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Anti-Hypertensive and Anti-Microbial Activity of Protein Hydrolysate Obtained from Seven Edible Insects

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Abstract:
The bioactive peptides derived from plants and insects have increased contemplation for their function in preventing numerous diseases, including, cardiovascular diseases and microbial infection. Edible insects comprise rich contents of bioactive peptides, which are recognized as antioxidant, anti-inflammatory, anti-diabetic, and anti-obesity properties. The current investigation was aimed to assess the antihypertensive and anti-microbial properties of protein hydrolysates obtained from renowned seven edible insects' after simulated gastrointestinal enzymatic digestion. Antihypertensive efficiency was determined by the inhibition of digestive enzymes viz., ACE inhibitory activity. Three active protein hydrolysate extracts (1.25, 2.5, and 5mg/dl) were selected based on the IC_{50} values and all tested extracts inhibited those enzyme activities in a dose-respective manner. Antimicrobial activity was analyzed by the Kirby–Bauer test using nine strains of Gram-positive/negative bacteria with *Candida albicans*. Based on the present study, we found the outcome of antihypertensive and antimicrobial activityof the protein hydrolysate obtained from the seven edible insects.

Keywords: Seven Edible Insects, Functional Proteins, Antihypertensive, Anti-Microbialactivities.

INTRODUCTION

Entomophagy has often been adopted in the human race for many centuries and still wide spread to numerous nations in Asia, Africa, America, and Australia. Food and Agriculture Organization have suggested that most common edible insect species have been used for their richness of the proteins and minerals, include bamboo worms, bees, beetles, caterpillars, cicadas, crickets, dragonflies, flies, grasshoppers, housefly, locusts, mealworms, silkworm, termites, wasps, and weaver ants, (Liceaga, 2019; Van Huis, 2020). Insects are generallyrichin protein, comprising most of the essential amino acids viz., tryptophan, methionine, valine, arginine, phenylalanine, tyrosine, threonine, histidine, isoleucine, leucine, and lysine (Hall et al., 2018; Coley et al., 2020). Depending upon the insects and their various stages of the life cycle, the content of protein might vary between 50 and 75% (Matheswaran et al., 2019, 2020). Apart from the protein, insects are abundant sources of carbohydrates, lipids, vitamins, and minerals (Van Huis, 2020). The office of Food and Drug Administration proclaims hydrolysates of protein as generally recognized as safe, thus, food chemists have been practiced protein hydrolysate from insects to improve the various physiological functions in human (Greenhalgh et al., 2019; Van Huis, 2020; Ssepuuya et al., 2020).

Protein hydrolysates are the products of the proteins, which produce various sizes of polypeptides, oligopeptides, and simple amino acids upon hydrolysis (Ochiai et al., 2020). The products are normally low molecular weight peptide groups (CO=NH), and polar (CO_2-NH_4+) that augment hydrophilic nature and alters the shape of the protein architecture resulting in functional changes of the protein (Liceaga and Hall, 2019). Alkalis or acids can be employed for hydrolysis, which can breakdown the protein to peptides hydrolysates that may yield poor functionality as well as nutritional quality (Do et al., 2020). Thus, the chosen technique is enzymatic degradation, which regulates hydrolysis, permits cleavage of the appropriate peptide bond, and eventually outcomes in better functional and nutritional stuff.

Hypertension or high blood pressure is the most common chronic clinical settings globally and is a keyjeopardy component for coronary heart disease, cerebrovascular disease, Aneurysm, congestive heart failure, metabolic syndrome, and kidney disorder (Pandian et al., 2006a,b; Kumar et al., 2008; Sarker et al., 2015). Cardiovascular diseases are the foremost causes of early demise in most of the Western and Asian countries (Ganesan et al., 2018).The hypertension is primarily healed by alteration in the lifestyle and conservative drug therapy with commercial agents of antihypertensive (Ganesan and Gani, 2014; Ganesan and Xu, 2018). The synthetic angiotensin-converting enzyme (ACE) inhibitors are often clinically practiced for hypertension,however,it can cause various side effects,including dizziness, skin rashes, inflammation, angioedema,and renal failure (Arendse et al., 2019). The natural constituents of ACE inhibitory peptidesare often found in both animals and plants, which offer alternatives for the synthetic agents. For instance, regular consumption of Calpis fermented sour milk has significantly decreased blood pressure in the hypertensive subject because of the occurrence of two ACE inhibitory peptides namely VPP and IPP(Abu Hasan et al., 2017; Pujiastuti et al., 2019). Hence,functional foods aid a great potential functionin the prevention and therapy of hypertension.

The impact of antihypertensive has been identified in proteins of several plant and animal sources that have been prepared upon hydrolysis using various enzymes (Cajado-Carvalho et al., 2016; Chen et al., 2018, 2019). ACE inhibitory peptides have been obtained from various protein food sources viz., soybean (Dellafiora et al., 2020), walnut(Liu et al., 2020), wheat bran (Zou et al., 2020), bovine casein (Bueno-Gavilá et al., 2019), peach seed (Vásquez-Villanueva et al., 2019), cashew (Shu et al., 2019) and Pearl Oyster (Liu et al., 2019). Insect protein as the main occurrence of ACE inhibitorypeptides that have not been examined yet. As insects hold vast biodiversity and characterizehugein numbers, they providegreater quantities ofnovel bioactive peptides.

As the research directions, the development of drug-resistant bacterial strains has produced the various dreaded infectious diseases, and prevention or treatment would be more challenging and complex. Hence, the finding of novel antimicrobial medicine is clinically very urgent (Xu et al., 2020). The existingantibiotics have been incompetent to drive the present requirement of urgent therapy; the exploration and progress of novel antibiotics cannot kill the speed of infectious diseases caused by pathogens. Hence, investigators are giving special consideration to the study and improvement of novel antimicrobial agents (Banu et al., 2009; Banu and Kumar, 2009). Insects are a significant part of traditional medicine, which has a greater benefit in the treatment of fever, throat infections, cough, seizure, pain, tetanus, tuberculosis, rubella, and other microbial infections (Ma et al., 2019). Recently in our lab, the antioxidant, anti-diabetic, anti-inflammatory,and anti-obesity effects of seven insects were reported (Matheswaran et al., 2019, 2020). Previous pieces of the literature showed that insects' peptides have a potential antimicrobial activity(Saviane et al., 2018; Brady et al., 2019; Melo-Braga et al., 2020).Based on the stimulation of the previous literature, the present study aimed to design antihypertensive and antimicrobial activity of selected seven edible insects.

MATERIALS AND METHODS

Microorganisms and Chemicals

Ten-Gram positive and Gram-negative bacterial isolates were used in the present study.The isolates were *Bacillus pumilus* (ATCC 14884), *Bacillus cereus* (ATCC 11778),*Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 8043), *Bordetella bronchiseptica* (ATCC 4617), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 9027), *Micrococcus luteus* (ATCC 9341), *Staphylococcus epidermidis* (ATCC 6538) and*Candida albicans* (ATCC 10231).Chemicals, Mueller-Hinton broth, and agar were all obtained from Chemico Glass & Scientific Company, Erode, Tamilnadu, India. N-[3-(2-Furyl)acryloyl]- L -phenylalanyl-glycyl-glycine was acquired from Sigma-Aldrich, MO, USA. All chemicals employed for the existing study were of analytical grade.

Insects samples

Seven common edible insects' viz., Bamboo worms (Larvae), crickets (Adult), house fly (Larvae), locusts (Adult), mealworms (Larvae), silkworm (Larvae), and weaver ants (Adults) were obtained from the local market trader and used for the current investigation. Appropriate diets were provided for different insects.

Preparation of edible insects and their proteins

Seven species of insects were prepared according to our previous publication (Matheswaran et al., 2019, 2020). The individual insects' species were divided into 3 groups according to the treatment viz. raw, boiling, and baking. The protein isolates were obtained from the listed edible insects' species according to the technique of Girón-Calle et al. (2010).

Assessment of antimicrobial activity

This test aided to find antibiotic sensitivity of bacteria, which was performed disc diffusion test according to the method of Kirby–Bauer(Brown and Kothari,1975). Firstly, microbial strains were spread on the suitable culture plate containing media, and 6 discs comprising antibiotics were kept on the surface of every plate; in addition, every disc was added 10 µg of different protein isolates of seven insects. Next, the diameter of the zone of inhibition was noted after 1-2 days of culture at 37°C to detect the growth proportion. Standard antibiotic ciprofloxacin (Cadila Pharmaceuticals, India) at 4 µg/ml concentration, was used as a positive control.

Assessment of ACE Inhibitory Activity

The ACE-inhibitory activity was measured by Murray et al. (2004) using a spectrophotometric analysis contain N-[3-(2-Furyl)acryloyl]- L -phenylalanyl-glycyl-glycine (FAPGG) as substrate. Briefly, the substrate FAPGG (0.8 mM) was hydrolyzed by ACE (175 units) and kept incubation at 37°C for 1 h with EDTA (100 mM) for inactivation of ACE. The hydrolysis and degradation of FAPGG to FAP and GG were calculated by measuring the decrease in wavelength at 340 nm.

Statistical Analysis

The outcome data are Mean ± S.D. The outcomes were generally related by one-way analysis of variance (ANOVA) and the significant differences among the test means were done by Tukey's method. The variances among the mean value at a 5% level ($P < 0.05$) were measured significant statistically.

RESULTS

Determination of ACE inhibitory activity

The highest ACE inhibitory activity (%) was found in the raw insects' fraction obtained from the locusts (91.1%) at higher levels of 5mg/ml (Table 1). Likewise, the lowest ACE inhibitory activity was found in the raw fraction obtained from the mealworms (31.26%) at the concentration of 1.25mg/ml. Similarly, boiled mealworms fractions (5mg/ml) had the highest ACE inhibitory activity (89.26%); and boiled locust fractions (1.25mg/ml) had the least ACE inhibitory activity (37.11%). By the same way, baked silkworm fractions (5mg/ml) exhibited the maximum ACE inhibitory activity (89.35%); and baked locust fractions (1.25mg/ml) had the minimum ACE inhibitory activity (37.56%). Hence, the heat treatment typically facilitated a maximum ACE inhibitory activity at a higher concentration when compared with raw fractions of insects' protein (Table 1).

Assessment of antimicrobial activity

The maximum antimicrobial activity of all bacterial and fungal strains was found in the raw insects' fraction obtained from the locusts at a higher concentration of 5mg/ml (Table 2). Likewise, the lowest inhibitory activity of all bacterial and fungal strains was found in the raw fraction obtained from the mealworms at the concentration of 1.25mg/ml. Similarly, boiled mealworms fractions (5mg/ml) had the highest antimicrobial activity of all bacterial and fungal strains; and boiled locust fractions (1.25mg/ml) had the least antimicrobial activity of all bacterial and fungal strains. By the same way, baked silkworm fractions (5mg/ml) exhibited the maximum antimicrobial activity; and baked locust fractions (1.25mg/ml) had the minimum antimicrobial activity. Hence, the heat treatment, as well as raw fractions of edible insects, typically facilitated a maximum antimicrobial activity (Table 2).

Table 1: The functional proteins of seven edible insects on ACE inhibitory activity

Edible insects	Type of heat treatment	Functional protein concentration (mg/ml)	ACE inhibitory activity (%)
Bamboo Worms	raw	1.25	41.10±5.15^{f}
		2.5	53.65±5.79^{e}
		5.0	69.77±5.56^{cd}
	boiled	1.25	41.97±4.67^{f}
		2.5	55.35±5.14^{e}
		5.0	69.20±6.89^{cd}
	baked	1.25	57.77±5.31^{de}
		2.5	71.17±6.37^{c}
		5.0	85.40±7.69^{b}
Crickets	raw	1.25	45.16±4.56^{f}
		2.5	59.15±4.52^{de}
		5.0	75.20±6.15^{c}
	boiled	1.25	57.24±5.63^{de}
		2.5	71.66±6.58^{c}
		5.0	85.69±6.57^{b}
	baked	1.25	41.64±3.47^{f}
		2.5	55.18±4.18^{e}
		5.0	69.66±5.33^{cd}
Housefly	raw	1.25	51.23±5.49^{e}
		2.5	63.77±5.18^{d}
		5.0	81.33±7.78^{b}
	boiled	1.25	53.69±4.97^{e}
		2.5	67.46±6.55^{cd}
		5.0	81.90±6.75^{b}
	baked	1.25	45.76±3.28^{f}
		2.5	59.73±4.62^{de}
		5.0	73.46±6.92^{c}
Locusts	raw	1.25	56.76±5.79^{e}
		2.5	71.70±6.49^{c}
		5.0	91.10±7.99^{a}
	boiled	1.25	37.11±3.55^{fg}
		2.5	51.55±4.17^{e}
		5.0	65.22±5.65^{d}

	baked	1.25	37.56 ± 2.69^{fg}
		2.5	51.35 ± 3.68^{e}
		5.0	65.86 ± 4.55^{d}
Mealworms	raw	1.25	31.26 ± 3.51^{g}
		2.5	45.15 ± 3.34^{f}
		5.0	59.53 ± 4.65^{de}
	boiled	1.25	61.12 ± 6.22^{d}
		2.5	75.77 ± 7.16^{c}
		5.0	89.26 ± 7.69^{ab}
	baked	1.25	54.63 ± 5.36^{e}
		2.5	67.38 ± 6.30^{cd}
		5.0	81.74 ± 7.43^{b}
Silkworm	raw	1.25	54.16 ± 4.16^{e}
		2.5	67.23 ± 5.70^{cd}
		5.0	85.69 ± 6.84^{b}
	boiled	1.25	49.59 ± 4.67^{ef}
		2.5	63.55 ± 5.95^{d}
		5.0	77.58 ± 5.95^{bc}
	baked	1.25	61.16 ± 6.62^{d}
		2.5	75.22 ± 7.44^{c}
		5.0	89.35 ± 8.85^{ab}
Weaver Ants	raw	1.25	37.75 ± 3.16^{fg}
		2.5	49.75 ± 3.25^{ef}
		5.0	63.19 ± 4.62^{d}
	boiled	1.25	45.71 ± 4.49^{f}
		2.5	59.44 ± 5.95^{de}
		5.0	73.24 ± 5.45^{c}
	baked	1.25	49.19 ± 4.55^{ef}
		2.5	63.41 ± 5.26^{d}
		5.0	77.32 ± 6.94^{bc}

Means ± SD. Variable letters designate significant variance ($p < 0.05$).

Table 2: Effect of function protein isolates (5 mg/ml) from seven edible insects on the growth of bacterial isolates

Edible insects	Type of heat treatment	*B. cereus*	*B. pumilus*	*B. subtilis*	*B. bronchiseptica*	*M. luteus*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*E. faecalis*	*C. albicans*
Control	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Ciprofloxacin	-	-	-	-	-	-	-	-	-	-	-	*
Bamboo Worms	raw	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	boiled	+++	+++	+++	++++	+++	++++	+++	+++	+++	+++	+++
	baked	+	++	+	+	++	+	+	++	++	++	+
Crickets	raw	+++	++	+++	+++	++	++	++	+++	+++	+++	++
	boiled	+	++	+	+	++	+	+	++	++	++	+
	baked	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
House fly	raw	+	++	+	+	++	+	+	++	++	++	+
	boiled	+	++	+	+	++	+	+	++	++	++	+
	baked	+++	++	+++	+++	++	++	++	+++	+++	+++	++
Locusts	raw	+	+	+	++	+	+	++	+	+	++	++
	boiled	+++	+++	+++	++++	+++	++++	+++	++++	+++	+++	+++
	baked	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Mealworms	raw	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	boiled	+	++	+	+	++	+	+	++	++	++	+
	baked	+	++	+	+	++	+	+	++	++	++	+
Silkworm	raw	+	++	+	+	++	+	+	++	++	++	+
	boiled	+++	++	+++	+++	++	++	++	+++	+++	+++	++
	baked	+	++	+	+	++	+	+	++	++	++	+
Weaver Ants	raw	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	boiled	+++	++	+++	+++	++	++	++	+++	+++	+++	++
	baked	+++	++	+++	+++	++	++	++	+++	+++	+++	++

Extent of growth: ++++ no inhibition;+++ 25% inhibition; ++ 50% inhibition; + 75% inhibition; - Complete inhibition;

DISCUSSION

The underlying molecular mechanisms of blood pressure-dropping effect of food-derived peptides and hydrolysate obtained from plants, animals, or insects that perform ACE inhibitory effects. Hence, the investigation of *in vitro* ACE inhibitory activity is the most common approach in the choice of antihypertensive proteins (Pattarayingsakul et al., 2017; Liu et al., 2017). In earlier literature, severalinvestigations on ACE inhibition effects of peptides derived from various plants and animals have been welldocumented (Liu et al., 2019, 2020; Chen et al., 2020). However, the ACE inhibition effects of peptides derived from insects are inadequate(Pattarayingsakul et al., 2017; Liu et al., 2017).

In the previous investigations, the authors found novel protein hydrolysates "APPPKK" obtained from the silkworm pupae, which had a peptide inhibitory activity. The protein was attached to His^{353}, Asp^{415}, Thr^{282}, Glu^{162},Asp^{453},and H-linkage to ACE active sac (Wang et al., 2010). Similarly, another silkworm study showed the ACE inhibition effects in *in vitro* and proved the mechanisms of a hydrolysate of proteins from silkworm pupae that decreased the blood of systolic pressure on impulsively hypertensive animals (Wang et al., 2008). The present study also has potential protein hydrolysate from all those seven insects and have strong ACE inhibitory activity.

In a previous investigation of Vercruysse et al. (2005) described whole insect enzymatic hydrolysis was essential to get a noteworthy upsurge, extending the range of 5-100 fold of ACE inhibition effects. In the present investigation, the GI enzymes induced human digestion through pepsin (pH 2) followed by trypsin and or α-chymotrypsin (pH 6.5)was established the greater outcomes with 100 times increase activity. Similarly, many researchers established the noteworthy hydrolysis in GI tract, including alcalase, thermolysin, collagenase, proteinase A, etc.,that have a great impact onGI digestion ensuing the bioactive peptide for inhibition of ACE (Arihara et al., 2001; Byun and Kim, 2001; Igarashi et al., 2006; Lo et al., 2006; Majumder and Wu, 2009). In the contemporary study, the hydrolysis of the enzyme was performed at 37°C in a dark environment with the treatment of succeeding GI enzymes viz., α-amylase, pepsin, pancreatin, and bile, which yield a strong protein hydrolysate that inhibits the enzyme activation of ACE.

Fascinatingly, insect extracts hydrolysis caused a negligible development of the ACE inhibition, as the value of IC50 ranged between 0.4-0.7 mg/ml. It is in great difference to numerousearlier publications (Arihara et al., 2001; Byun and Kim, 2001; Igarashi et al., 2006; Majumder and Wu, 2009) that established that food protein hydrolysis especially enzymatic degradation is a vital stage for attaining ACE inhibition. In the present study, after enzymatic hydrolysis, the activity of ACE inhibition was achieved employing as the IC50 values ranged between 1.25-5 mg/ml.

Many functional compounds have been isolated from insects, including, chitin, polyphenols, antioxidant enzymes, and antimicrobial peptides (Zielińska et al., 2018). In global research, insects produce about 280 antimicrobial peptides which have been identified and characterized earlier(Yi et al., 2014). They have varied configurations, and however, most antimicrobial peptides have general features including, short-chain amino acids as well as positively charged aminoacids (Li et al., 2016). Antimicrobial peptides are also identified as potential substances that have possible broad-spectrum antimicrobial activity on various Gram-negative and positive bacteria, yeasts and molds, and lipid-coated viruses (Hashemi et al., 2017). Furthermore, they revive the activity against various antibiotic-resistant bacterial strains and did not freely provoke resistance (Brogden and Brogden, 2011). Several antimicrobial peptides and immune reactivecompoundshave been extracted from houseflies (Dang et al., 2011; Fu et al., 2009; Guo et al., 2017). In addition, a number of antimicrobial peptides genetic factors have also been found in the chromosome of the housefly (Scott et al., 2014). In the present study, we found that protein isolates from seven insects have potential antibacterial activity against diseases causing gram-positive/negative bacteria. Similarly, those protein isolates have a strong antifungal activity against *C.abicans* based on the variable dose of the compounds (1.25-5 mg/ml). These outcomes recommend that insects proteins can be consumed as food preservers as it has strong antimicrobial activities.

CONCLUSION

Based on the current study, we accomplishedthat the seven edible insects and their protein hydrolysates have potential ACE enzyme inhibitory activities and thusevidenced as an antihypertensive activity. These outcomes recommend the function for insect protein as an antihypertensive and antimicrobial constituent which is known to be as significant functional foods and nutraceuticals for the food industry. For further consideration, more detailed research is required to characterize the protein hydrolysate, which has exact molecular mechanisms involved in the antihypertensive and antimicrobial activities.

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