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PENGARUH EKSTRAKSI DENGAN PELARUT PADA TOTAL FAVONOID BUAH ADALIMAN (*Zanthoxylum acanthopodium* DC)

[Effect of extraction solvent on total flavonoid content of andaliman fruit (Zanthoxylum acanthopodium DC)]

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ABSTRACT

Hypertension is a disease where blood pressure increases chronically beyond normal limits. Hypertension needs to be watched out because it includes asymptomatic diseases and can cause complications. One alternative treatment for hypertension that has been proven effective in the pharmaceutical industry is the use of ACE-I (Angiotensin-I Converting Enzyme Inhibitors). At present, ACE-I synthesis has been produced commercially as an antihypertensive drug, for example captopril, enalapril, alcacepril, and lisinopril. However, there are many studies that show the existence of ACE-I synthesis side effects for its users, both physiological and non-physiological effects such as coughing, angioedema, and disorders in pregnancy. ACE-I obtained naturally is safer than ACE-I synthesis. Research on ACE-I activity from natural ingredients is still very limited. Andaliman (Zanthoxylum acanthopodium DC) or better known as Batak Pepper has the potential as a natural source of ACE-I. Natural ACE-I in Andaliman can be obtained from the flavonoid content of Andaliman fruit extract. The purpose of this study was to determine total flavonoid content in andaliman fruit by comparing the types of solvents used for extraction. The solvents used were N-hexane, ethyl acetate, ethanol, and water. The extraction results were then compared using a completely randomized design (CRD) to determine the success of this study.

Keywords: *Antihypertensive, flavonoids, flavonoid, Zanthoxylum acanthopodium DC*

ABSTRAK

Hipertensi adalah penyakit di mana tekanan darah meningkat secara kronis di luar batas normal. Hipertensi perlu diwaspadai karena termasuk penyakit tanpa gejala dan dapat menyebabkan komplikasi. Salah satu pengobatan alternatif untuk hipertensi yang telah terbukti efektif dalam industri farmasi adalah penggunaan ACE-I (Angiotensin-I Converting Enzyme Inhibitors). Saat ini, sintesis ACE-I telah diproduksi secara komersial sebagai obat antihipertensi, misalnya captopril, enalapril, alcacepril, dan lisinopril. Namun, ada banyak penelitian yang menunjukkan adanya efek samping sintesis ACE-I bagi penggunanya, baik efek fisiologis dan non-fisiologis seperti batuk, angioedema, dan gangguan pada kehamilan. ACE-I yang diperoleh secara alami lebih aman daripada sintesis ACE-I. Penelitian tentang aktivitas ACE-I dari bahan-bahan alami masih sangat terbatas. Andaliman (Zanthoxylum acanthopodium DC) atau lebih dikenal dengan sebutan Batak Lada berpotensi sebagai sumber alami ACE-I. ACE-I alami di Andaliman dapat diperoleh dari kandungan flavonoid dari ekstrak buah Andaliman. Tujuan dari penelitian ini adalah untuk menentukan total flavonoid dalam buah andaliman dengan membandingkan jenis pelarut yang digunakan untuk ekstraksi. Pelarut yang digunakan adalah N-heksana, etil asetat, etanol, dan air. Hasil ekstraksi kemudian dibandingkan dengan menggunakan desain acak lengkap (CRD) untuk menentukan keberhasilan penelitian ini.

Kata kunci: Antihipertensi, flavonoid, flavonoid, Zanthoxylum acanthopodium DC

INTRODUCTION

Indonesia has a high prevalence of hypertension, about 25.8% people of ≥18 years suffer from hypertension.This value does not include 63.2% undiagnosed hypertension cases in Indonesia (Riskesdas, 2013). Therefore, it could be concluded that hypertension is a serious health problem in Indonesia. Generally, hypertension treatment is carried out by taking antihypertensive drugs

such as captopril, enalapril, alcacepril and lisinopril which are synthetic ACE-inhibitors. The mechanism of ACE-inhibitors in preventing hypertension is to inhibit the convertion of angiotensin I to angiotensin II that caused the vasoconstriction of arteries. However, there have been many studies that show the side effects of synthetic ACE-inhibitor drugs on patients, such as cough, angioedema, and pregnancy problem (Düsing, 2016). Further research is needed to find a safer alternative ACE-I from natural resources.

Meanwhile, Indonesia has a local plant known as andaliman or merica batak. Andaliman (*Zanthoxylum acanthopodium*) is a wild spice from North Sumatra and is a part of Rutaceae. It is mostly used in meat dishes because local people believes that andaliman could reduce blood cholesterol and prevent hypertension. However, there hasn't any studies or research that prove andaliman as antihypertension. The studies about andaliman are mostly focused on the antibacterial activity as in a study conducted by Muzafri et al. (2018) regarding the use of andaliman as a natural preservative in *Pangasius sutchi* fish fillets. Andaliman contains flavonoid, especially quercetin that have ACE-I activity> 30% (Guerrero et al., 2012). Hence, this study aims to examine andaliman potential as a natural ACE-inhibitor that could be used as ingredient of antihypertension drug or supplement.

The activity of ACE-I from flavonoids is caused by the presence of 2-3 unsaturated bonds which are conjugated with 4 groups = O. The Zanthoxylum genus contains flavonoids which are mostly flavonoons, flavonols, and flavonoids (Patino et al., 2008). These compounds have been shown to act as ACE-I which can inhibit ACE activity by up to 50%. Andaliman also has quercetin flavonoids which have ACE-I activity> 30% (Guerrero et al., 2012).

MATERIALS AND METHODS

Materials

Fresh andaliman fruit (Sidikalang, North Sumatra), n-hexane, ethylacetate, ethanol, mineral water, methanol, quercetin. All other reagents were analytical grades.

Preparation of Andaliman Fruits

Andaliman is washed using tap water and dried overnight for approximately 18 hours. Then Andaliman is dried using an cabinet dryer at 40^{o}C. The next step is andaliman ground using a blender and was filtered using a 60 mesh filter to obtain a homogeneous andaliman powder.

Extraction of Andaliman

Andaliman extraction uses the maceration method with the addition of different solvents. The solvents used were n-hexane, ethylacetate, ethanol, and water. Andaliman solvent and powder ratio is four to one. Maseration is done for 4 hours then filtered twice.

Total flavonoid content

The total flavonoid content (TFC) of the extract was determined by the aluminium chloride colorimetric method (Baba and Malik, 2014) with modifications. The quercetin solutions used are 15 ppm, 20 ppm, 25 ppm, 30 ppm and 35 ppm. The wavelength used is the maximum wavelength of quercetin solution which is 435 nm. 1 ml of standard quercetin or extract solution was put into a 10 ml volumetric flask containing 4 ml of distilled water. 0.3 ml of $NaNO_2$ solution was added and then allowed to stand for 5 minutes. Next 0.3 ml of AlCl3 solution was added and left for 4 minutes. 2 ml of 1 M NaOH solution was added and the volume was made into 10 ml with distilled water. The absorbance value was recorded at 370 nm wavelength using a UV-VIS spectrophotometer. The analysis was done in triplicate. The total flavonoid content was reported as total quercetin equivalent per g sample (mg QE/g).

RESULTS AND DISCUSSIONS

Sample Preparation

Dry samples were obtained as much as 177 g. The dried sample was mashed using a blender and filtered with a 60 mesh filter to obtain a powder sample of 42.26 g.

Extraction of Andaliman

Andaliman fruit extraction in this study used maceration method. According to Sie (2013) the content of antioxidants is the content of compounds that are not heat resistant so that the maceration method which is cold extraction will be more optimal in extracting antioxidant compounds. The solvents used in this study were n-hexane, ethyl acetate, ethanol, and water. The purpose of using four solvents with different polarity is to find out the yield and get the active compounds from andaliman fruit based on their level of polarity. Extraction using solvents with different polarity will produce different components of flavonoids so that the antioxidant properties possessed by each compound obtained from the extraction are also different (Pambayun et al., 2007). The n-hexane extract has a yield of %, ethyl acetate extract has a yield of 2,962%, ethanol extract has a yield of 3,329%, and water extract has a yield of 0,746%. This result is in accordance as stated by Priyanto (2010) that the extract yields of maceration with different solvents will produce different yields.

Determination of Total Flavonoid Content

Determination of flavonoid levels using Chang's method in 2002 and as a comparison used quercetin standard. Then the wavelength optimization is carried out to determine the maximum λ that will be used in measurements on UV-Vis spectrophotometry. The measurement results obtained by the maximum wavelength is 370 nm.

The measurement results of absorbance of the standard quercetin solution at several concentrations (ppm), namely 15, 20, 25, 30 and 35 obtained a linear relationship between absorbance and concentration which is equal to 0.650. From the calculation results, the intercept value is 0.0034 and the slope value is 0.032 so that the standard curve equation is y = 0.0038x - 0.0352. The equation was used as a comparison in the quantitative analysis on the measurement of the content of quercetin flavonoids on n-hexane, ethyl acetate, ethanol, and water extracts in andaliman fruit.

Table 1. The results of the standard absorbance measurements of quercetin

Concentration (mg/l)	Absorbance 435 nm
15	0.024
20	0.034
25	0.067
30	0.084
35	0.095

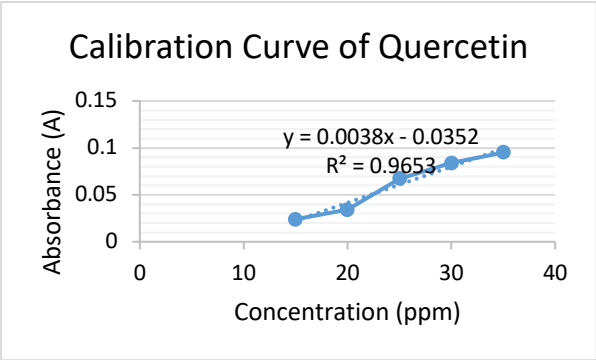

Figure 1. Calibration curve of quercetin concentration at 435 nm.

Table 2. Total flavonoid content of extracts.

Extract	Absorbance (A)	Total flavonoid content (mg QE/g)
n-heksana	0,487	137,421
Etil asetat	0,783	215,316
Etanol	0,806	221,368

Total flavonoids in andaliman fruit extract were obtained by entering the absorbance value on the standard quercetin curve so that the results of the total amount of total flavonoid hexane extract were equal to 137,421 mg/L, ethyl acetate extract namely 215,316 mg/L, and ethanol extract namely 221,368 mg/L.

The result of this study showed that ethanol extract from Andalusia gave the highest total flavonoid content (TFC). This present study supports the amount of flavonoids that are dependent on the type of plant parts and solvents used for extraction.

CONCLUSION

Based on the results of the research that has

been done, it can be concluded that the best solvent to extraxt flavonoid from andaliman fruit is ethanol which total amount of flavonoid that can be extracted is 221,368 mg/L calculated against or as quercetin.

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