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Evaluation Potency of *Boesenbergia rotunda* as Antioxidant Achieved by Free Radicals Scavenging Activities

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ABSTRACT

Boesenbergia rotunda is an ethnomedicinal plant with potential free radical scavenging activity. Antioxidant activity was determined based on DPPH, ABTS, and CUPRAC free radical scavenging assays. The experimental results revealed that the dichloromethane extract of *B. rotunda* exhibited better free radical scavenging activity compared to the hexane extract. The dichloromethane extract showed inhibition percentages of 49.868 ± 0.331 for DPPH and 87.594 ± 0.065 for ABTS, while the CUPRAC assay yielded a TEAC value of 0.586 ± 0.007 mmol/g.

1. Introduction

The *Zingiberaceae* family comprises plants with various benefits that naturally grow in tropical and subtropical regions. One species that grows abundantly in Indonesia and is frequently used as a traditional medicine is *Boesenbergia rotunda*, locally name as 'temu kunci'. This plant has elongated leaves, oval rhizomes with yellowish-brown coloration, and emits a distinctive aroma [1, 2]. Several botanical names have been used to refer to this plant, such as *Boesenbergia pandurata* (Roxb.) Schltr., *Boesenbergia cochinchinensis* (Gagnep.) Loes., *Curcuma rotunda* L., *Gastrochilus panduratus* (Roxb.) Ridl., *Gastrochilus rotundus* (L.) Alston, *Kaempferia cochinchinensis* Gagnep., *Kaempferia ovata* Roscoe, and *Kaempferia pandurata* Roxb. Currently, it is officially recognized as *Boesenbergia rotunda* (L.) Mansf. [3].

Boesenbergia rotunda is an ethnomedicinal plant known for its role in wound healing and treating stomach disorders. Moreover, its rhizomes are widely used by local communities as a traditional culinary spice. This is particularly interesting as the use of organic or natural ingredients may help prevent various serious diseases [4]. The rhizomes of *B. rotunda* have been proven to possess antimicrobial, anti-inflammatory, analgesic, antitumor, and antioxidant

properties. This is attributed to the results of phytochemical screening, which revealed that *B. rotunda* contains flavonoids, alkaloids, phenolic compounds, along with small amounts of tannins and saponins [5, 6].

The antioxidant potential of *B. rotunda* rhizomes can inhibit free radical oxidation processes in the body that may lead to degenerative diseases [7]. Mat Yasin et al. (2018) conducted an antioxidant test using ethanol extract of *B. rotunda* with DPPH free radical scavenging activity, yielding an IC_{50} value of 738.3 ± 0.43 µg/mL, and ABTS free radical scavenging activity, yielding an IC_{50} value of 63.87 ± 0.76 µg/mL. In this experiment, the extraction of *B. rotunda* rhizomes involved solvents of varying polarity, namely n-hexane and dichloromethane. Due to differences in extraction solvents compared to Mat Yasin et al.'s (2018) experiment, further tests on DPPH, ABTS, and Cuprac free radical scavenging activity are necessary to better evaluate its antioxidant potential.

2. Materials and methods

2.1 Plant materials

The plant used in this experiment was *Boesenbergia rotunda*. The selected part for the experiment was its rhizomes. The plant species was obtained from Magetan, East Java.

2.2 Chemical reagents and instrument

DPPH (*1,1-diphenyl-2-picrylhydrazyl*) and ABTS *(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)* were procured from Tokyo Chemical Industry Co. Ltd. Potassium persulfate, ammonium acetate, neocuproine, copper (II) chloride, and ascorbic acid (Vitamin C) as positive standard were purchased from Sigma Chemical Co (St. Louis, MO, USA). Absorbance measurements were taken using a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader.

2.3 Plant extraction

The *Boesenbergia rotunda* rhizomes were washed with distilled water and dried at room temperature. The dried material was ground into powder and extracted by maceration in three repetitions using solvents of varying polarity, including non-polar (hexane), semi-polar (dichloromethane and ethyl acetate), and polar (ethanol) solvents. The filtrates obtained from each solvent extraction were concentrated using a rotary evaporator (Rotavapor R100, BUCHI). All extracts were then stored at 4°C. However, only hexane and dichloromethane extracts were used for further testing.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

DPPH free radical scavenging activity was measured following the method used by Ramadhan et al. (2019), with slight modifications. Specifically, 20 µL of extract at various concentrations (0.01 – 0.5 mg/mL) was added to 0.1 mM DPPH solution. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a 96-

well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader. The antioxidant activity of the samples was expressed as percentage inhibition (PI), calculated using the formula:

% percentage inhibition = [(Abs control – Abs sample)/Abs control] x 100.

2.4.2 ABTS radical scavenging activity

ABTS free radical scavenging activity was measured according to the method described by Ramadhan et al. (2019). The ABTS solution was prepared by mixing a 7 mM ABTS solution with a 2 mM potassium persulfate solution and allowing it to stand for 24 hours at room temperature. ABTS free radical scavenging activity was tested by mixing 20 µL of extract at various concentrations (0.01 – 0.5 mg/mL) with the ABTS·+ solution. The mixture was incubated in the dark for 60 minutes, and absorbance was measured at 750 nm using a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader. The antioxidant activity of the samples was expressed as percentage inhibition (PI), calculated using the formula:

% percentage inhibition = [(Abs control – Abs sample)/Abs control] x 100.

2.4.3 Cupric Reducing Antioxidant Capacity (CUPRAC)

The antioxidant capacity was determined using the CUPRAC assay following the method by Tunnisa et al. (2022), with slight modifications. Specifically, 50 µL of extract at various concentrations (0.01 to 0.5 mg/mL) was added to 50 µL of $CuCl_2$ solution, 50 µL of neocuproine, and 50 µL of NH_4Ac buffer. The mixture was incubated for 60 minutes, and absorbance was measured at 450 nm using a 96-well Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader. The antioxidant capacity was expressed in Trolox Equivalent Antioxidant Capacity (TEAC (mmol/g of extract)) and compared with the standard trolox which has high reducing potential.

2.5 Data analysis

All data were presented as the average of three experiments. Each experiment was conducted in triplicate, and the results were reported as the mean ± standard deviation. The quantitative data obtained were displayed in graphical form and analyzed descriptively.

3. Results and discussion

This experiment aimed to determine the antioxidant potential of *B. rotunda* hexane and dichloromethane extracts with DPPH, ABTS, and Cuprac free radical scavenging activities, with absorbance measured using a Thermo Scientific Multiskan SkyHigh RE 6.1.1 microplate reader. Before antioxidant testing, the *B. rotunda* samples were extracted by maceration using solvents with varying polarities. The results of the experiment are presented in terms of antioxidant activity percentage (inhibition percentage) and TEAC, as shown in Table 1. The inhibition percentage indicates the ability of antioxidant compounds in the sample to capture free radicals and convert them into stable molecules, as marked by a color change. The higher the inhibition percentage, the greater the ability to inhibit free radicals [11]. In this experiment,

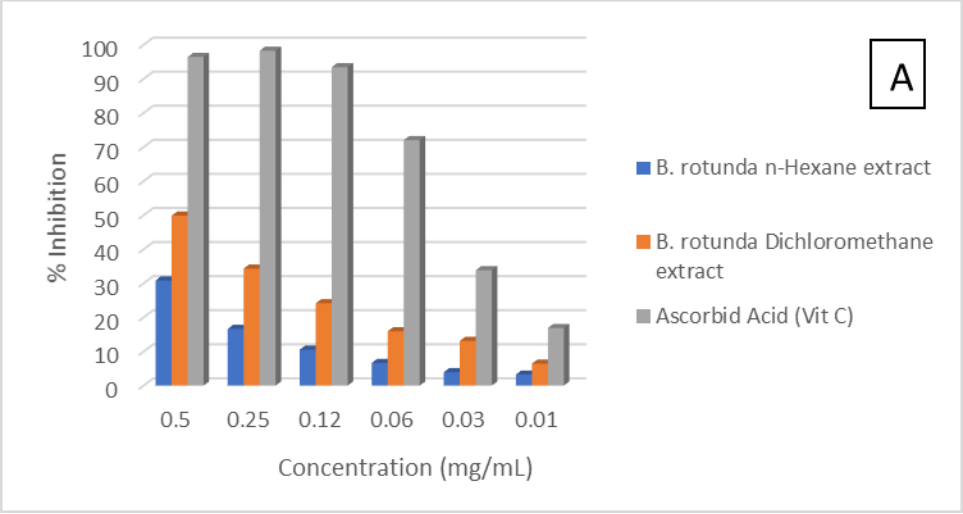
the dichloromethane extract of *B. rotunda* at a DPPH radical scavenging concentration of 0.5 mg/mL showed an inhibition percentage of 49.868 ± 0.331, which was higher than that of the hexane extract. Similarly, for ABTS free radical scavenging at the same concentration (0.5 mg/mL), the dichloromethane extract of *B. rotunda* had a higher inhibition percentage of 87.594 ± 0.065.

Table 1. Antioxidant properties of B. *rotunda*

Samples	Percent inhibition		Cuprac
	DPPH	ABTS	TEAC (mmol/g)
B. *rotunda* (hexane)	30.864 ± 0.277	54.293 ± 0.121	0.350 ± 0.003
B. *rotunda* (dichloromethane)	49.868 ± 0.331	87.594 ± 0.065	0.586 ± 0.007
Ascorbic Acid (Vit C)	96.422 ± 0.738	99.854 ± 0.392	-

Values are presented as mean ± SD, concentration 0.5 mg/mL
DPPH and ABTS free radical scavenging activities are expressed as percentage inhibition.
CUPRAC was expressed as Trolox equivalent antioxidant capacity (TEAC (mmol/g))

Several studies on the antioxidant activity of *B. rotunda* have been reported. Atun et al. (2018) stated that the ethanol extract of *B. rotunda* rhizomes exhibited moderate antioxidant activity. Similarly, Saah et al. (2021) reported that the water extract of *B. rotunda* showed weak antioxidant activity. The differences in antioxidant activity observed in this experiment are likely due to the type of solvent used during extraction, which affects the total content of bioactive compounds because of solvent polarity differences [14]. Additionally, according to Li et al. (2020), variations in the growing location of a plant influence the biosynthetic precursors of secondary metabolites, which in turn affect its bioactivity. Figure 1. presents the results of hexane and dichloromethane extracts of *B. rotunda* for DPPH, ABTS, and Cuprac free radical scavenging activities at various concentrations (0.01-0.5 mg/mL).



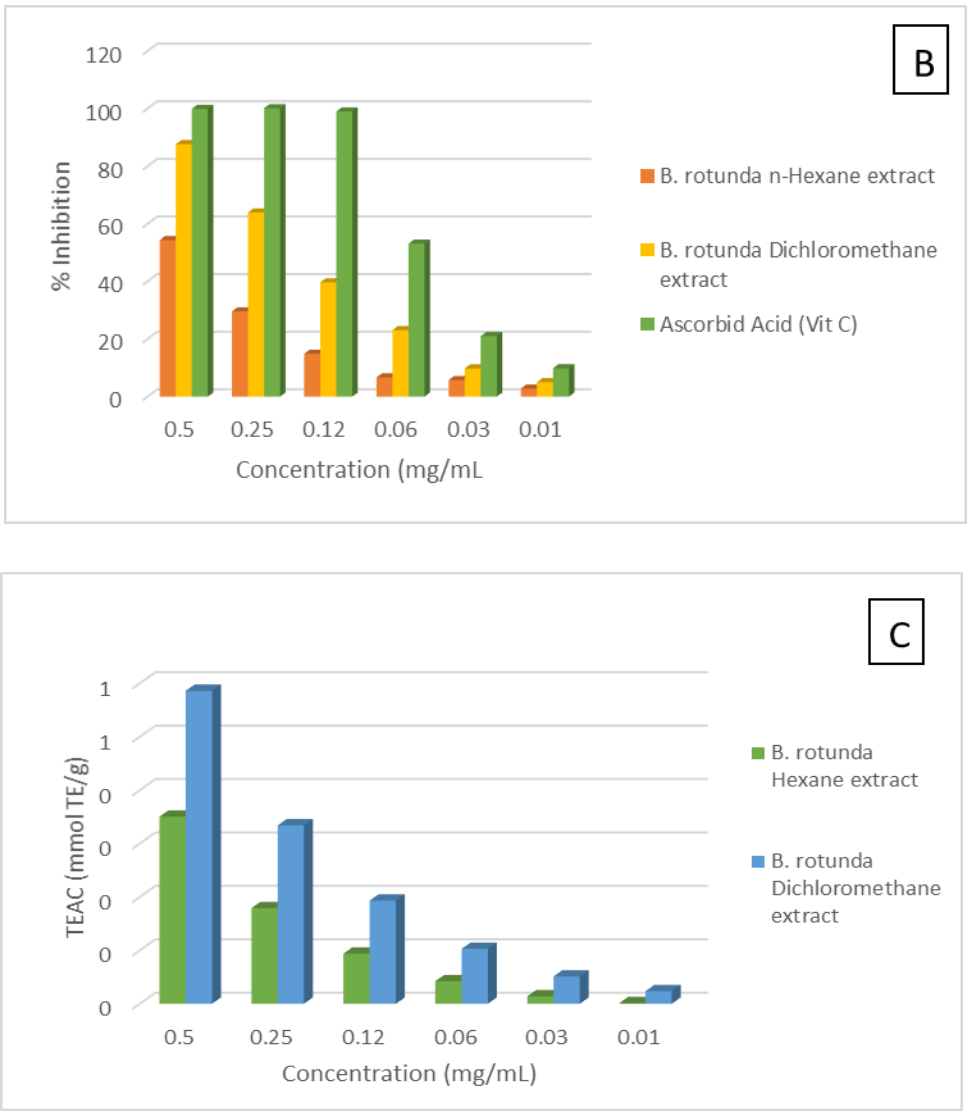


Figure 1. antioxidant activities of *B. rotunda* hexane and dichloromethane extracts: DPPH (A), ABTS (B), and CUPRAC (C).

Figures 1A. and 1B. show that the percentage of DPPH and ABTS free radical inhibition by hexane and dichloromethane extracts of *B. rotunda* increased with higher concentrations. However, the ABTS radical scavenging activity of the dichloromethane extract of *B. rotunda* demonstrated an inhibition percentage nearly equal to that of the positive control, ascorbic acid. This suggests that the dichloromethane extract of *B. rotunda* has a relatively high sensitivity when reacting with ABTS. ABTS is a free radical used to measure antioxidant activity through an electron transfer mechanism involving the reduction of a colored oxidant. The ABTS assay is performed based on the formation of a blue-green ABTS radical [16].

The antioxidant activity of *B. rotunda* was also evaluated using the CUPRAC method. The formation of the CUPRAC reagent involves a reaction between $CuCl_2$, neocuproine, and ammonium acetate, resulting in a chelating complex compound (yellow in color). The antioxidant inhibition mechanism between the CUPRAC reagent and antioxidant agents

involves a reduction reaction, indicated by a color change from bluish-green to yellow. The antioxidant results of hexane and dichloromethane extracts of *B. rotunda* are presented in Table 1 and Figure 1C. The dichloromethane extract of *B. rotunda* showed antioxidant potential of 0.586 ± 0.007 mmol/g, which was higher than that of the hexane extract. This indicates that the dichloromethane solvent extract contains compounds that act as antioxidant agents. Antioxidant agents function as proton and electron donors. Therefore, bioactive compounds such as flavonoids, alkaloids, and phenolic groups can serve as alternative antioxidant agents derived from plants. Jitvaropas et al. (2012) reported that *B. rotunda* contains flavonoids, alkaloids, phenolics, and tannins based on phytochemical screening.

4. Conclusions

The antioxidant potential of hexane and dichloromethane extracts of *B. rotunda*, tested through DPPH, ABTS, and CUPRAC free radical scavenging assays, indicates that the dichloromethane extract *B. rotunda* exhibits better free radical scavenging activity than the hexane extract. The dichloromethane extract showed inhibition percentages of 49.868 ± 0.331 for DPPH and 87.594 ± 0.065 for ABTS. Meanwhile, the CUPRAC test showed a TEAC value of 0.586 ± 0.007 mmol/g. Therefore, further research on the dichloromethane extract of *B. rotunda* is necessary, including isolation and other bioactivity tests.

Acknowledgements

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