**ANTIOXIDANT POTENTIAL OF EXTRACT OF BLACK RICE, BAJAKAH WOOD ROOT AND KEBIUL SEED**

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ABSTRACT

Exposure to free radicals and oxidative stress are major factors in various degenerative diseases, such as cardiovascular disease, diabetes, cancer, and neurodegenerative diseases. Therefore, the use of natural antioxidant sources is important to reduce the negative effects of free radicals. This study compares the natural antioxidant potential of black rice, bajakah root, and kebiul seeds, whereas previous studies have only identified the antioxidant potential of a single natural ingredient. This study aims to measure the antioxidant potential, such as total polyphenols, total flavonoids, and antioxidant activity, of these three natural materials using distilled water, 70% ethanol, and 96% ethanol as solvents. The experimental research method involved testing antioxidant activity and measuring total phenolics and flavonoids. The results showed that the 70% ethanol extract of black rice had the highest total polyphenol

content at 6.83 ± 0.28 mgGAE/g ($0.007 \pm 0.00\%$), the 70% ethanol extract of bajakah wood root had the highest flavonoid content at 8.77 ± 0.02 mgQE/g ($0.009 \pm 0.00\%$), and the distilled water extract of kebiul seeds showed the strongest antioxidant activity based on an IC₅₀ value of 5.96 ± 0.01 ppm. This study concluded that black rice has potential as a primary source of phenolics and anthocyanins, while bajakah wood root and kebiul seeds are sources of flavonoids with strong antioxidant activity.

INTRODUCTION

Exposure to free radicals and oxidative stress has become a major concern in modern health research. Free radicals, especially reactive oxygen species (ROS) and reactive nitrogen species (RNS), are produced from various internal and external sources. Internal sources include normal metabolic processes, while external sources include pollution, cigarette smoke, radiation, and consumption of processed foods. When free radical production exceeds the body's capacity to neutralize them with antioxidants, cellular damage occurs, which can contribute to various degenerative diseases. In cardiovascular disease, oxidative stress plays an important role in the pathogenesis of atherosclerosis, myocardial infarction, and heart failure through excess ROS production that exceeds the capacity of endogenous antioxidants, causing damage to cells and blood vessels. In diabetes mellitus, ROS imbalance causes pancreatic beta cell dysfunction and insulin resistance, and contributes to vascular complications such as retinopathy, nephropathy, and neuropathy (Dubois-deruy et al., 2020; Kibel et al., 2020).

In the context of cancer, ROS can trigger genetic mutations, DNA damage, and activate signaling pathways that support tumor development, but at high concentrations it can also cause cancer cell death. In neurodegenerative diseases such as Alzheimer's and Parkinson's, oxidative stress is involved in the damage of proteins, lipids, and neuronal DNA, resulting in mitochondrial dysfunction, glial activation, and damage to the blood-brain barrier and proteasome (Hayes et al., 2020; Sienes Bailo et al., 2022).

In this context, antioxidants play a very important role in neutralizing free radicals and protecting cells from damage. Foods rich in antioxidants, such as fruits and vegetables, are very important for maintaining redox balance in the body. Several studies have shown that a diet rich in antioxidants can provide protection against various diseases. For example, consuming foods rich in antioxidants can reduce the risk of cardiovascular disease by increasing serum antioxidant capacity and reducing oxidative stress (Ginter, 1994; Sharifi-Rad et al., 2020). In addition, antioxidant supplementation can help improve glycemic control in diabetic patients by reducing oxidative stress levels (Gupta et al., 2023; Martins et al., 2022; Moini Jazani et al., 2022). Consuming foods rich in polyphenols can reduce the risk of cancer by reducing oxidative damage to the body's cells (Rudrapal et al., 2022). In the context of neurodegenerative diseases such as Alzheimer's, research shows that antioxidant supplementation can slow the progression of the disease by reducing the accumulation of beta-amyloid proteins associated with oxidative stress (Cassidy et al., 2020).

Black rice (*Oryza sativa* L. indica) has been recognized as one of the strongest sources of antioxidants among other rice varieties. Research shows that black rice contains more than 23 types of compounds with high antioxidant activity, including anthocyanins and flavonoids. A study by Zheng et al. shows that black rice can reduce oxidative stress and inflammation, and has a positive effect in overcoming diabetes complications (Zheng et al., 2020). Anthocyanins, which give black rice its dark color, have various health benefits, including anti-inflammatory and anti-cancer effects. Recent clinical and preclinical studies highlight the positive effects of anthocyanins and phenolic compounds in black rice on vascular endothelial protection, reduction of oxidative stress, and

improvement of blood vessel function. Regular consumption of black rice is also associated with benefits in preventing coronary heart disease and improving other cardiometabolic risk biomarkers (Mendoza-Sarmiento et al., 2023). Research by Sahewalla et al. shows that black rice has a high flavonoid content, which acts as a free radical scavenger, thereby reducing oxidative damage to the body's cells (Sahewalla et al., 2023).

Bajakah wood root (*Spatholobus littoralis*) is known for its bioactive compounds such as flavonoids, saponins, and tannins, which have antioxidant, antidiabetic, and anticancer properties. Recent studies show that bajakah wood root extract not only has therapeutic potential but can also be used as a copigment to enhance the stability and antioxidant activity of anthocyanins in food products and supplements (Istiqomah & Safitri, 2021; Novalia Rahmawati Sianipar et al., 2023). In addition to bajakah tree roots, another natural ingredient used as a copigment agent is kebiul seeds (*Caesalpinia banduc*), which are rich in bioactive compounds, including flavonoids, tannins, and saponins, that have antioxidant potential. Research shows that kebiul seed extract has significant scavenging activity against free radicals (Yani & Dirmansyah, 2021). These three materials offer great potential as sources of antioxidants. However, we need to study the ability of polar solvents such as distilled water, 70% ethanol, and 96% ethanol to extract bioactive compounds in these natural materials in order to select the appropriate polar solvent for use in further research.

Based on this background, this study specifically aims to determine the total polyphenol content, total flavonoid content, and antioxidant activity of polar solvent extracts (distilled water, 70% ethanol, and 96% ethanol) from black rice, bajakah root, and kebiul seeds.

RESEARCH METHOD

Experimental research method, where this research was conducted at the Pharmacy and Applied Sciences Laboratory of Fort De Kock University.

Materials

The tools used were glassware (Pyrex®), an oven (Mettler®), an incubator (Mettler®), a rotary evaporator (IKA®), and a UV-VIS spectrophotometer (Merck Pro 300®). The materials used were samples consisting of black rice (*Oryza sativa* L. *indica*), bajakah root (*Spatholobus littoralis*) and kebiul seeds (*Caesalpinia bonduc*); 70% ethanol and 96% ethanol (Emsure); DPPH (2,2-diphenyl-1-picrylhydrazyl) (Merck), and distilled water (Medstuff), filter paper (Whatman).

Procedure

1. Sample Preparation and Extraction

The extract is made by dissolving 1000 grams of each sample (black rice, bajakah root, and kebiul seeds) in 1L of solvent (distilled water, 70% ethanol solvent, 96% ethanol solvent). The samples are then macerated for 3x24 hours and homogenized for 1x6 hours. Next, filtration is performed and the macerate is stored. The 70% ethanol and 90% ethanol macerates are rotary evaporated using a rotary vacuum evaporator at a temperature of 40°C

until a thick extract is obtained. Specifically for the distilled water macerate, freeze drying is performed to obtain a dry extract.

2. Determination of Total Polyphenol Content

Total phenols were determined in each polyherbal formula combination using the Folin-Ciocalteu (FC) assay, following previous research by Akmal T et al., 2023. Total polyphenol content was measured by reacting 1 ml of polyherbal formula (1000 µg/ml) with 5 ml of FC reagent (1:10 in water for injection) and incubating for 5 minutes. Then, 4 ml of 7.5% Na₂CO₃ solution was added. After 45 minutes of incubation in the dark at room temperature, the absorbance was measured at 743 nm using a UV-VIS spectrophotometer (Merck Prove 300). Gallic acid was used as a standard. Gallic acid was used to create a calibration curve, and its concentration ranged from 10 to 50 µg/ml. The total polyphenol content of each PHF was determined using a regression equation and absorbance. These values were then converted to milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g) (Akmal et al., 2023).

3. Determination of Total Flavonoid Content

a. Preparation of blank solution

Add 3.0 mL of ethanol p.a; 10% AlCl₃; 0.2 mL of 1M CH₃COOK; add distilled water to 10 mL.

b. Preparation of 1000 ppm quercetin standard solution

Add 10.0 mg of quercetin standard reference material to a 10 mL volumetric flask and fill with ethanol p.a. to the mark, resulting in a concentration of 1000 ppm. Determination of quercetin operating time The operating time was determined in a 7 ppm quercetin working standard solution, measured at a wavelength of 432 nm from 0 to 45 minutes at 1-minute intervals. A curve showing the relationship between absorbance and time was obtained.

c. Determination of maximum wavelength

The quercetin working standard solution was left to stand in a dark place for the operating time, then measured at λ 400-475 nm. A curve showing the relationship between wavelength and absorbance was obtained.

d. Determination of the quercetin standard curve

The stock standard solution was pipetted in amounts of 0.04 mL, 0.05 mL, 0.06 mL, 0.07 mL, 0.08 mL, 0.09 mL, and 0.10 mL. Next, they were placed in a 10.0 mL measuring flask. The solution was added with 3 mL of p.a ethanol, 0.2 mL of 10% AlCl₃ and 1M CH₃COOK. The volume was adjusted with distilled water up to the mark, so that a series of standard solutions with concentrations of 4, 5, 6, 7, 8, 9, 10 ppm was obtained. The solution can be measured on a spectrophotometer after operating time at the maximum wavelength, starting from the smallest concentration.

e. Determination of flavonoid content

Add 0.25 grams of concentrated extract to 25.0 mL of distilled water and shake until dissolved. Take 1 mL, add 3.0 mL of p.a ethanol, 0.2 mL of 10% AlCl₃, 0.2 mL of 1M CH₃COOK, and adjust the volume with distilled water to 10 mL. Place the solution in a

dark place until it reaches operating time, then measure the absorbance at the maximum wavelength using a UV-Vis spectrophotometer (Suharyanto & Hayati, 2021).

4. Determination of Antioxidant Activity using the DPPH Method

The DPPH (2,2-diphenyl-1-picrylhydrazil) free radical test is used to determine how well the Polyherbal formula cleanses free radicals. A total of 1 mL of polyherbal formula with concentrations of 12.5, 25, 50, 100, and 200 g/mL was mixed with 2 mL of DPPH methanol solution (40 g/mL). The final mixture was thoroughly mixed and left at room temperature in the dark for 45 minutes. Then, a UV-Vis spectrophotometer was used to capture this mixture at 517 nm. Methanol was used as a blank. The cleansing capacity of the Polihherbal formula was measured, and the IC₅₀ value was determined (Akmal et al., 2023).

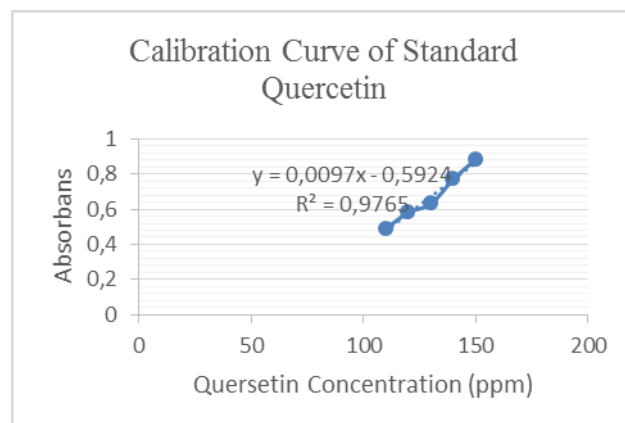
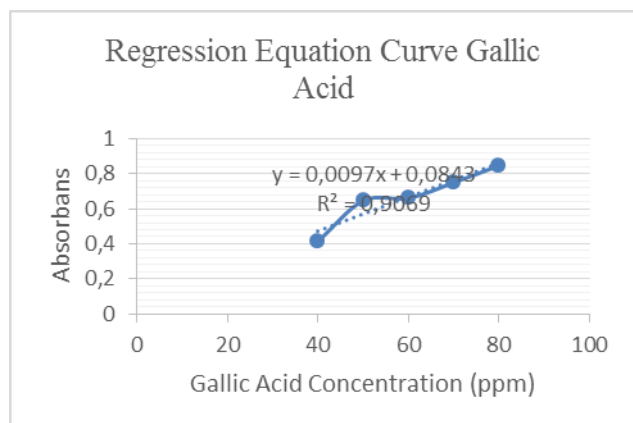
RESULTS AND DISCUSSION

1. Preparation of Raw Materials and Extraction Process

The study used three main raw materials, namely black rice (*Oryza sativa* L. *Indica*), bajakah root (*Spatholobus littoralis* Hassk), and kebiul seeds (*Caesalpinia bonduc* (L.) Roxb.). Extraction was carried out using the maceration method for 3x24 hours, followed by remaceration until a clear filtrate was obtained. The solvents used included distilled water, 70% ethanol, and 96% ethanol for each sample. Each extraction result mentioned above underwent a process of removing water solvents using a freeze dryer at a temperature of -43°C. Meanwhile, ethanol solvents were removed through an evaporation process using a rotary evaporator at a temperature of 40°C.

2. Testing the Antioxidant Potential of Each Sample

Antioxidant potential testing included determining the total phenolic concentration using a gallic acid standard at a maximum wavelength of 743 nm, measuring the total flavonoid concentration using a quercetin standard at a maximum wavelength of 432 nm, and analyzing antioxidant activity using the DPPH method at a maximum wavelength of 517 nm. All measurements were performed using a UV/VIS spectrophotometer. Based on the regression curve presented above, the Total Phenolic content can be determined using the regression equation from the Gallic Acid Standard Solution, namely $y = 0.0097x + 0.0843$ (Figure 1). Meanwhile, the Total Flavonoid content can be calculated using the regression equation from the Quercetin Standard Solution, namely $y = 0.0097x - 0.5924$ (Figure 2). The data on the determination of Total Phenolic and Total Flavonoid content are presented in Table 1.

**Figure 1.** Calibration Curve of Standard Gallic Acid**Figure 2.** Calibration Curve of Standard Quercetin**Table 1. Antioxidant Test Results for Each Extract**

Sample		Total Phenolics		Total Flavonoid		IC ₅₀ (ppm)
		Concentration	Content	Concentration	Content	
		(mg GAE/g)	g/100 g (%)	(mg QE/g)	g/100 g (%)	
Black rice	Aquadest extract	6.60 ± 0.24	0.007 ± 0.00	7.77 ± 0.06	0.008 ± 0.00	6.70 ± 0.02
	Etanol 70% extract	6.83 ± 0.28	0.007 ± 0.00	7.67 ± 0.02	0.008 ± 0.00	7.26 ± 0.01
	Etanol 96 % extract	5.08 ± 0.33	0.005 ± 0.00	7.71 ± 0.04	0.008 ± 0.00	6.83 ± 0.01
Bajakah root	Aquadest extract	5.72 ± 0.21	0.006 ± 0.00	8.75 ± 0.06	0.009 ± 0.00	8.66 ± 0.02
	Etanol 70% extract	5.90 ± 0.13	0.006 ± 0.00	8.77 ± 0.02	0.009 ± 0.00	8.67 ± 0.05
	Etanol 96 % extract	5.80 ± 0.17	0.006 ± 0.00	8.68 ± 0.02	0.009 ± 0.00	9.79 ± 0.01
Kebiul Seed	Aquadest extract	5.74 ± 0.27	0.006 ± 0.00	7.78 ± 0.04	0.008 ± 0.00	5.96 ± 0.01
	Etanol 70% extract	5.05 ± 0.29	0.005 ± 0.00	7.81 ± 0.03	0.008 ± 0.00	7.97 ± 0.02

	Etanol 96 % extract	4.81 ± 0.23	0.005 ± 0.00	7.66 ± 0.07	0.008 ± 0.00	7.13 ± 0.02
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Extraction of black rice using 70% ethanol solvent produced the highest total phenolic content of 6.83 ± 0.28 mgGAE/g ($0.007 \pm 0.00\%$), which was superior to extracts using distilled water and 96% ethanol. The total phenolic content in this black rice extract is also higher than the phenolic content in extracts from bajakah root and kebiul seeds. These findings indicate that black rice is a primary raw material rich in polyphenols and acts as a major source of anthocyanins in the polyherbal formulation studied. This is in line with previous studies showing the efficiency of 70% ethanol in extracting phenolic compounds and anthocyanins from black rice (Bae et al., 2017; Devi et al., 2024).

70% ethanol shows higher efficiency in extracting polyphenolic compounds from black rice compared to 96% ethanol and distilled water, due to its polarity balance. The combination of ethanol and water at a concentration of 70% has optimal polarity that can dissolve phenolic compounds with moderate polar properties. The presence of water in 70% ethanol plays a role in opening the plant tissue structure, allowing for maximum extraction of phenolic compounds and anthocyanins. Conversely, 96% ethanol, which is more non-polar, is less effective at dissolving polar phenolic compounds, while distilled water has too high polarity to optimally extract semi-polar compounds such as polyphenols. Research by Sholihah et al. (2021) using the Ultrasonic-Assisted Extraction method on Sirampog Black Rice showed that the highest total phenolic content was achieved with ethanol at a concentration of around 48.5%, which provided the best solvent balance for the extraction of phenolic compounds and anthocyanins (Sholihah et al., 2021). In addition, Kurnia Hartati (2016) reported that 70% ethanol extract from black rice produced higher total phenolic content and antioxidant activity compared to extracts using distilled water (Hartati, 2016). Mechanistically, the 70% ethanol mixture balances the solubility of polar and non-polar compounds and increases the penetration of the solvent into plant tissue, allowing polyphenolic compounds to be extracted more optimally, resulting in extracts with higher concentrations and more effective antioxidant activity. However, this pattern differs for total flavonoid content. Black rice showed the highest flavonoid content in extracts with distilled water as the solvent, namely 7.77 ± 0.06 mgQE/g ($0.008 \pm 0.00\%$). Meanwhile, the highest overall flavonoid content was found in the 70% ethanol extract from bajakah wood roots, at 8.77 ± 0.02 mgQE/g ($0.009 \pm 0.00\%$), which supports the role of bajakah wood roots as an important co-pigment agent that can increase antioxidant activity in this polyherbal formulation. This is reinforced by reports noting the ability of 70% ethanol extract of bajakah wood root to optimally extract flavonoids and enhance their antioxidant activity (Anisa et al., 2022; Fitriyani & Fatahillah, 2022). In addition, kebiul seed extract also has good antioxidant potential with the highest total flavonoid content in 70% ethanol extract, which is 7.81 ± 0.03 mgQE/g ($0.008 \pm 0.00\%$), which is also higher than the flavonoid content of Black Rice extract. These findings illustrate that kebiul seeds have significant flavonoid potential, supporting their role in polyherbal formulations as copigments, which are sources of bioactive compounds that contribute to antioxidant activity (Ayuni Kurrata, 2020; Rahmi, 2020).

Therefore, these data confirm that black rice plays a major role as a source of phenolic compounds and anthocyanins, while bajakah root and kebiul seeds serve as significant sources of flavonoids in polyherbal formulations. This combination can synergistically enhance the antioxidant activity of the product through the copigmentation mechanism proposed in this study. Copigmentation is a mechanism that stabilizes and enhances the activity of natural pigments, especially anthocyanins, through interaction with other copigment compounds, which can be colored or colorless molecules, metal ions, or phenolic compounds. In this process, copigment compounds interact non-covalently with anthocyanins, replacing or strengthening group bonds in the anthocyanin structure, thereby forming a more chemically stable complex and increasing color stability. This copigmentation mechanism also contributes to a synergistic increase in antioxidant activity by protecting the anthocyanin structure from oxidative degradation. For example, copigment compounds can protect anthocyanin chromophores from water nucleophilic attacks that typically cause color loss and reduced antioxidant activity. Additionally, copigmentation can occur through electron transfer, where anthocyanins act as electron acceptors and copigments as electron donors, thereby increasing the antioxidant capacity of the system (Gençdağ et al., 2022).

When viewed from the results of antioxidant activity, the IC_{50} of Kebiul seeds extracted with distilled water showed a very strong value of 5.96 ppm. This indicates that Kebiul seeds have very high potential as a copigment agent with the strongest antioxidant activity compared to black rice and bajakah root extracts due to the content of specific phenolic and flavonoid compounds with high antioxidant activity that are effectively extracted in aquades and 70% ethanol solvents. In extraction with aquades and 70% ethanol, bioactive compounds such as flavonoids, tannins, and alkaloids are optimally extracted because these solvents have the appropriate polarity to extract polar and semi-polar compounds, which are responsible for very strong free radical activity. Unlike concentrated ethanol (96%), solvents with moderate water content such as 70% ethanol and aquades are more capable of extracting compounds that play an active role in antioxidants because their polarity balance supports penetration into plant tissues and the solubility of active compounds. Several studies support this, showing that red bajakah root stems have an IC_{50} of 26.29 ppm, confirming strong antioxidant activity related to high flavonoid content (Amiani et al., 2022).

In addition, the synergistic mechanism of various bioactive compounds in bajakah root enhances antioxidant activity, whereby flavonoids not only act as direct free radical scavengers but also as copigments that increase the stability and durability of anthocyanin molecules. This proves that bajakah root has higher potential as a copigment with stronger antioxidant activity. Thus, in polyherbal formulations containing anthocyanin-rich black rice and bajakah root as well as kebiul seeds as a source of flavonoids (copigments), this copigmentation mechanism synergistically increases the color stability and antioxidant activity effectiveness of the product through pigment structure protection and increased electron transfer. The results showed that the 70% ethanol extract of black rice had the highest total polyphenol content, namely 6.83 ± 0.28 mgGAE/g ($0.007 \pm 0.00\%$), 70% ethanol extract of bajakah root had the highest flavonoid content, namely 8.77 ± 0.02 mgQE/g ($0.009 \pm 0.00\%$), and distilled water extract of kebiul seeds showed the strongest antioxidant activity based on an IC_{50} value of 5.96 ppm.

When viewed from the results of antioxidant activity, the IC_{50} of Biji Kebiul aquadest extract is the strongest at 5.96 ± 0.01 ppm. This indicates that Biji Kebiul seed extract has very high antioxidant potential with the strongest antioxidant activity compared to black rice and bajakah root extracts due to the content of specific phenolic and flavonoid compounds with high antioxidant activity that are effectively extracted in aquades and 70% ethanol solvents. In extraction with aquades and 70% ethanol, bioactive compounds such as flavonoids, tannins, and alkaloids are optimally extracted because these solvents have the appropriate polarity to extract polar and semi-polar compounds, which are responsible for very strong free radical activity. Unlike concentrated ethanol (96%), solvents with moderate water content such as 70% ethanol and aquades are more capable of extracting compounds that play an active role in antioxidants because their polarity balance supports penetration into plant tissues and the solubility of active compounds (Amiani et al., 2022).

CONCLUSION

Research focusing on the antioxidant potential of black rice extract, bajakah root, and kebiul seeds specifically revealed that ethanol extract from black rice had the highest total polyphenol content, namely 6.83 ± 0.28 mgGAE/g ($0.007 \pm 0.00\%$). The 70% ethanol extract from bajakah wood root showed the highest total flavonoid content, reaching 8.77 ± 0.02 mgQE/g ($0.009 \pm 0.00\%$). The strongest antioxidant activity was found in the distilled water extract of kebiul seeds, with a value of 5.96 ± 0.01 ppm.

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