Isolation of Antioxidant Compounds from Ethanol Extract of Temu Kunci (*Boesenbergia pandurata* Roxb.) Rhizomes

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Abstract

Temu kunci (*Beosenbergia pandurata* Roxb.) is traditionally used to treat various diseases, and antioxidants are one of their utilization. The aim of this study was to isolate and identify the antioxidant compounds of *temu kunci* rhizomes. *Temu kunci* rhizomes was extracted by maceration with ethanol 95%. Etanol extract was then fractionated by liquid-liquid extraction, vacuum liquid chromatography, and classical column chromatography. Monitoring and testing the antioxidant activity used thin-layer chromatography (TLC) with 0.2% DPPH (1,1-diphenyl-2-picrylhydrazyl) reagent. Purity test was performed by a single development TLC using three different kinds of mobile system and two-dimensional TLC. Isolate BP-1 was isolated from the ethanol extract and active as an antioxidant. Based on ultraviolet-visible and infrared spectrums, isolate BP-1 was identified as flavanone in the absence of hydroxyl groups at the ortho position (o-diOH), with substitution of -OH at C5 and C7.

Keywords: *Temu kunci*, *Boesenbergia pandurata*, DPPH, Antioxidant.

Abstrak

Temu kunci (*Beosenbergia pandurata* Roxb.) secara tradisional digunakan untuk mengobati berbagai macam penyakit, salah satunya adalah sebagai antioksidan. Tujuan dari penelitian ini adalah untuk mengisolasi dan mengidentifikasi komponen antioksidan yang dimiliki temu kunci. Rimpang temu kunci diekstraksi dengan cara maserasi menggunakan etanol 95%. Ekstrak etanol kemudian difraksinasi dengan ekstraksi cair-cair, kromatografi cair vakum, dan kromatografi kolom klasik. Aktivitas antioksidan kemudian dipantau dan diuji menggunakan kromatografi lapis tipis (KLT) menggunakan reagen 0,2% DPPH (1,1-diphenyl-2-picrylhydrazyl). Uji kemurnian dilakukan dengan pengembangan tunggal KLT menggunakan 3 macam fase gerak yang berbeda dan KLT dua-dimensi. Isolat BP-1 merupakan hasil isolasi dari ekstrak etanol dan aktif sebagai antioksidan. Berdasarkan spektrum UV-Vis dan inframerah, isolat BP-1 teridentifikasi sebagai flavanone karena adanya gugus hidroksil yang hilang pada posisi orto (o-diOH), dengan substitusi –OH pada C5 dan C7.

Kata kunci : Temu kunci, *Boesenbergia pandurata*, DPPH, antioksodan.

Introduction

An amount of evidence showed that free radical-mediated damage plays an important role in several human diseases such as cancer and cardiovascular diseases. Free radical which continuously generated during normal metabolism eventually triggers the onset of degenerative diseases. Exogenous antioxidants derived from dietary components sometimes required preventing this condition. The study about isolation and activity testing of plant-origin antioxidants was significantly increased in recent years. Potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, barks, roots and crude plant drugs.

Temu kunci (*Boesenbergia pandurata* Roxb.) rhizomes is one of Zingiberaceae family which used to treat some diseases such as stomach disorders, reproductive infections, kidney stones, or laxative (Subarnas 2001). *In vitro* test showed that *Temu kunci* (*Boesenbergia pandurata* Roxb.) rhizomes could increase the number of lymphocytes, specific antibodies, and kill cancer cells (Fahey and Stephenson 2002). *Boesenbergia pandurata* Roxb. which has been widely used as a medicinal plant, was reported to possess significant anti-oxidative properties (Shindo *et al.* 2006; Seal 2011). *Temu kunci* rhizomes contain essential oil component such as metylcinnamate, champhor, cineole (Norajit *et al.* 2007). *Temu kunci* rhizomes also contain saponin and flavonoid such as pinostrobin, pinocembrin, alpinetin, cardamonin, and panduratin (Chairul and Harapini 1996; Win *et al.* 2008; Ching *et al.* 2007). Phenolic compounds such as flavonoid which contain hydroxyls, are responsible for the radical scavenging effect in the plants (Seal 2011; Ren *et al.* 2003).

According to its benefits, especially in the treatment of various degenerative diseases, it is necessary to conduct further study to find antioxidant component of *temu kunci* rhizomes. In this study, an activity-guided purification was conducted to isolate the free

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radical scavenging components from *Temu kunci* rhizomes.

Materials & Methods

Plant material

Rhizomes of *Boesenbergia pandurata* Roxb. were collected from Bandung, Indonesia. The specimens were identified at Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology. The rhizomes were cut into slices and dried at temperature ± 42˚C on the drying cabinet. Dried sample was grind in a comminution mill.

Extraction and Fractionation of Rhizomes

Dried powder (900 g) of *Boesenbergia pandurata* Roxb. rhizomes was extracted by maceration method using ethanol 96% for 3 x 24 hours (1000 mL each). The extract was filtered through Whatman filter paper and the filtrate was concentrated by rotavapor. Total extracts was 95.98 g. Ethanol extract was partitioned by liquid-liquid extraction using n-hexane, ethyl acetate, and ethanol. N-hexane fraction was fractionated by vacuum liquid chromatography, using silica gel 60H as stationary phase and n-hexane-ethyl acetate mixture as mobile phase with gradient elution.

Antioxidant activity test

Isolation of antioxidant compound was guided by antioxidant activity. Antioxidant component of *Boesenbergia pandurata* Roxb. was determined qualitatively by TLC using silica gel GF 254 as stationary phase and n-hexane-ethyl acetate (9:1) as mobile phase with DPPH (1,1-diphenyl-2-picrylhydrazyl) as visualizing agent.

Purification

Purification was performed by column chromatography using silica gel 60 as stationary phase and n-hexane-ethyl acetate (9:1) as mobile phase. Purified isolate was obtained by washing the isolate by n-hexane. Purity test performed by a single development TLC using three different kinds of mobile phases and two-dimensional TLC.

Characterization and Identification of Isolate

Isolate was characterized and identified by UV-Vis Spectrophotometer using shifting reagent and IR Spectrophotometer.

Result and Discussion

Fractions which obtained from liquid-liquid extraction showed antioxidant activity (figure 1). Fraction of n-hexane showed strong yellow and rapid colour change, so that fraction used for further fractination with vacuum liquid chromatography. Thin layer chromatogram of 11 fractions from Vacuum liquid chromatography (figure 2) showed that fraction number 4, 5, and 6 showed strong yellow and rapid colour change.

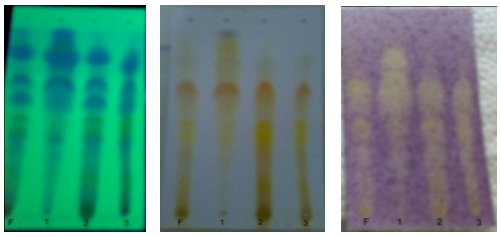

Figure 1. Thin Layer Chromatogram of Extract (F), n-hexane fraction (1), ethyl acetate fraction (2), and metanol-H_2O fraction (3); with silica gel GF 254 as stationary phase and hexane-EtOAc (9:1) as mobile phase.

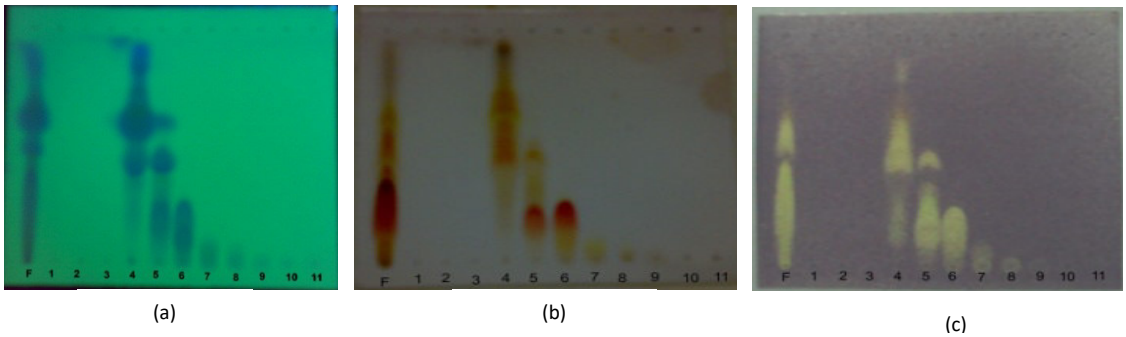

Figure 2. Thin Layer Chromatogram of Extract (F), fractions from VLC (1-11); with silica gel GF 254 as stationary phase and hexane-EtOAc (9:1) as mobile phase using detector/visualizing agent (a) UV λ 254 nm; (b) H_2SO_4; (c) DPPH 0.2%.

Fraction number 6 has one big spot but less spot if compared to fraction number 4 and 5. Further separation by coloumn chromatography gave 31 fraction (1a-31a). Fraction 3a-23a had antioxidant activity. Crystal from fraction 17a was washed by n-hexane, and gave isolate BP-1. Purity test was performed by a single development TLC using three different kinds of mobile phase and two-dimensional TLC showed that BP-1 was pure. Isolate BP1 indicated as flavonoid because it gave yellow-green fluoresens when identified by TLC using $AlCl_3$ 5% as spotting agent.

Isolate characterization by spectrophotometer UV-Vis showed two band (figure 4), $\lambda_{1\text{-MeOH}}$ 320 nm and $\lambda_{2\text{-MeOH}}$ 289 nm. Isolate was indicated as flavanon or dihydroflavonol. UV-Vis spectrum of Isolate with NaOH showed that band II shifted from 289 to 321 nm (bathochromic 32 nm). This result indicated OH-substitution on C5 and C7.

OH-subtitution on C5 also indicated by Natrium Acetate (MeOH/NaOAc) UV-Vis spectrum which showed that band II shifted from 289 to 324 nm (bathochromic 34 nm). This result indicated BP1 was flavanon with OH-substitution on C5 and C7.

UV-Vis spectrum of Isolate + $AlCl_3$ 5% showed that band II shifted from 289 to 308 nm (bathochromic 19 nm). It was indicated OH-substitution on C5.

There was no OH-substitution on ortho position. Which was indicated by MeOH/$AlCl_3$ and MeOH/$AlCl_3$/HCl UV-Vis spectrum of BP1 which did not give any shift. This result supported by MeOH/NaOAc/H_3BO_3 spectrum which showed no shifting relative to MeOH spectrum.

Infrared spectrum showed there were C-O (1087 cm^{-1}), C=C (1488 cm^{-1}), C=O (1639 cm^{-1}), O-H (3093 cm^{-1}) and aromatic (3016 cm^{-1}). Isolate BP1 was predicted as Pinocembrin (figure 3) (Ching *et al.,* 2007).

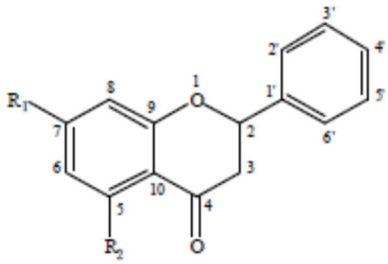

Figure 3. Structure of Pinocembrin. R1, R2 : OH.

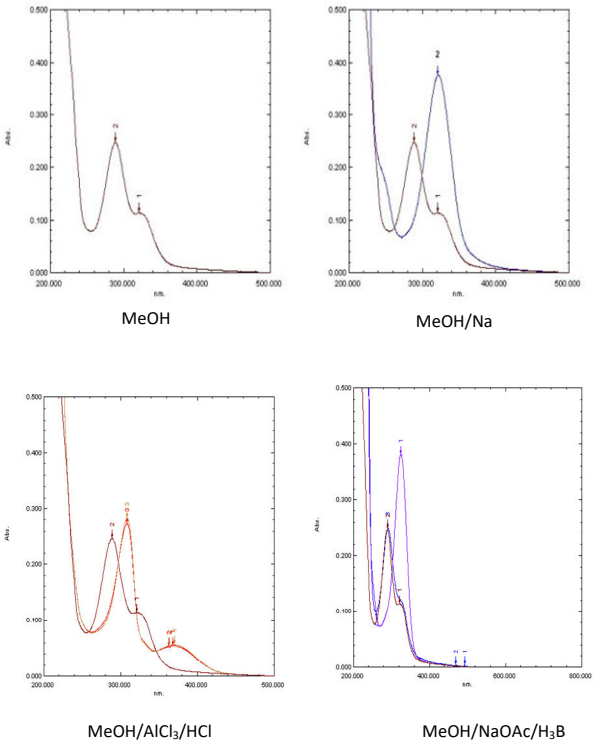

Figure 4. UV-Vis spectrum of Isolate BP-1

Conclusion

Isolate BP-1 was isolated from the ethanol extract and active antioxidant. Based on ultraviolet-visible and infrared spectrum, isolate BP-1 was identified as flavanone in the absence of hydroxyl groups at the ortho position (o-diOH), with substitution of -OH at C5 and C7.

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