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Research Article

Potent antioxidant activity of black grass jelly (*Mesona palustris* BL) leaf extract and fractions

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Abstract

Black grass jelly (*Mesona palustris* BL) is an Indonesian traditional food that is rich in antioxidants and believed to have potential for treating various diseases such as diabetes, hypertension, and cancer. The present study aimed to determine the antioxidant activity of black grass jelly leaf extract as well as fractions using ABTS, DPPH and FRAP methods. Following ethanol extraction and subsequent fractionations, total flavonoid level was determined, followed by antioxidant activity tests using the ABTS, DPPH and FRAP with vitamin C as references. The tests revealed the following order of antioxidant activities, ethyl acetate fraction>extract>water fraction>n-hexane fraction. All test substances had IC_{50} of <50 ppm, which categorized them as having very high antioxidant activities. In line with this data, on the basis of reducing power, ethyl acetate extract was shown to be the most potent antioxidant, having a value of 20.24 mgAAE/g sample. Overall, results of the present study suggest the potential use of black grass jelly as a part of the therapeutic armamentarium for oxidative stress-related diseases.

Keywords

Antioxidant, black grass jelly, *Mesona palustris* BL, ABTS, DPPH, FRAP

Introduction

Oxidative stress is a condition in which the body produces more free radicals, such as hydroxyl radicals, superoxide radicals, and lipid peroxides that are capable of being reduced by natural antioxidant system. This is a natural process that occurs in our body, however when excessive redox imbalance happens, life-threatening diseases can ensue (Li et al. 2022; Rey et al. 2023).

Black grass jelly (*Mesona palustris* BL) leaf has been used traditionally as a food ingredient which contains high levels of antioxidants. Empirically, black grass jelly leaf is believed to be efficacious in preventing oxidative stress (Widyaningsih 2012). Phenolic compounds are bioactive components of leaf which have antioxidant properties, classified as exogenous antioxidants (Galano et al. 2016; Zeng et al. 2023). Structurally, antioxidants contain one or more hydroxyl groups in their aromatic rings, and the derivatives of these compounds include a large number of flavonoids, alkaloids, tannins, as well as other phenolic compounds (Hendratama et al. 2020).

There are several methods for assessing antioxidant activity. The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method measures the number of free radicals that can be dampened. The ABTS free radical compound originates from the oxidation of potassium persulfate with ABTS diammonium salt in ethanol which can be analyzed by spectrophotometry at a wavelength of 753 nm (Dawidowicz and Olszowy 2013; Dong et al. 2015; Ilyasov et al. 2020). The other method is 2,2-diphenyl-1-picrylhydrazyl (DPPH) where the interaction of antioxidants with DPPH either by transferring electrons or hydrogen

radicals to DPPH neutralizes the free radical character of DPPH to form reduced DPPH (Kedare and Singh 2011; Baliyan et al. 2022). The FRAP (Ferric Reducing Antioxidant Power) method is also used in research, which directly measures antioxidants in ingredients. This method measures antioxidants by reducing the blue ferric to yellow ferrous complex (Payne et al. 2013; Fernandes et al. 2016).

In the present study antioxidant activities of black grass jelly extract and fractions were assessed using ABTS, DPPH, and FRAP methods.

Materials and methods

Collection and determination of plant materials

Black grass jelly (*Mesona palustris* BL) leaves were obtained from the Manoko plantation Lembang, West Java and determined at the Taxonomy and Plant Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, West Java, Indonesia.

Extraction

One-and-a-half kilograms of black grass jelly leaves were extracted by maceration with 96% ethanol solvent for 3 days, with periodic stirring, and then concentrated with a rotary evaporator.

Fractionation

The concentrated extract was fractionated using liquid-liquid extraction method with three different solvents having increasing polarity, namely n-hexane, ethyl acetate, and water. The ethanol extract was diluted with water in a 1:1 ratio. The homogenous solution was then fractionated with n-hexane with the same extract to solvent ratio, followed by collection of the supernatant. This procedure was repeated three times. The residue was then subjected to the same procedure of fractionation using ethyl acetate and water, consecutively.

Phytochemical sreening

Phytochemical screening was carried out on the pulverized dried plant (crude drug), extracts and fractions to determine the presence of secondary metabolites including alkaloids, flavonoids, saponins, terpenoids, steroids and tannins.

Total flavonoid determination

The total flavonoid content in black grass jelly leaf extract was quantified according to the protocol as previously described by Shraim et al. (2021). The method is based on the formation of Al(III)-flavonoids chelates. The absorbance at 430 was measured to determine the total flavonoid. Based on the quercetin calibration curve, the total amount of flavonoids was expressed as milligram quercetin equivalents per gram of each extract (mg QE/g extract).

Identification of metabolites by HPLC

Chromatographic analysis was performed on a Waters Alliance e2695 HPLC system (Waters Corporation, Milford, MA, USA) equipped with column Merck LiChroCART (250 mm × 4.6 mm). The chromatograph was equipped with a UV-Vis 2489 detector (Waters Corporation, Milford, MA, USA). The injection volume was 10 µL. The mobile phase was composed of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B), with a flow rate of 1 mL/min. The spectrum was measured at a wavelength of 254 nm. Peak areas were calculated using Empower 3 software.

Antioxidant activity testing using the ABTS method

ABTS solution was prepared by weighing 7,100 mg of ABTS, dissolved in 5 ml of ethanol, and incubated for 24 hours. An amount of 3,500 mg of $K_2S_2O_8$ was weighed, dissolved in 5 ml of ethanol, and incubated for 24 hours. The solutions were then mixed in light-protected chamber and added with ethanol to the final volume of 25 ml. The test was carried out on a series of ascorbic acid and quercetin concentrations (3; 4; 5; 6; and 7 ppm) and that of black grass jelly leaf fraction (10; 20; 30; 40 and 50 ppm). One mililiter of each concentration of sample was mixed with 1 mL of ABTS reagent, and the final solution was checked for the absorbance at 750 nm (Rohmah 2022; El-Guourrami et al. 2023).

Antioxidant activity testing using the DPPH method

The test was carried out on a series of ascorbic acid solution (1, 2, 3, 4, and 5 ppm) and those of black grass jelly leaf fraction (10, 20, 30, 40 and 50 ppm). Two mililiters of each of the test solutions were placed in a test tube. They were then added to 2 mL of 0.1 mM DPPH solution, homogenized, and kept for 30 min in a light-protected chamber. The absorbance of the final solution was measured at 515 nm (Liu et al. 2015; Ahmad Nejhad et al. 2023).

Antioxidant activity testing using the FRAP method

A standard curve of $FeSO_4.7H_2O$ was prepared from a series of concentrations (20, 40, 60, 80 and 100 ppm). An amount of 0.1 mL of each concentration was then added to 1.5 mL of FRAP reagent and left for 20 minutes. The absorbance of the final solution was then observed at 595 nm. For antioxidant measurement, similar procedure was repeated but instead of the ferric solution, ascorbic acid or black grass jelly was mixed with FRAP reagent (Wahjuningsih et al. 2021; El-Guourrami et al. 2023).

Results

Plant determination

The determination carried out at the Taxonomy and Plant Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor, West Java, Indonesia, confirmed that the plant used was black grass jelly (certificate No.25/HB/06/2022).

Phytochemical screening

Results of phytochemical screening are presented in Table 1. Crude drug and extract tested positive for alkaloids, flavonoids, saponins, tannins, phenols. In general, the screening on fractions revealed similar results to those of crude drug and extract, with the exception of the n-hexane fraction where saponins was absent.

Total flavonoid

The measurement result showed a total flavonoid content of 4.9 mg QE/g extract. This quantity was relatively small, but was considerably higher compared to other plants with known antioxidant activity.

Characterization of metabolites in extract

The HPLC spectrogram of black grass jelly, showing the composing metabolites, is presented in Fig. 1 . Further identification revealed several metabolites of *Mesona palustris* which were also found in *Mesona sinensis*, as presented in Table 2. The corresponding retention time indicated caffeic acid (14.738 min), quercetin 3-o-galactoside (20.185 min), isoquercetin (21.850 min), astragalin (27.341 min), and rosmarinic acid (29.128 min) (Hung and Yen 2002).

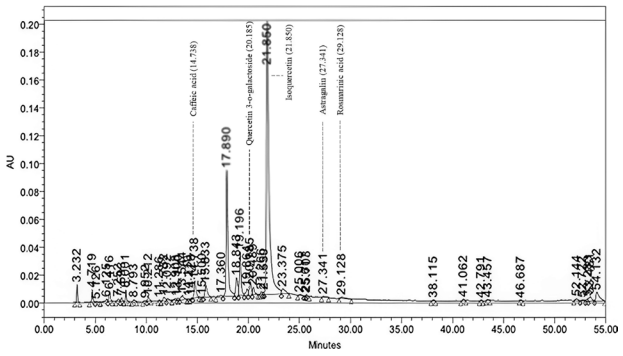

Figure 1. HPLC Spectrogram of black grass jelly extract.

Table 1. Results of phytochemical screening of crude drug, extract and fraction.

Metabolite	Crude drug	Extract	n-Hexane fraction	Ethyl acetate fraction	Water fraction
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	-	+	+
Tannins	+	+	+	+	+
Phenols	+	+	+	+	+

Table 2. Results of extract constituents' identification.

Retention time (min)		Compound
Mesona sinensis	*Mesona palustris*	
14.647	14.738	Caffeic acid
20.040	20.185	Quercetin 3-o-galactoside
21.860	21.850	Isoquercetin
27.560	27.341	Astragalin
30.553	29.128	Rosmarinic acid

Antioxidant activity tests

Results of the measurement of antioxidant activity using ABTS (Fig. 2A) showed that test substance with the lowest IC_{50} was ethyl acetate fraction (2.512 ppm), meanwhile, the one with the highest IC_{50} was n-hexane fraction (3.504 ppm). When the activity was measured with DPPH method, as presented in Fig. 2B, similar pattern of result was obtained. Ethyl acetate fraction had the lowest (17.74 ppm), and that of n-hexane showed the highest IC_{50} (25.14 ppm). However, as shown in Fig. 3, with FRAP method, the opposite was found. Quantitative measurement results showed

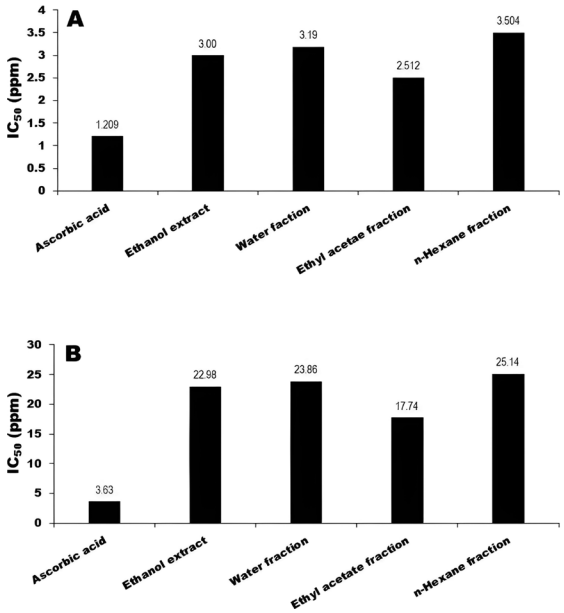

Figure 2. Results of antioxidant activity test using ABTS (**A**) and DPPH (**B**). The activity is represented by IC_{50}.

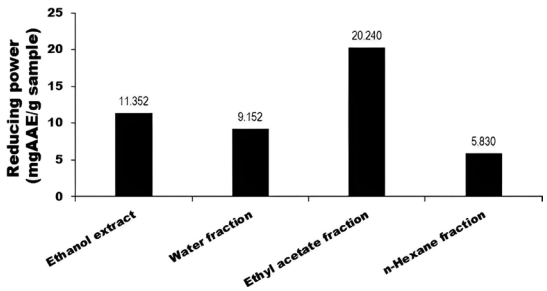

Figure 3. Results of antioxidant activity test using FRAP. The activity is represented by reducing power.

that the antioxidant capacity was equivalent to ascorbic acid. As shown in Fig. 3, the respective values for extract, water fraction, ethyl acetate fraction, and n-hexane fraction were 11.352, 9.152, 20.24, and 5,830 mgAAE/g sample. Based on FRAP, therefore, the order of reducing power was ethyl acetate fraction>extract>water fraction>n-hexane fraction.

Discussion

The present study extended the investigation of the antioxidant activity of black grass jelly extract by further assessing the reducing power of n-hexane, ethyl acetate and water fractions of the extract. Many pharmacological effects have been known to involve oxidative pathways. Antioxidant activy has well been known to involve in the mechanisms of several pharmacological effects (Mohieldein et al. 2015; Khutami et al. 2022).

Among others, secondary metabolites derived from plants known to have health benefits are phenolics and flavonoids (Liu 2004; Lin et al. 2016; Desgagné-Penix 2017). These metabolites have been shown to scavenge free radicals and lower oxidative stress. The production of phenolic compounds is commonly tied to stressors to which plants are exposed (Liu 2004; Larbat et al. 2012). In general, the quantitative order of the secondary metabolites contained in plant is terpenoids<flavonoids<alkaloids<phenolics (Nantongo et al. 2018). With the observation of total flavonoid of 4.9 mg QE/g extract, the extract obtained in this study could be considered as containing a low amount of this particular metabolite. An earlier study has indicated that the extracting solvent polarity dictated the levels of flavonoid content (Jing et al. 2015). Although the afore-mentioned amount of total flavonoid level might be considerably small, the value was relatively higher compared to other plants with known antioxidant activity. Thus, Teucrium takoumitense, which was shown to have potent reducing power, had only a total flavonoid content of 2.99 mg QE/g extract (El-Guourrami et al. 2023).

Hung and Yen (2002) have found that there were several phenol derivatives in *Mesona procumbens* Hemsl leaves, including caffeic acid, protocatehuic acid, α-tocopherol, ρ-hydrobenzolic acid, vanilic acid, and syringic acid. This data was in line with the HPLC spectrogram observed in our present study which revealed the presence of caffeic acid, quercetin 3-o-galactoside, isoquercetin, astragalin, and rosmarinic acid, among others. Caffeic acid as a phenolic derivative has been shown to demonstrate potent antioxidant activity (Alam et al. 2022). It is soluble in ethanol and ethyl acetate, and this physicochemical characteristic might explain the highest reducing power of ethyl acetate fraction. Ethanol extract came in second in terms of activity due to higher variety of components, some of which might possibly have opposite activities.

The results further showed that n-hexane fraction had the lowest reducing power. This might be tied to the lack of saponins as observed from the phytochemical screening data. Early studies showed that this metabolite had significant antioxidant activity. Thus, Chen et al. (2014) revealed strong reducing power of saponin from *Radix trichosanthis* extract, and Khan and colleagues (2022) demonstrated that anti-inflammatory and antiangiogenesis effects of saponin were associated with its antioxidant activity.

Conclusion

Tests for antioxidant activity using ABTS, DPPH, and FRAP methods show that ethanol extract, as well as its water, ethylacetate, and n-hexane fractions have strong antioxidant activity, with ethyl acetate fraction being the strongest. This fraction is, thereore, worth studying further for pharmacological activities.

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