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Solvent optimization enhanced phenolic content and antioxidant activity in ultrasound-assisted extraction of *Boesenbergia rotunda* rhizomes

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Abstract The study revealed that using 100% acetone as the solvent in ultrasound-assisted extraction yielded the highest levels of total phenolic content (TPC) and ferric reducing antioxidant power (FRAP), with values of 10.63±0.48 mg GAE/g DW and 34.27±1.82 µmol TE/g DW, respectively. In contrast, the solvent combination of ethanol and acetone (50:50) produced the highest antioxidant capacity based on the ABTS assay, reaching 100.31±4.80 µmol TE/g DW. A strong positive correlation (r = 0.9388) was observed between TPC and FRAP antioxidant activity. The findings highlighted that solvent selection significantly affected the extraction efficiency of phenolic compounds and antioxidant properties from *Boesenbergia rotunda* rhizomes. Notably, acetone was most effective for extracting TPC and FRAP, while a 50:50 ethanol-acetone blend was ideal for ABTS. This is underscored the pivotal role of solvent choice in optimizing the extraction of bioactive compounds, enhancing the therapeutic potential of herbal sources.

Keywords: Acetone, Antioxidant, *Boesenbergia rotunda*, Ethanol, Total phenolic

Introduction

Free radicals are highly reactive molecules with unpaired electrons, making them inherently unstable (Meo and Venditti, 2020). These unstable species can initiate chain reactions by stealing electrons from other molecules, leading to cellular damage and compromising body tissues (Unsal *et al.*, 2021). Continuous exposure to free radicals is associated with various diseases, including genetic mutations and uncontrolled cell proliferation, which are hallmarks of cancer (Shrivastava *et al.*, 2019). Furthermore, free radicals can damage the endothelial lining of blood vessels, promoting inflammation and atherosclerosis, thereby

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increasing the risk of cardiovascular diseases such as heart attacks and strokes (Chen *et al.*, 2021). Additionally, these reactive species can impair neural cells, potentially contributing to neurodegenerative disorders like Alzheimer's and Parkinson's disease (Ahmadinejad *et al.*, 2017). Antioxidants, however, have the capacity to neutralize these harmful effects (Sharma, 2014).

Antioxidants protect cells from oxidative stress-induced damage, as evidenced by several studies (Adwas *et al.*, 2019; Aziz *et al.*, 2019; Gulcin, 2020). They act through various mechanisms, such as donating electrons (Chen *et al.*, 2020), neutralizing free radicals (Choudhari *et al.*, 2014), replenishing other antioxidants (Cömert and Gökmen, 2017), and inhibiting oxidative reactions (Santos-Sánchez *et al.*, 2019). For instance, enzymes like glutathione and superoxide dismutase (SOD) convert free radicals into less harmful forms (Sharma, 2014). Moreover, vitamins C and E can regenerate other antioxidants, perpetuating a cycle of cellular protection (Pehlivan, 2017; Shakeri *et al.*, 2020). By disrupting the chain reactions initiated by free radicals, these compounds help minimize cellular damage (Gulcin, 2020). Antioxidants are naturally abundant in fruits, vegetables, and herbal plants (Yadav *et al.*, 2016; Xu *et al.*, 2017).

Herbal plants, which are rich in bioactive compounds, have long been utilized in traditional medicine to promote health (Ekor, 2014; Ali-Shahveh *et al.*, 2013). Throughout history, various cultures have harnessed these plants for their healing properties (Ozioma and Chinwe, 2019). Among these, fingerroot (*Boesenbergia rotunda*) has demonstrated significant potential as a source of antioxidants (Saah *et al.*, 2021).

Boesenbergia rotunda, commonly known as fingerroot, belongs to the Zingiberaceae family and is widely found in regions such as Indonesia, India, Sri Lanka, and Malaysia (Kadir *et al.*, 2013; Atun, 2014; Wang *et al.*, 2023). This perennial plant, characterized by yellow rhizomes, is renowned for its anticancer properties, particularly its capacity to induce apoptosis in cancer cells (Adhikari *et al.*, 2020). The rhizomes contain kaempferol, which exhibits antioxidant, anti-inflammatory, and anticancer effects (Ruttanapattanakul *et al.*, 2021). Traditionally, fingerroot rhizomes have been employed as spices and remedies for conditions like fever, inflammation, and digestive issues (Eng-Chong *et al.*, 2012). These rhizomes are rich in bioactive substances, including flavonoids and phenolics (Ongwisespaiboon and Jiraungkoorskul, 2017).

Maximizing the extraction of these bioactive compounds requires careful selection of solvents, as effective solvents can significantly enhance the extraction yield of phenolics and antioxidant compounds. This research aimed to determine the most efficient solvents for extracting phenolics and to evaluate the antioxidant capacity of *B. rotunda* rhizomes.

Materials and methods

Study location and duration

This research was conducted at the Biochemistry Laboratory of IPB University in Bogor, Indonesia, from February to May 2023. *B. rotunda* plants were obtained from the Biopharmaca Collection Garden of the Tropical Biopharmaca Research Center, IPB University (coordinates: -6.5470915, 106.711514). The rhizomes of these plants were used as the study's samples.

Solvent selection and combinations

Three solvents—water, ethanol, and acetone—were utilized to create seven different solvent combinations: water 100% (W), water-ethanol 50%:50% (WE), water-acetone 50%:50% (WA), ethanol 100% (E), ethanol-acetone 50%:50% (EA), acetone 100% (A), and water-acetone-ethanol 33.33%:33.33%:33.33% (WAE). Each combination was tested in triplicate.

Sample preparation and extraction

Plant materials obtained from the Biopharmaca Collection Garden were dried at 45°C for a period of 48 hours before being ground into a fine powder using an 80-mesh sieve. The ultrasound-assisted extraction technique, modified from the procedure described by Nurcholis *et al.* (2022), involved combining 4 g of the powdered sample with 20 ml of the selected solvent. The mixture was subjected to sonication at room temperature for 30 minutes. Subsequently, the mixture underwent centrifugation at 10,000 × g for 15 minutes at 4°C, yielding a supernatant with a final concentration of 0.2 g/mL.

Evaluation of total phenolic content

Total phenolic content (TPC) was quantified using a modified procedure based on the method described by Nurcholis *et al.* (2022). In a microplate, 20 µL of the sample extract was combined with 120 µL of 10% (v/v) Folin-Ciocalteu reagent and allowed to react in the dark at room temperature for 5 minutes. Following this, 80 µL of 10% (w/v) Na_2CO_3 solution was added, and the mixture was further incubated in darkness at room temperature for an additional 30 minutes. The absorbance was then measured at 750 nm using a SPECTROstarNano BMG LABTECH spectrophotometer. Gallic acid was used as a calibration standard, with concentrations ranging from 0 to 300 ppm. Results

were expressed as mg GAE (gallic acid equivalent) per gram of dry weight (g DW).

Determination of antioxidant capacity

The antioxidant capacity was assessed using both FRAP and ABTS methods with slight modifications from previous studies (Liwanda *et al.*, 2023; Seno *et al.*, 2023). For the FRAP assay, a reagent was prepared by mixing TPTZ, $FeCl_3$, and acetate buffer. The sample (10 µL) was combined with 300 µL of FRAP reagent and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 593 nm using a spectrophotometer, with Trolox as the standard (0–600 µM), and results were expressed in µmol TE/g dry weight. For the ABTS assay, ABTS and $K_2S_2O_8$ were mixed, then diluted to achieve an absorbance of 0.7 at 734 nm. A mixture of 20 µL sample and 280 µL ABTS reagent was incubated for 6 minutes before measuring absorbance at 734 nm. Trolox (0–500 µM) served as the standard, with results expressed in µmol TE/g dry weight.

Data analysis

Data were analyzed using ANOVA in IBM SPSS Statistics 25, with a significance threshold set at $\alpha = 0.05$. Significant differences between groups were identified using Tukey's HSD test. Additionally, Pearson correlation analysis in GraphPad Prism 8 was applied to examine the relationship between TPC and antioxidant capacity.

Results

Significant differences were observed in the total phenolic content (TPC) across various solvent combinations. The acetone solvent yielded the highest TPC, recording 10.63±0.48 mg GAE/g DW (Figure 1A), while the acetone-water mixture showed the lowest content at 0.84±0.11 mg GAE/g DW.

For antioxidant capacity, notable variations were found among the different solvent mixtures, as evaluated using the FRAP and ABTS assays. The highest FRAP antioxidant capacity was achieved with pure acetone (34.27±1.82 µmol TE/g DW), whereas the lowest was found in the water-ethanol mixture (1.73±0.05 µmol TE/g DW) (Figure 1B). Similarly, the ethanol-acetone combination demonstrated the highest antioxidant capacity in the ABTS assay at 100.31±4.80 µmol TE/g DW, with the water-ethanol mixture showing the lowest value at 1.57±2.01 µmol TE/g DW (Figure 1C).

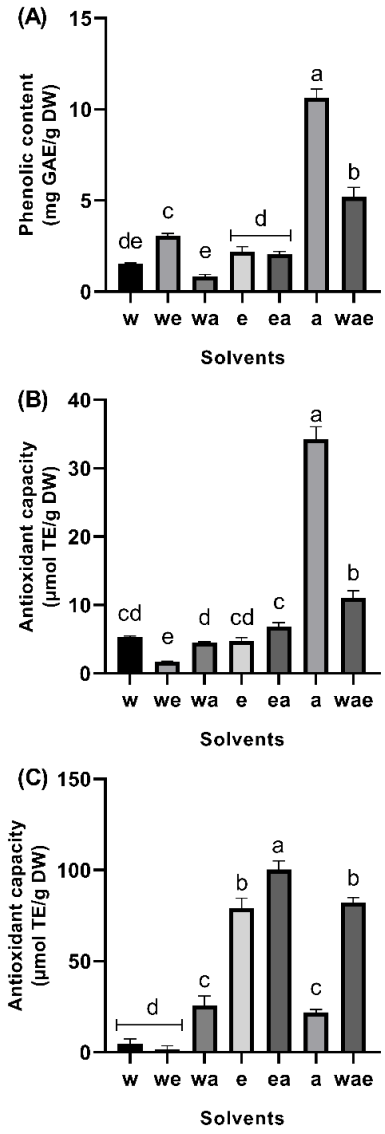

Figure 1. Analysis of total phenolic content (A), FRAP antioxidant activity (B), and ABTS antioxidant capacity (C) in *B. rotunda*rhizomes. Data represent the mean ± standard deviation (SD) from three independent replicates, Distinct letters denote statistically significant differences ($p < 0.05$) based on Tukey's test. Solvent combinations used include water (w), ethanol (e), and acetone (a)

A Pearson correlation analysis was conducted to examine the relationship between TPC and antioxidant capacity. A strong positive correlation was found

between TPC and FRAP antioxidant capacity (r = 0.9388, Figure 2A). However, the correlation between TPC and ABTS capacity was weak and not statistically significant (r = -0.09045, Figure 2B).

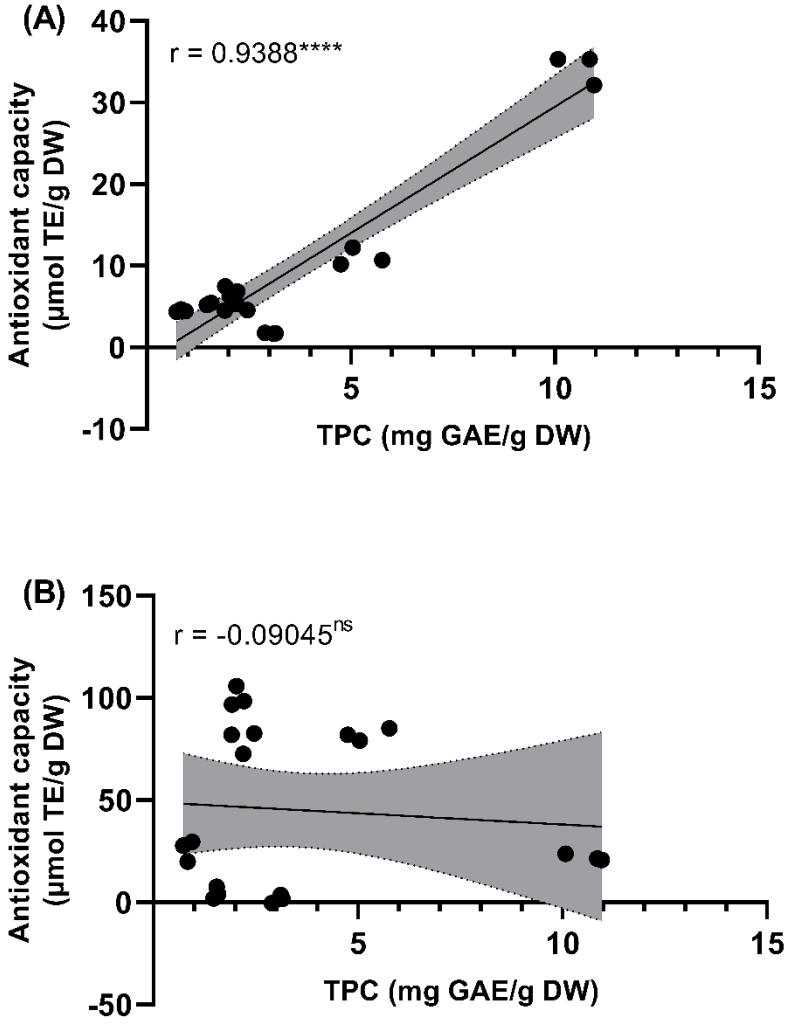

Figure 2. Relationship between total phenolic content and antioxidant capacity using the FRAP (A) and ABTS (B) assays for *B. rotunda* extract: Pearson correlation coefficients (r) are shown, with **** indicating a very strong correlation ($p < 0.01$) and "ns" representing non-significant correlations

Discussion

Phenolic compounds significantly contribute to the antioxidant properties of plant extracts (Aryal *et al.*, 2019). In this study, TPC was assessed using the Folin-Ciocalteu method, which detects phenolics through a colorimetric reaction involving electron transfer (Dai and Mumper, 2010). The choice of solvent is crucial for the efficient extraction of phenolic compounds, influenced by differences in solvent polarity and solubility (Nguyen *et al.*, 2022). Phenolics, with their hydroxyl groups, show greater solubility in organic solvents (Wang and Weller, 2006). Our results indicated that acetone, a semi-polar solvent, was the most effective for extracting phenolics from *B. rotunda* (Figure 1A), aligning with previous studies on the effectiveness of semi-polar solvents in phenolic extraction (Hidayati *et al.*, 2020).

The antioxidant capacity of *B. rotunda* extracts was evaluated using the FRAP and ABTS assays. The FRAP method measures the reduction of Fe^{3+} to Fe^{2+}, reflecting antioxidant potential (Balaji *et al.*, 2015), while the ABTS assay assesses the ability of antioxidants to neutralize ABTS radicals (Ratnavathi and Komala, 2016). Acetone extracts exhibited the highest FRAP antioxidant capacity, whereas the ethanol-acetone mixture showed the highest ABTS capacity (Figures 1B and 1C). Extracts prepared with acetone showed the highest TPC and FRAP values, while the water-ethanol mixture yielded the lowest antioxidant capacities in both assays. This difference can be attributed to variations in solubility and interaction between solvents and phenolic compounds (Alam *et al.*, 2019; Jakob *et al.*, 2021).

A strong positive correlation was found between TPC and FRAP capacity ($r = 0.9388$), suggesting that phenolics play a key role in antioxidant activity. These compounds can scavenge free radicals by forming phenoxyl radicals, thus enhancing antioxidant defence (Bors and Michel, 2002). In contrast, the correlation between TPC and ABTS capacity was weak and not statistically significant ($r = -0.09045$), indicating the presence of other non-phenolic antioxidants, such as vitamins, heteropolysaccharides, or polypeptides, that may interact with ABTS radicals (Jakubczyk *et al.*, 2019).

Overall, acetone was identified as the most effective solvent for extracting TPC and FRAP capacity, achieving the highest values of 10.63±0.48 mg GAE/g DW and 34.27±1.82 µmol TE/g DW, respectively. The ethanol-acetone mixture exhibited the highest ABTS capacity at 100.31±4.80 µmol TE/g DW. These results emphasize the importance of solvent selection in optimizing the extraction of bioactive compounds, highlighting the critical role of TPC in enhancing antioxidant properties.

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