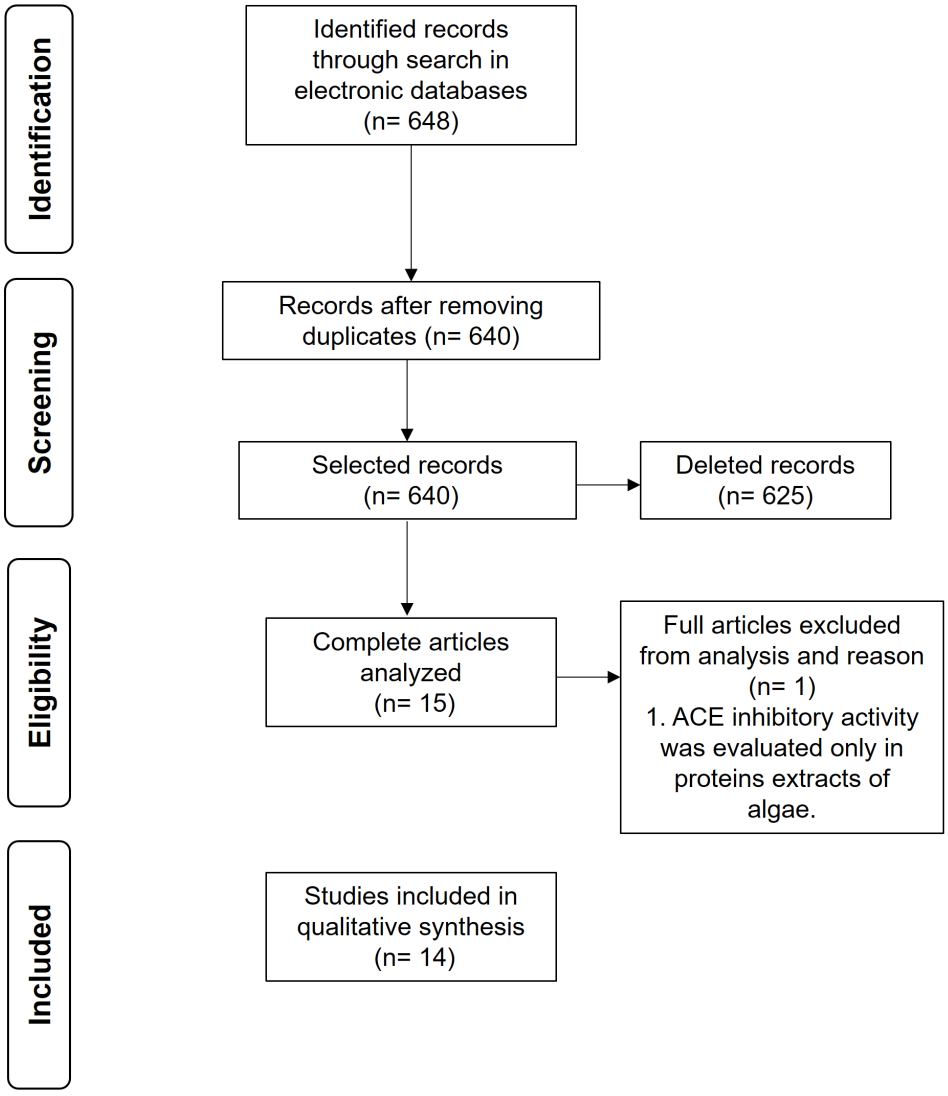

Figure 1. Flow diagram for study identification and selection adapted from Moher et al. (2009).

and 45 articles from MDPI, totalizing 648 articles. A total of 640 articles were identified after removing duplicate. The title and abstract were examined and only 15 articles were selected for full-text assessment. Of these, 1 article was excluded because ACEi activity was evaluated using protein extracts of algae instead of purified peptides.

A total of 14 eligible articles were submitted to an assessment of the risk of bias on the MetaAnalysis of Statistics Assessment and Review Instrument (MASTARI) protocol (The Joanna Briggs Institute Reviewers' Manual 2014, Gadioli et al. 2018) with some modifications. All studies were considered with "low risk of bias", because the frequency of "yes" was higher than 70%. Thus, no study was removed from the review (Table I). As shown in Table II, the quality criteria 1, 2, 4, 6 and 7 were attended by more than 70% of the studies, being classified as "good", while that criteria 3 and 5 were answered by 50% of the studies, obtaining "moderate" quality.

A total of 13 species of algae have been screened for their potential ACEi activities (Table III). The macroalgae (n = 9) belongs to division Chlorophyta (Green Algae) (n = 5), predominantly genus *Ulva*, followed by division Rhodophyta (Red Algae) (n = 4). Microalgae (n = 5) includes the main division Chlorophyta (Green Algae), being all of the genera *Chlorella*, followed by division Ochrophyta (n = 1) and Haptophyta (n = 1).

The peptides were produced by enzymatic hydrolysis using a single enzyme or enzymatic combinations. The enzymes were obtained from gastrointestinal tract (pepsin, trypsin and chymotrypsin), microbial origin (alcalase and thermolysin) and/or plant proteases (bromelain and papain). Trypsin has been widely used to hydrolyze proteins derived from algae (n = 6). Some authors (n = 7) reported ACEi activities after the enzymatic hydrolysis and the highest inhibition degrees were in hydrolyzed using thermolysin or bromelain.

Many different techniques can be used alone or in combination to purify the peptides (Table III). The peptides were purified from algae proteins by ultrafiltration (UF) (n = 9), Sephadex G-25 gel filtration column (n= 6), Sephadex G-15 gel filtration column (n= 5), Hydrophobic interaction chromatography (HIC) (n= 2) and/or reverse-phase high performance liquid chromatography (RP-HPLC) (n= 10).

As shown in Table III, peptide sequences derived from algae proteins are short-chained and contains aromatic amino acids in their sequence. In addition, there are correlation between low molecular weight peptides and high bioactivities. The activity of peptides is expressed in IC_{50} value, which is the peptide concentration that inhibits 50% of ACE activity.

The *in vitro* inhibition mode of ACEi peptides was evaluated by Lineweaver–Burk plots. Non-competitive (n= 5) and competitive (n= 4) inhibition were the mechanisms of inhibition of ACEi peptides. Among these, the non-competitive inhibitors have been shown to inhibit ACE at lower IC_{50} values when compared to other mechanisms of inhibition (Table III).

Several *in vitro* assays have been used for the determination of ACE inhibition. The method most used (n= 11) is described by Cushman & Cheung (1971) which quantity hip-puric acid from hippuryl-L-histidyl-L-leucine (HHL) catalyzed by ACE, followed by the method using furanacroloyl-Phe-Glu-Glu (FA-PGG) as subtract to ACE (n= 2) and, finally, by the kit of detection of 3-hydroxybutyrate (3-HB) produced from 3-hydroxybutyryl-Gly-Gly-Gly (3HB-GGG) (n= 1).

Purified peptides and protein hydrolyzed with activity antihypertensive were investigated in spontaneously hypertensive rats (SHRs) (Table IV). Most of the peptides and protein hydrolyzed from microalgae (n= 3), mainly of genus *Chlorella* and *Gracilariopsis* macroalgae (n= 1), showed antihypertensive effects *in vivo*. The doses used within the studies varied from 5 to 60 mg/kg and, in all cases, positive effects were found.

Table I. Assessment of risk of bias and quality criteria of the studies selected for the systematic review about ACEi peptides of algae.

Reference	Does the article reports the procedure to obtain bioactive compounds?	Does the article reports the description of the process of carrying out biological activity in vitro?	Does the article reports the molecular weight of peptides?"	Does the article characterizes the ACEi peptides	Does the article describes the inhibition mode of ACEi peptides?	Does the article uses different sequence of methods to identify the peptides	Does the article determines the IC50 of peptide?	Percentage of positive answers (yes) for each study that attained the risk of bias
Pan et al. (2016)	Y	Y	Y	Y	Y	Y	Y	100
Wu et al. (2016)	Y	Y	N	Y	N	Y	Y	71.4
Paiva et al. (2016)	Y	Y	N	Y	Y	Y	Y	85.7
Furuta et al. (2016)	Y	Y	N	Y	N	Y	Y	71.4
Cao et al. (2017)	Y	Y	N	Y	N	Y	Y	71.4
Joel et al. (2018)	Y	Y	N	Y	Y	Y	Y	85.7
Garcia-Vaquero et al. (2018)	Y	Y	Y	Y	N	Y	N	71.4
Deng et al. (2018)	Y	Y	Y	Y	Y	N	Y	85.7
Sun et al. (2019)	Y	Y	N	Y	N	Y	Y	71.4
Ko et al. (2012)	Y	Y	Y	Y	Y	Y	Y	100
Samarakoon et al. (2013)	Y	Y	Y	Y	N	Y	Y	85.4
Wu et al. (2014)	N	Y	N	Y	Y	Y	Y	85.4
Lin et al. (2018)	Y	Y	Y	Y	N	Y	Y	71.4
Xie et al. (2018)	Y	Y	Y	Y	Y	N	Y	71.4
Percentage of positive answers (yes) for each quality criteria (%)	100	100	50	100	50	85	92	-

Y = Yes; N = No; U = Unclear; NA = Not applicable.

Table II. Percentage of positive answers (yes) for each quality criteria.

Quality criteria	Percentage (%)
Does the article reports the procedure to obtain bioactive compounds?	100
Does the article reports the description of the process of carrying out biological activity *in vitro*	100
Does the article reports the molecular weight of peptides?	50
Does the article characterizes the ACEi peptides?	100
Does the article describes the inhibition mode of ACEi peptides?	50
Does the article uses different sequence of methods to identify the peptides?	85
Does the article determines the IC50 of peptide?	92

Table III. ACE inhibitory peptides derived from algae.

Species	Enzyme	Purification Method	Peptide	IC_{50}	Inhibition Pattern	Reference
Macroalgae						
Enteromorpha clathrata	Alcalase	Ultrafiltration (UF), sephadex G-15 gel and reverse-phase high performance liquid chromatography (RP-HPLC)	PAFG	35.9 μM	Non-competitive	Pan et al. (2016)
Bangia. fusco-purpurea	Pepsin and trypsin	Sephadex G-15 column chromatograph, RP-HPLC	ALLAGDPSVLEDR, VVGGTGPVDEWGIGAR	57.2 ± 5.0 and 66.2 ± 4.2 μg/mL	-	Wu et al. (2016)
Ulva rigida	Pepsin and bromelain	UF, sephadex G-25 size exclusion column, RP-HPLC	IP, AFL	0.020 and 0.023 mg/mL	Non-competitive, Competitive	Paiva et al. (2016)
Palmaria palmata	Thermolysin	UF, Sephadex G-25 gel fltration column, RP-HPLC	LRY	0.044 μM	-	Furuta et al. (2016)
Gracilariopsis lemaneiformis	Trypsin	Sephadex G-15 gel fltration column, Hydrophobic interaction chromatography (HIC)	QVEY	474.36 μM	-	Cao et al. (2017)
Caulerpa lentillifera	Thermolysin	UF, RP-HPLC	FDGIP, AIDPVRA	58.89 ± 0.68 and 65.76 ± 0.92μM	Competitive, Competitive	Joel et al. (2018)

Table III. Continuation.

Ulva luctua	Papain	UF, HPLC	48 differents peptides	-	-	Garcia-Vaquero et al. (2019)
Gracilariopsis lemaneiformis	Trypsin	UF	FQINMCILR, TGAPCR	9.64 ± 0.36 and 23.94 ± 0.82 μM	Non-competitive, Non-competitive	Deng et al. (2018)
Ulva intestinalis	Trypsin	UF, Sephadex G-25 gel fltration column, Sephadex G-15 gel, RP-HPLC	FGMPLDR, MELVLR	219.35 and 236.85 μM	-	Sun et al. (2019)
Microalgae						
Chlorella ellipsoidea	Alcalase	UF, Sephadex G-25 gel fltration column, RP-HPLC	VGGY	128.4 μM	Competitive	Ko et al. (2012)
Nannochloropsis oculata	Pepsin	UF, Sephadex G-25 gel fltration column, RP-HPLC	GMNNLTP, LEQ	123 and 173 μM	-	Samarakoon et al. (2013)
Isochrysis galbana	Trypsin	Sephadex G-25 column, HIC	YMGLDLK	36.1 μM	Non-competitive	Wu et al. (2014)
Chlorella sorokiniana	Protease N	Sephadex G-15 gel fltration column, HPLC	WV, VW, IW, LW	307.61, 0.58, 0.50, and 1.11 μM,	-	Lin et al. (2018)
Chlorella vulgaris	Pepsin, trypsin and chymotrypsin (*in silico*)	-	TTW, VHW	0.61 ± 0.12 and 0.91 ± 0.31 μM	Non-competitive, Non-competitive	Xie et al. (2018)

Table IV. Effects of administration of hydrolysates and peptides derived of algae in the systolic blood pressure (SBP) of spontaneously hypertensive rats.

Species	Peptide	SBP maximum reduction (mmHg)	Reference
Chlorella ellipsoidea	VEGY	- 22.8 mmHg after 4 h	Ko et al. (2012)
Chlorella vulgaris	TTWT VHW	- 35 mmHg after 2 h - 50 mmHg after 2 h	Xie et al. (2018)
Chlorella sorokiniana	-	-11.1 mmHg after 6 h -21 mmHg after 6 h	Lin et al. (2018)
Gracilariopsis lemaneiformis	TTWT VHW	- 34 mmHg after 2 h - 27 mmHg after 2 h	Deng et al (2018)

DISCUSSION

Macroalgae and microalgae are considered sources of proteins, carbohydrates, minerals, vitamins (Fitzgerald et al. 2011, Larsen et al. 2011) and can accumulate > 50% of protein in their dry matter (Becker 2007). Due to these properties, peptides with antihypertensive activity have been isolated from several algae and cyanobacteria (He et al. 2013). Among algae species, *Ulva* and *Chlorella* (Chlorophyta) are main sources of ACEi peptides (Table III). Indeed, algae of division Chlorophyta are the most promising to be exploited as sources of bioactive peptides, due to their high protein contents when compared to other divisions (García-Vaquero 2018).

Bioactive peptides are defined as peptide sequences within a protein with beneficial effects on body functions and/or with positive impacts on human health (Kitts & Weiler 2003). It is released by enzymatic hydrolysis and by fermentation process (Abdel-Hamid et al. 2017, Babini et al. 2017, Wang et al. 2013). However, the enzymatic hydrolysis method has advantages when compared to fermentation, because it is easy to scale up and generally has a shorter reaction time than microbial fermentation (Daliri et al. 2017).

Bioactive peptides released from enzymatic hydrolysis showed multi-bioactivities, including antioxidant (Jang et al. 2017), anti-inflammatory (Joshi et al. 2016) and antihypertensive (Lin et al. 2018). Different enzymes are reported to hydrolyze proteins from algae biomass and extract to produce ACEi peptides. Species of algae, enzymes, purification method, peptide, IC_{50} value and inhibition pattern can be seen in Table III.

Several studies describe the type and concentration protease to obtain specific bioactive peptides. In addition, is possible to use a single enzyme or two or more enzymes (Kim & Wijesekara 2010). Physicochemical conditions of enzymes in the process and hydrolysis conditions, such as temperature and pH, are important factors in the peptide composition and in the ACEi activity (García-Vaquero 2018).

Algae protein digested by thermolysin produce peptide with high ACEi activities. Furthermore, previous studies using other natural resources have also revealed that thermolysin are one of the most efficient proteases to produce hydrolysates with high ACEi activity (García et al. 2015, Nakashima et al. 2002, Vásquez-Villanueva et al. 2015). Indeed, these enzymes have advantages if compared to other enzymes due their high degree of specificity in the production of short chain peptides with residues of hydrophobic amino acids, such as Phe or Leu, that are important to peptides activity (Pank et al. 1982).

Bromelain is the second more used enzyme to obtain algae extract with high ACEi activity. The use of only bromelain in *Ulva rigida* algae obtained 1.4-fold higher than the use in combination with trypsin (Paiva et al. 2016). This suggest that thermolysin and bromelain has potential to be used to produce ACEi peptides derived from algae, since the hydrolysates released have high ACE inhibition when released by other enzymes.

However, the selection of the enzyme has a fundamental role in amino acids composition. The use of the same enzyme in different species of algae may cause a profile of different peptides (Table III), since red algae have low amounts of leucine and isoleucine, while brown algae often have limited methionine and cysteine (Dawczynski et al. 2007, Mišurcová et al. 2014). Therefore, the selection of the algae species is also an important factor to peptides produce with high ACEi activity.

The molecular weight of peptide influence in the ACEi activity (Pujiastuti et al. 2019). With the purpose to separate the peptides based on their molecular weight, one of the methods to fractionation is the UF, which consists to separate the peptide according to its size using membrane with different cut-off (Cao et al. 2017, Sun et al. 2019). Ko et al. (2012) reported that <5 kDa fraction from *Chlorella ellipsoidea* hydrolysates had higher ACEi activity (IC_{50} = 0.89 ± 0.04 mg mL^{-1}), while that protein digested from *Ulva rigida* in fraction < 1kDa obtained IC_{50} value of 0.095 ± 0.003 mg mL^{-1}. Similar studies using other natural sources had demonstrated the highest antihypertensive effect in fractions with low molecular weight (Ug et al. 2019, Vásquez-Villanueva et al. 2015).

To enhance bioactivity, the fractions with higher ACEi activity are purified after the UF process (Furuta et al. 2016, Paiva et al. 2017). Different chromatography methods, such gel filtration column, HIC and HPLC (Cao et al. 2017), are used alone or in combinations (Table III). Pan et al. (2016) reported a decrease on the IC_{50} value of peptides from *Enteromorpha clathrata* of 12.14 mg mL^{-1} in the extract hydrolyzed by enzymes to 0.255 mg mL^{-1} at last purification step. Samarakoon et al. (2013) reported that the fraction <5kDa of the hydrolysates of *Nannochloropsis oculata* presented an IC_{50} of 2.721 ± 0.13 mg mL^{-1} and, after the purification process, the IC_{50} value decreased to 0.090 ± 0.002 mg mL^{-1}. *C. sorokiniana* hydrolysates with IC_{50} of 0.035 ± 0.002 decrease to 0.015 mg mL^{-1} after size exclusion chromatographic purification steps and RP-HLC (Lin et al. 2018). These results emphasize that the addition of purification is an important step to provide peptides with high ACEi activity.

The amino acids profile can contribute to ACEi activity. The peptides LRY, IP and VHW showed the lowest IC_{50} values (0.044 μM, 0.020 mg mL^{-1} and 0.31 μM, respectively) (Table III). These three peptides have a hydrophobic amino acids content on C-terminal which contributes to high activity. The composition of amino acids residues from C and N-terminals of ACEi peptides has significant effects on ACE inhibition level (Lin et al. 2018), especially when the C-terminal residue is Tyr, Phe, Pro, Trp or Leu and the N-terminal residue is a hydrophobic amino of aliphatic branched-chain. In addition, LRY, IP and VHW are composed of 2 to 3 amino acids, which can also play a crucial role on ACEi activities. According to previous studies, the most of peptides are short, with 2 to 12 amino acids residues and can more easily fit into the ACE active site, improving inhibitory activity (Li et al. 2018, Wu et al. 2006).

The ACE inhibition pattern of the purified peptide derived from algae was estimated by Lineweaver – Burk plots. The peptides can have non-competitive or competitive inhibition pattern. Non-competitive inhibition pattern is defined when the inhibitor binds to a different site than the enzyme active site, changes the structural conformation and decreases the activity (Kim & Wijesekara 2010), while that in the competitive inhibition pattern, the inhibitor binds on enzyme active site, preventing the binding of substrate. Non-competitive peptides have lower IC_{50} values when compared to competitive peptides (Table III). However, there is no well knowledge between the ACEi activity and the inhibition pattern – therefore, further investigations are necessary to clarify this relationship.

The ACEi activity can be assessed by different *in vitro* methods. The method described by Cushman & Cheung (1971) is the most utilized. Other methods have been listed to overcome the lower selectivity and sensitivity (Shalaby et al. 2006). The use of FAPGG substrate has been an alternative to determination of the ACEi

activity (Henda et al. 2013, Shalaby et al. 2006). In addition, others analytical techniques can be used to measure ACE activity, as 3-HB produced from (3HB-GGG) furanocryloyl.

Bioavailability of peptides can be altered after oral administration. It is important that these peptides being resistant to the action of gastrointestinal tract enzymes to reach the bloodstream (Jao et al. 2012). Lin et al. (2018) examined the antihypertensive effects of *C. sorakiniana* hydrolyzed on SHRs at two doses, 30 and 60 mg/kg body weight, and observed a higher activity at 60 mg/kg. A single dose (10 mg/kg BW) of the VEGY peptide from *C. elipsoidea* was administered on a SHRs and resulted in a reduction of arterial pressure after 4 hours of oral administration (Ko et al. 2012). However, among the peptides evaluated by *in vivo* assays (Table IV), two peptides derived from *C. vulgaris* proteins, TTWT and VHW, had a more effective antihypertensive activity (Xie et al. 2018). In the VHW peptide, the presence of Val residues at N-terminus and aromatic amino acid at C-terminus in the short peptide chain could contribute to a higher ACE inhibition when compared to other peptides. Although significant antihypertensive effects had been obtained, there are still limited information about effects *in vivo* of peptides derived from algae. Therefore, additional studies on *in vivo* bioavailability are needed to better understand the effects of these peptides.

CONCLUSION

ACEi peptides derived from macroalgae and microalgae have potential antihypertensive. However, only a few studies with proteins of microalgae were published, indicating that more research using microalgae are necessary. The results suggest that thermolysin or bromelain enzymes are promising to produce hydrolysates with high ACEi activity. Additionally, it indicated that short peptides with hydrophobic amino acids in the C-terminal and with non-competitive inhibition pattern are more effective. Some studies using *in vivo* models showed significant antihypertensive effects. However, much more *in vivo* research is needed to understand the efficacy of ACEi peptide derived from algae.

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ANDREZA P. DE AMORIM[1]
https://orcid.org/0000-0001-7371-6278

GABRIELLY H. DA SILVA[1]
https://orcid.org/0000-0003-4564-3686

ROMERO M. P. BRANDÃO[2]
https://orcid.org/0000-0001-7045-2975

ANA LÚCIA F. PORTO[1]
https://orcid.org/0000-0001-5561-5158

RAQUEL P. BEZERRA[1]
https://orcid.org/0000-0002-1801-2945

[1]Universidade Federal Rural de Pernambuco/UFRPE, Laboratório de Tecnologia de Bioativos (LABTECBIO), Departamento de Morfologia e Fisiologia Animal, Av. Dom Manoel de Medeiros, s/n, 52171-900 Recife, PE, Brazil

[2]Universidade de Pernambuco (UPE), Instituto de Ciências Biológicas, Campus Recife, Av. Governador Agamenon Magalhães, s/n, Santo Amaro 50100-010, Recife, PE, Brazil

Correspondence to: **Raquel Pedrosa Bezerra**
E-mail: raquel.pbezerra@ufrpe.br

Author contributions

(I) Conception and design: AP de Amorim, RP Bezerra; (II) Provision of study materials: Literary data; (III) Collection and assembly of data: AP de Amorim, GH da Silva; (IV) Data analysis and interpretation: All authors; (V) Manuscript writing: All authors; (VI) Final approval of manuscript: All authors.