Prosiding Novel Antihypertension

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Novel Antihypertension Bioactive Compounds from Rusip

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Abstract. Bioactive compounds from *rusip* has function as a antihypertension which inhibit Angiotensin Converting Enzyme (ACE). They was obtained by fractionation methode. Fractionation by molecular weight of *rusip* was to separate its bioactive compounds. Steps taken for this research was extraction and fractionation of *rusip,* assayed their ACE inhibition and peptide profile analysis. The results showed that there were four fractions from *rusip* extract, i.e F0 (free supernatant), F1 (Molecular wight fraction > 10 kD), F2 (Molecular wight fraction 1-10 kD), and F3 (Molecular wight fraction > 1 kD) . There are three similar peptides in the Fo and F1 rusip fraction, i.e 44.78 kD, 25.90 kD and 17.18 kD, however there were no peptide in the F2 and F3 fractions. The ACE inhibitor activity of rusip fraction range for 34.40% to 97.52%, where F2 Fraction had highest ACE inhibitor activity. This research revealed those bioactive compounds which have highest activity of ACE inhibition from *rusip* was low molecular weight (under 10 kD) peptides or other biaoctives compounds.

1. Introduction

Hypertension is one of the degenerative desease, where caused by constriction of blood vessels. There cause increasing hearth activity to the movement of blood through the vessels and it cause high blood pressure or hypertension. Hypertension can be caused by some factore, i.e. genetics, obesity, stress and unhealthy eating patterns. In addition, in abnormal conditions Angiotensin Converting Enzym (ACE) can trigger a rise in blood pressure[1].

Many studies have done to reduce hypertension, one of them by consuming functional food. Some functional foods can reduce blood pressure, they are yogurt and whey kefir[2,1], eggwhite fermented[3] and fish Fermented products such as heshiko[4] and narezushi[5]. They have antihypertensive activity caused by their ability to inhibit Angiotensin Converting Enzym (ACE) activity[6,7].

Lactic acid bacteria during the fermentation process are known to produce bioactive compounds which have a role in inhibiting ACE enzyme activity[8] and HMG-CoA reductase inhibitor[9]. Rusip as one of the fish fermented product from Indonesia is known o be a potential functional food as a antihypertensive, but the bioactive compounds that act as antihypertensive agents from rusip are unknow. Therefore this study examines the presence of bioactive compounds that function as antihypertensive agents from rusip.

2. Materials and methods

2.1. Materials

Rusip was obtained from Bangka traditional market, Bangka-Belitung Island-Indonesia. Angiostensin Converting Enzyme (ACE) were purchased from Sigma Aldrich (USA) and a standard

molecular weight protein marker (Low Range Protein Ladder) were purchased from Thermo Scientific (Lithuania). All other chemicals were analytical grade and purchased from the local representative of Sigma and Merck.

2.2. Extraction of rusip

Extract of *rusip* was prepared according to the following method which used by Rinto *et al.* 2015[9].

2.3. Fractionation of rusip extract

The purpose of fractionation was to separate bioactive peptides from other compounds in the *rusip* extract. Fractionation was based on the molecule size using filtration membrane and the following method Rinto, *et al*. 2017[10].

2.4. Profile peptides assay of rusip extract fractions

The peptide profile was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[9].

2.5. ACE inhibition assay

Determination of ACE inhibitor activity was carried out according to the Wikandari and Leny, 2016[8].

3. Results and discussion

3.1. Rendement of rusip extracts

Rusip from Bangka-Belitung Island was extracted by aquabides, its caused the most bioactive compounds in rusip are hidrophylic. The yield of extract rusip was 60%. This shows that bioactives compound from rusip can extracted by aquabides and this methode can produce more than 50% extracts. Rusip extract contain some bioactive compounds, i.e: peptides, amino acid, and organic compounds[11]. Fractionation of the rusip extract that based on molecule weigth showed that the most of yield rusip fraction was rusip fraction with molecule weight more than 10 kD and least of all was rusip fraction with molecule weight under 1 kD. This showes that the bioactive compounds in rusip extract dominated by bioactive compounds with molecule weight more than 10 kD. Nevertheless, the most yield (rendement) does not show higher inhibition activity for Angiostensin Converting Enzyme (Table 1).

Table 1. The yield of rusip fraction, peptides and inhibition of rusip extract fraction for ACE enzyme activity

No.	Sample	Yield (%)	Peptides Content (%)	Peptides (kD)	Inhibition of ACE (%)
1.	F0 (extract free supernatant)	60.00	6.49	17.18 25.90 44.78	50,70
2.	F1 (MW > 10 kD)	14.50	4.57	17.18 25.90 44.78	34,40
3.	F2 (MW 1-10 kD)	13.40	4.34	ND	97,52
4.	F3 (MW < 1 kD)	12.00	5.88	ND	62,77

Note : ND = Not Detected

3.2. The content of peptide in the rusip fraction

Peptides are one of the bioactive compounds in rusip extract. The rusip fermentation process at several monts caused the change of proteins to peptides or amino acids. Rusip extraxts contained

6.49% peptides and the highest content of peptide in rusip have molecular weight under 1.000 D or under 1 kD (Table 1). The fermentation process at several mounts caused continous change of protein to be low molecular weigth peptides. This result is different from the content of peptide in bekasam that highest content of peptide in bekasam have molecular weight more than 10 kD[9]. The fermentation process of bekasam only 7 days, it is shorter than rusip[11], with the result that protein and peptide aren't further degradation.

3.3. Peptides profile of rusip fraction

The presence of peptides in rusip extract can detected from band peptides by SDS-PAGE. Band peptide from SDS-PAGE showed that the presence peptides from rusip extract in range 17.18 kDa - 44.78 kDa (Figure 1 and Table 1). There is a similarity of peptides in rucip extract (F0) and rusip fraction with molecular weight more than 10 kDa (F1). There are 3 peptides, i.e. 44.78 kDa, 25.90 kDa and 17.18 kDa. But in the rusip fraction with molecuar weight 1-10 kD (F2) and under 1 kD (F3) there were no peptide band (Not Detected). There is show that rusip fraction (F2) and (F3) contain peptides 4.34% and 5.88%, respectively (Tabel 1), but their molecular weight are under 5 kD so they are no detected (Fig. 1).

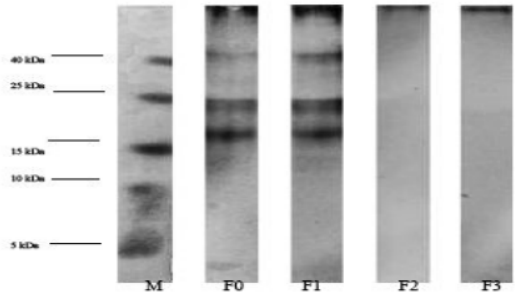

Figure 1. Peptide profile of rusip fraction.

Marker (M), (F0: extract free supernatant; F1: fraction of *rusip* extract with molecule weight > 10 kD; F2: fraction of *rusip* extract with molecule weight 1-10 kD; F3: fraction of *rusip* extract with molecule weight < 1 kD).

3.4. ACE inhibition activity

Angiotensin converting enzyme (ACE) inhibition activity of each fraction for rusip extract resulted different inhibition, which range for 34.4 to 97.57 (Figure 2). The fractionation will separate the bioactive peptides in rusip into fractions extract free supernatant (F0), fractions > 10 kDa (F1), fractions 1-10 kDa (F2) and fractions <1 (F3). The results showed that the highest of inhibited of ACE enzyme activity from rusip extract was fraction 1-10 kDa (F2). The average value of ACE inhibitory (ACEI) activity from fractionated samples were be higher compared then not fractionated sample (extract free supernatant). The highest ACEI value was second fraction with ACEI values 97.52%. This shows that the ACEI activity of peptide from *rusip* fraction will be higher if working individually (Fig. 2).

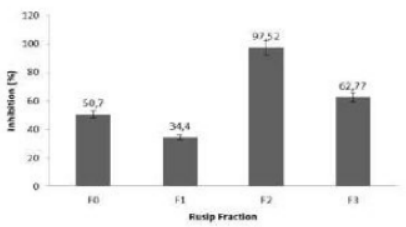

Figure 2. ACE Inhibition activity of *rusip* fraction

F0: extract free supernatant; F1: fraction of *rusip* extract with molecule weight > 10 kD; F2: fraction of *rusip* extract with molecule weight 1-10 kD; F3: fraction of *rusip* extract with molecule weight < 1 kD)

4. Conclusion

Rusip is a fermented fish product that has the potential to be developed as a source for antihypertensive bioactive compounds. Fractionation of rusip extract produced fractions 1-10 kD which had the highest antihypertensive ability. Detection of the type of bioactive compound in rusip as an antihypertensive agent shows that the bioactive compound from rusip fraction that has the highest inhibitory ability was not peptide.

Acknowledgement

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