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Solvent selection for extraction of bioactive compound from *bekasam* with antioxidant and anticholesterol activities

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Solvent selection for extraction of bioactive compound from *bekasam* with antioxidant and anticholesterol activities

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Abstract. Heart disease and hypertension are two of the main causes of human mortality worldwide. Both can be caused by the oxidation of cholesterol, causing plaque and narrow blood vessels. The purpose of this research was to determine the solvent for the extraction of antioxidant and anticholesterol bioactive compounds from *bekasam* extracts. *Bekasam* was extracted by polar solvents, i.e., aquabides, methyl alcohol (methanol), and ethyl alcohol (ethanol). The parameters observed in this research were the yield of crude extract, antioxidant assay, lovastatin assay, and peptide profile analysis (SDS-PAGE). The result of this study indicated that the yields of crude extract of *bekasam* using aquabides, methanol, and ethanol were 17.00%, 9.00%, and 2.83%, respectively. The antioxidant activities of bekasam extracts produced using methanol, ethanol, and aquabidest were 69.09%, 64.09%, and 53.43%, respectively. Lovastatin contents of *bekasam* extracts produced using ethanol, aquabides, and methanol were 442.53 ppm, 168.67 ppm, and 158.19 ppm, respectively. Peptides were detected on *bekasam* extract by aquabidest in the range of 5.60-29.26 kD but undetected when extracted using the other solvents.

Keywords: Bekasam, bioactive compound, solvent

1. Introduction

Bekasam [1] and *rusip* [2] are fermented fish products from Indonesia that contain bioactive compounds. They are potentially functional food for inhibiting degenerative diseases (hypertension and hypercholesterolemia). Lovastatin and peptide are both anticholesterol bioactive compounds from *bekasam* [3]; a bioactive peptide from *bekasam* functions as a hypertension reducer [4]. Lovastatin is one of the statin drugs used to decrease cholesterol levels in the blood by inhibiting cholesterol biosynthesis. Besides, bioactive peptides from *bekasam* function as cholesterol reducers like heshiko and narezushi, the Japanese fermented fish products [5,6].

Some bioactive peptides function as an antioxidant. An antioxidant inhibits the oxidation of other molecules in a living organism. Peptides extracted from ngari (fermented fish from India) can prevent degenerative diseases that block free radicals compounds [7]. Ngari is one of a fermented fish product like *bekasam* from Indonesia, so *bekasam* potentially contains bioactive antioxidant compounds.

The presence of anticholesterol and antioxidant compounds from *bekasam* can be detected with extraction methods. Lovastatin and bioactive peptide from *bekasam* that function as an anticholesterol can be extracted using aquabides [8]. Aquabides is one of the polar solvents; lovastatin and bioactive peptides can be extracted with other polar solvents, e.i. Ethyl alcohol and methyl alcohol [9,10]. We

studied the utilization of polar solvents (aquabides, ethyl alcohol, and methyl alcohol) to extract anticholesterol and antioxidants from *bekasam*.

2. Materials and methods

2.1 The production of bekasam with Lactobacillus acidophillus as a starter culture
Minnows fish (*Rasbora argyrotenia*), the local name is seluang fish was used to manufacture *bekasam*. A total of 1 kg of minnows fish was gutted and washed. After that, it was soaked in a starter culture of *Lactobacillus acidophilus* (1 L). It was soaked in the starter culture for 30 minutes in cold conditions. Then, seluang fish were separated from *L. acidophilus* starter culture. The fish was added some salt and rice as much as 15% respectively and fermented until seven days to become *bekasam* (fig.1).

Figure 1. Bekasam from Minnows fish (*Rasbora argyrotenia*).

2.2 Extraction of bekasam
Bekasam was extracted by three types of polar solvents, i.e., aquabides (H_2O), ethyl alcohol (C_2H_5OH), and methyl alcohol (CH_3OH) with a single maceration extraction method. Extract of *bekasam* was prepared according to a method described by [8]. Bekasam (10 g) was homogenized with 40 mL of distilled water, and then the homogenate was centrifuged at 2,000 *x* g, 4°C for 15 minutes. After that, the first supernatant was separated and the residue was added with 50 mL of distilled water to get second supernatant in the same way. The two supernatants (first and second) were mixed and filtered through a 0.45 m membrane (Biotechlab, Bulgaria).

2.3 Parameters assay
The filtrate was used to analyze yield extract, lovastatin, antioxidant, and peptide profile. The yield of *bekasam* extract can be calculated by the ratio between extract (w) with sample/*bekasam* (w). Lovastatin content was determined by spectrophotometer (UV-Mini-1240, Shimadzu) according to the following method [8]. Antioxidant Activity was estimated by ABTS assay, according to [11] and peptide profile according to [8].

2.4 Statistical analysis
Data were presented as the mean of three measurement for yield, lovastatin content and antioxidant activity. The significance of differences among samples were determined by Analysis of variance (ANOVA) used Minitab 16. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1 Rendement of bekasam extract
The yield of bekasam extract is a comparison between the yield of extract and sample (bekasam). There is an identification of solvent's effectiveness in bioactive compounds extraction from *bekasam*. The averages of yield from three types of solvent are provided in Table 1.

Table 1. The average of rendement, lovastatin content, and antioxidant activity of *bekasam* extract.

Solvent	Yield (%)*	Lovastatin Content (ppm)*	Antioxidant Activity (%)
Aquabides	17.00 c	168.67 a	53.43
Methanol	09.00 b	158.19 a	69.09
Ethanol	02.83 a	442.53 b	64.09

*Result are means of three experiments, different superscript letters have significant differences

The yield of *bekasam* extract produced by aquabides (17%) was significantly higher than those produced by methanol (9%) and ethanol (2.83%). Such differences were due to difference in polarity of the solvents. [12] revealed that polarity could be determined by the dielectric constant where aquabidest (80.4) was more than methanol (33.1) and ethanol (24.3).

3.2 Lovastatin content as a anticholesterol bioactive compound

Lovastatin is one inhibitor of HMG-CoA reductase, which decrease cholesterol in plasma. Lovastatin content in the *bekasam* extract ranged between 158.19 – 442.53 ppm (Table 1). Statistical analysis confirmed that extraction by different solvents (aquabides, methanol, and ethanol) significantly increased lovastatin content in bekasam extract, whereas lovastatin in bekasam extract by ethanol more than methanol and aquabides. Lovastatin had two pools, i.e., polar (hydroxyl) and non-polar (benzene ring), so lovastatin better binds with ethanol than methanol and aquabides, respectifely where [13].

3.3 Antioxidant activity

An antioxidant is a natural substance that may prevent or delay some cell damage [14]. Antioxidant bioactive compounds in natural substances can be known by conducting antioxidant assay. Antioxidant assay by DPPH method for *bekasam* extract indicated that antioxidant activity of extract ranged from 53.43 to 69.09, where the antioxidant activity of bekasam extract by methanol was higher than the extract produced by aquabides and ethanol, respectively. Methanol is a better solvent than aquabides and ethanol for antioxidant extraction from *bekasam*.

3.4 Profile of peptide

Peptide is bonding of amino acids by covalent bond. Molecule weight of peptides from *bekasam* extract range from 5.60 kD to 29.26 kD (Fig. 1). Extract *bekasam* produced by aquabide solvent had four (4) peptides, i.e., 5.60 kD; 9.72 kD; 14.51 kD and 29.26 kD, but there was no peptide in the extract produced by ethanol and methanol.

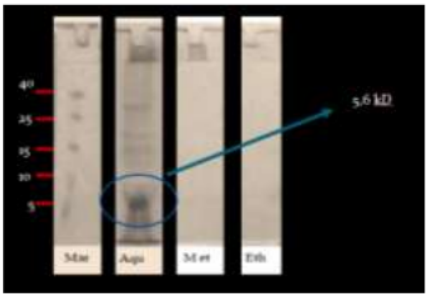

Figure 2. Peptide profile of *bekasam* extract by different solvents (Mar: marker; Aqu: Aquabides; Met: methyl alcohol; Eth: ethyl alcohol)

Extraction of *bekasam* by aquabides as a solvent had four (4) peptides which their weight molecule i.e 5.60 kD; 9.72 kD; 14.51 kD, and 29.26 kD, but extraction by methyl alcohol and ethyl alcohol did not result in the peptide (Fig. 1). Differences in the results were caused by methyl alcohol and ethyl alcohol that promote coagulation of protein or peptide, so they sedimented in the primary tubes when being extracted (no extract).

4. Conclusions

Bekasam was extracted by methyl alcohol resulted higher lovastatin content. *Bekasam* extract had low antioxidant activity. Peptide was not detected in the extract produced using ethanol and methanol, so that antioxidant from *bekasam* does not contain any peptide.

5. Acknowledgements

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