

The antioxidant activity of several antidiabetic herbal products

by Ichwan Ridwan Rais

Submission date: 28-Feb-2023 08:21AM (UTC+0700)

Submission ID: 2024801494

File name: Pharmaciaana_Vol_12_no_2_th_2022.pdf (506.67K)

Word count: 4970

Character count: 26756

The antioxidant activity of several antidiabetic herbal products

Ichwan Ridwan Rais*, Andhika Septiawan, Meta Ayuni,
Dhega Agung Wichaksono, Nanik Sulistyani

²
Faculty of Pharmacy, Universitas Ahmad Dahlan
Jl. Prof. Soepomo, S.H., Warungboto, Umbulharjo, Yogyakarta, Indonesia

Submitted: 03-01-2022

Reviewed: 24-03-2022

Accepted: 14-06-2022

ABSTRACT

The oxidative stress mechanism in the body involves the balance between increased ROS and decreased antioxidant agents. ROS can damage DNA, proteins, and lipids, leading to pathology and impaired insulin secretion by dysregulation of several genes expression. High concentrations of ROS are often followed by a low activity of antioxidants from endogenous sources. As a result, exogenous antioxidants are required to neutralize ROS. As reported, medicinal plants, which have had various traditional applications, contain large amounts of secondary metabolites, proven to be exogenous antioxidant agents. *Jamu*, a traditional medicine, also known as an alternative medication, can be widely and easily found in traditional markets. Antidiabetic herbal products are among the most popular of *jamu*. A ¹¹diabetic mechanism has a strong relationship with antioxidant roles in many biological systems. The aim of this research was to figure out ¹⁴the potential antioxidant activity of antidiabetic herbal products. Eight antidiabetic *jamu* samples were analyzed for their antioxidant activity by measuring the DPPH ¹⁴radical scavenging activity, phosphomolybdenum antioxidant activity, and FRAP, as well as for their total phenolic and flavonoid contents. Good linearity was a general finding for the quantitative analysis, with an average correlation coefficient of 0.999 for the standards. The highest total flavonoid content found was 8.66 ± 0.11 mgQE/g, and the highest total phenolic content was 22.66 ± 0.15 mgGAE/g. With regard to antioxidant activity, all of the samples demonstrated weak DPPH radical scavenging activity, while phosphomolybdenum antioxidant activity and FRAP were the highest at 48.58 ± 0.45 mgQE/g extract and 226.98 ± 0.19 mgQE/g extract, respectively. These results indicated that the claimed antidiabetic herbal products could be prepared as exogenous antioxidant sources, irrespective of being with or without antioxidant activity, for diabetes treatment.

Keywords: antioxidant, DPPH, FRAP, *Jamu*, Phosphomolybdenum

*Corresponding author:

²Ichwan Ridwan Rais

Faculty of Pharmacy, Universitas Ahmad Dahlan

Jl. Prof. Soepomo, S.H., Warungboto, Umbulharjo, Yogyakarta, Indonesia

Email: ichwan.rais@pharm.uad.ac.id



INTRODUCTION

A free radical is an unstable molecule with one highly reactive unpaired electron (Agarwal et al., 2006). It is produced by air pollution, industrial combustion residue, smoke, and solar radiation. As a reactive molecule, a free radical will be highly attractive to electrons from other molecules or body cells. This reaction will be sustained until there is damage to body cells, thus frequently associated with various diseases, including diabetes (Kothari et al., 2010). Among notable free radicals are reactive oxygen species (ROS), which include hydrogen peroxide, hydroxyl radicals, and superoxide anion. They are frequently associated with the oxidative stress mechanism. ROS can damage DNA, proteins, and lipids, leading to pathology (Schieber & Chandel, 2014) and impaired insulin secretion by dysregulation of several genes expression (Ofosu et al., 2020). The oxidative stress mechanism in the body involves the balance between increased ROS and decreased antioxidant agents (Kunwar, 2011). High concentrations of ROS are often followed by a low activity of antioxidants from endogenous sources, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes. As a result, exogenous antioxidants are required to neutralize ROS (Zulaikhah, 2017).

One popular method for assessing antioxidant activity is that method that uses diphenylpicrylhydrazyl (DPPH), a stable free radical currently easy to use in the interpretation of an antioxidant experiment (Molyneux, 2004). Other methods are phosphomolybdenum and ferric reducing antioxidant power (FRAP) assays. These are colorimetric methods that measure the ability of a sample to reduce the intense color of the main reagent, hence changing the absorbance value (Badarinath et al., 2010).

Plant-based flavonoid and phenolic compounds have been reported to significantly contribute to the protection of the body from oxidative stress with their antioxidant activity (Chandrasekara & Shahidi, 2011). This antioxidant activity is involved in several pathological mechanisms of diabetes, such as stimulating insulin production from the β -cells of the pancreas, promoting insulin sensitivity, increasing GLUT2 and PPAR γ , and enhancing β -cells' survival (Vinayagam & Xu, 2015).

As reported previously, medicinal plants, which have seen various traditional applications, contain large amounts of secondary metabolites that have evidently been proven to be exogenous antioxidant agents. Various studies indicated that these metabolites have a role in counteracting ROS. One of the most recognized antioxidant agents, phenol, in addition to the flavonoid, has been demonstrated to be a promising oxidative stress neutralizer (Shi et al., 2019). These most prominent secondary metabolites are widely known, and they have exhibited impact in antioxidant assays such as DPPH, phosphomolybdenum, and FRAP assays, by which their ability to suppress free radicals is investigated.

Jamu is a traditional medicine known in Indonesia to maintain health and fitness; it is also used in disease treatments (Elfahmi, et al., 2014). It is renowned and popular as an alternative medication and as a complement. It is even at times used as the main medication along with modern drugs. As an alternative medicine, *jamu* is vastly and readily available in traditional markets. Empirical evidence showed that, in general, Indonesian people are long time consumers of *jamu*; they drink macerated medicinal plants as *jamu* (Pan et al., 2013). Meanwhile, based on scientific evidence, it is highly expected that traditional medicines be developed and integrated with modern medicines in the public health care system (Mulyani et al., 2016). It is deemed necessary to collect, report, and deliver both empirical and scientific evidence for *jamu*. This present study in particular, was conducted to collect scientific evidence of the possibility of using *jamu* as a source of natural exogenous antioxidants.

MATERIALS AND METHODS

Materials

Eight *jamu* products were used as samples in this research. These were crude herbal compositions from several medicinal plants randomly obtained from Beringharjo Traditional Market, Yogyakarta, Indonesia. All of the samples were claimed by the sellers to be anti-diabetic herbal

medicines. They were coded A to H, respectively. The crude herbal medicines were finely powdered and macerated using 96% ethanol at a ratio of 1:10 (w/v) based on the method by (FHI, 2017) with a slight modification. Maceration was performed for 48 hours and repeated three times. Each of the macerated medicines was filtered with grade 1 Whatman® filter paper, then collected, mixed, and evaporated with a rotary evaporator at 50 °C under pressure to get viscous extracts. This evaporation process was followed by a water bath, where the extracts were kept in sealed containers at 4 °C. Analysis of levels of solvents, chemicals, and reagents was performed in this research.

Qualitative analysis of phytochemical contents

Total phenolic content test

One mL of 10% FeCl₃ was mixed with ethanolic extract. A deep blue color in the samples indicated the presence of phenolic compounds (Salamah & Farahana, 2014).

Total flavonoid content test

A solution of ethanolic extract was dropped on a piece of Whatman® filter paper and evaporated with ammonia. A yellow color that appeared on the paper indicated the presence of flavonoid compounds (Salamah & Farahana, 2014).

DPPH (2,2-diphenylpicrylhydrazyl) test

One mL of 100 ppm DPPH reagent was added to 1.0 mL of ethanolic extract. A yellow color indicated antioxidant potential (Salamah & Farahana, 2014).

Phosphomolybdenum test

Each 1.0 mL of ethanolic extract was mixed with 1.0 mL of phosphomolybdenum reagent. Antioxidant ability was indicated by a green color (Salamah & Farahana, 2014).

FRAP (ferric reducing antioxidant power) test

Each 1.0 mL of ethanolic extract was mixed with FRAP reagent at a 1:3 ratio and incubated for 30 minutes at 37 °C. Antioxidant ability as indicated by a blue color (Sukweenadhi et al., 2020).

Quantitative analysis of total phenolic and flavonoid contents

Standard curve of gallic acid

A stock solution of 1,000 ppm gallic acid was made by dissolving 25 mg of gallic acid in 1 mL of ethanol, added with distilled water to get 25 mL of solution, in a volumetric flask. Five different series concentrations—5, 10, 15, 20, and 25 ppm were prepared by diluting the solution. As much as 0.3 mL of the solution of each series concentration was mixed with 1.25 mL of Folin-Ciocalteu (10%) in an appropriate flask. The mixture was shaken for a minute and left for 3 minutes. Next, 1.2 mL of 7.5% Na₂CO₃ was added, followed by incubation for 54 minutes. The absorbance was measured using a UV-Vis spectrophotometer (Shimadzu UV-1900) at a wavelength of 751 nm. The measurement of each concentration was replicated three times (Alfian & Susanti, 2012).

Determination of total phenolic content

All sample solutions were prepared by dissolving 16 mg of ethanolic extracts in 10 mL of methanol and distilled water (at a 1:1 ratio). As much as 0.3 mL of each sample solution was added with 1.5 mL of 10% Folin-Ciocalteu in an appropriate flask. All sample mixtures were then processed as described above (Alfian & Susanti, 2012) in three replications.

Standard curve of quercetin

A stock solution of 1,000 ppm quercetin was prepared by dissolving 25 mg of quercetin in 25 mL of ethanol. Solution series concentrations of 6, 7, 8, 9, and 10 ppm were made and diluted from the stock solution. Two mL of each concentration was then mixed with 2 mL of 2% AlCl₃, shaken, and

incubated for 11 minutes. Measurement using a UV-Vis spectrophotometer (Shimadzu UV-1900) was performed for each concentration at 437 nm (Rais, 2015).

Determination of total flavonoid content

One thousand ppm of sample solutions were obtained by dissolving 10 mg of ethanolic extracts in 10 mL of ethanol. Two mL of sample was added with 2 mL of 2% AlCl₃. The mixture was then shaken and incubated for 11 minutes. A UV-Vis spectrophotometer (Shimadzu UV-1900) was used to measure the absorbance of the solution of each concentration at 437 nm in three replications (Rais, 2015).

Antioxidant activity

DPPH assay

Using the method by (Rais, 2016) with a slight modification, a standard solution of quercetin was prepared by dissolving 10 mg of quercetin powder in 10 mL of methanol until reaching a 1,000 ppm concentration. Solutions of series concentrations of 10, 11, 12, 13, and 14 ppm were prepared from the standard solutions for a calibration curve. All extracts were prepared as sample solutions by dissolving 25 mg of each extract in 25 mL of ethanol (1000 ppm). These solutions were then diluted to obtain 400, 410, 420, and 430 ppm sample solutions. Each concentration of standard and sample solution was mixed with 100 ppm DPPH at a 1:1 ratio. All mixed solutions were then shaken and incubated in a dark place at room temperature for 30 minutes. The scavenging activity of free radicals was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

Where the DPPH solution absorbance (t = 0 min) was denoted by A₀ and the sample solution absorbance (t = 30 min) was denoted by A₁. Linear regression was conducted based on the concentration of the standard or sample solutions versus the percentage of scavenging activity to obtain the value of 50% DPPH scavenging activity (IC₅₀ value).

Phosphomolybdenum assay

A phosphomolybdenum reagent was made by dissolving 3 mL of sulphuric acid, 199 mg of sodium phosphate, 247 mg of ammonium molybdate, and distilled water of up to 50 mL. A calibration curve was prepared by diluting the quercetin solution to 40, 45, 50, 55, and 60 ppm of series concentrations. Sample solutions were prepared by diluting each extract solution in ethanol to obtain 210, 800, and 900 ppm of concentrations. One mL of each sample and the standard solution were added with 1 mL of phosphomolybdenum reagent. The mixture was then heated for 60 minutes at 95 °C, added with ethanol of up to 5 mL, and left for 10 minutes at room temperature. A UV-Vis spectrophotometer was used to measure the absorbance of each solution at a wavelength of 695 nm (Shahwar & Raza, 2012).

FRAP assay

Three solutions were prepared to form a FRAP reagent by dissolving 187 mg of sodium acetate trihydrate in 16 mL of acetic acid (pH 3.4), added with distilled water to reach a volume of 250 mL, 270 mg of ferric chloride (FeCl₃.6H₂O) in 100 mL of distilled water, and 150 mg of TPTZ in 50 mL of 40 mM H₂SO₄. As much as 2.5 mL of FeCl₃.6H₂O solution (20 mM), 25 mL of sodium acetate trihydrate solution, 2.5 mL of TPTZ solution (10 mM), and distilled water were mixed to obtain 100 mL of FRAP reagent solution. Standard solutions were made by diluting quercetin solution to 60, 65, 70, 75, and 80 ppm of series concentrations. Sample solutions were made by dissolving each extract in ethanol

to obtain 750, 800, 850, and 900 ppm of concentrations. Each standard and sample solution was mixed with the FRAP reagent at a 1:3 ratio, incubated for 30 minutes at 37 °C, and measured using a UV-Vis spectrophotometer at a wavelength of 596 nm (Sukweenadhi et al., 2020).

Data Analysis

The total flavonoid content, total phenolic content, and antioxidant activity were figured out from the slope of the standard curve, which presented the relationship between concentration and absorbance. Linear regression of standards from each assay was then used to obtain the equivalence of flavonoid and phenolic contents (mgQE and mgGAE). Then, antioxidant activity assays were performed. The equivalence of total flavonoid and phenolic contents was then analyzed and correlated with the antioxidant activity assays.

RESULTS AND DISCUSSION

In this present study, eight different *jamu* products that were claimed to be antidiabetic were collected. The samples were subjected for identification of their herbal compositions and coded A to H, respectively, as listed in Table 1. Most of the contents of the samples were identified as antidiabetic with several specific mechanisms, including antioxidant one. For example, *Andrographis paniculata* with contents of flavonoids was reported by scientific studies to exhibit antioxidant and hypoglycemic activities (Nugroho et al., 2014; Rais, 2015). In addition, *Phaleria macrocarpa* and *Sonchus arvensis* with their polyphenols were able to suppress oxidative stress and directly affected diabetes (Hanggaresty & Warditiani, 2021).

There were correlations between diabetes and oxidative stress (Unuofin & Lebelo, 2020) through glucose autoxidation, non-enzymatic glycation of proteins, and the polyol-sorbitol pathway (Hanggaresty & Warditiani, 2021). Some research reports have provided evidence at in vitro, cellular, and molecular levels. These research works reported the potential of phytochemicals in diabetes treatment, in which case they can easily be obtained from *jamu*.

The maceration method was performed to obtain the samples because it is simple, easy, and widely used for the traditional process of extracting active compounds. This method can be used to extract thermolabile compounds, and it is effective for phenolic compounds extraction (Zhang et al., 2018). Ethanol was chosen as the extraction solvent. It has an alkyl group with a nonpolar site and a hydroxyl group with a polar site; therefore, ethanol is commonly used to extract compounds of various polarities (Choung et al., 2013). The yield percentages of the samples are shown in Table 2. The yield percentage can be used as a gain parameter of the extraction process.

Flavonoid and phenolic contents were reported to be generally distributed in all parts of the plant. These secondary metabolites are commonly known as free radical scavengers and oxidation inhibitors in body protection from oxidative stress (Ofosu et al., 2020). It is common to predict the presence of flavonoid and phenolic contents as well as antioxidant activity with qualitative analysis of samples. As shown in Figure 1, the chromatography profile of flavonoids appeared with an intensive yellow color after spraying with ammonia. Meanwhile, phenolic compounds appeared as a deep blue after tainting with FeCl₃ spray reagent for the standards and as a poor color expression for the samples. These were also found with a tube test with specific reagents.

Table 1. Sample product codes and herbal compositions

Product Code	Herbal Compositions
A	<i>Citrus hystrix</i> , <i>Phaleria macrocarpa</i> , <i>Curcuma zedoaria</i> , <i>Zingiber officinale</i> , <i>Garcinia mangostana</i> L., <i>Sonchus arvensis</i>
B	<i>Piper cubeba</i> L., <i>Baeckea frutescens</i> , <i>Phaleria macrocarpa</i> , <i>Jasminum officinale</i> , <i>Caesalpinia sappan</i> L., <i>Orthosiphon aristatus</i> , <i>Curcuma aeruginosa</i> Roxb., <i>Curcuma xanthorrhiza</i> , <i>Cinnamomum zeylanicum</i> , <i>Melaleuca fructus</i>
C	<i>Sonchus arvensis</i> , <i>Melaleuca fructus</i> , <i>Baeckea frutescens</i> , <i>Piper cubeba</i> L., <i>Caesalpinia sappan</i> L., <i>Tinospora cordifolia</i> , <i>Curcuma aeruginosa</i> Roxb., <i>Curcuma xanthorrhiza</i> , <i>Helicteres isora</i>
D	<i>Baeckea frutescens</i> , <i>Curcuma xanthorrhiza</i> , <i>Piper cubeba</i> L., <i>Cinnamomum zeylanicum</i> , <i>Amomum compactum</i> , <i>Melaleuca fructus</i> , <i>Tinospora cordifolia</i> , <i>Foeniculum vulgare</i> , <i>Jasminum officinale</i> , <i>Caesalpinia sappan</i> L., <i>Sonchus arvensis</i>
E	<i>Caesalpinia sappan</i> L., <i>Curcuma xanthorrhiza</i> , <i>Cinnamomum zeylanicum</i> , <i>Syzygium aromaticum</i> , <i>Sonchus arvensis</i> , <i>Andrographis paniculata</i>
F	<i>Melaleuca fructus</i> , <i>Baeckea frutescens</i> , <i>Andrographis paniculata</i> , <i>Sonchus arvensis</i> , <i>Jasminum officinale</i> , <i>Caesalpinia sappan</i> L., <i>Piper cubeba</i> L., <i>Curcuma longa</i> L., <i>Curcuma aeruginosa</i> Roxb., <i>Curcuma xanthorrhiza</i> , <i>Phaleria macrocarpa</i>
G	<i>Caesalpinia sappan</i> L., <i>Andrographis paniculata</i> , <i>Melaleuca leucadendra</i> , <i>Curcuma xanthorrhiza</i> , <i>Curcuma aeruginosa</i> Roxb., <i>Zingiber officinale</i> , <i>Melaleuca fructus</i> , <i>Baeckea frutescens</i> , <i>Phaleria macrocarpa</i>
H	<i>Curcuma xanthorrhiza</i> , <i>Jasminum officinale</i> , <i>Caesalpinia sappan</i> L., <i>Phaleria macrocarpa</i> , <i>Andrographis paniculata</i> , <i>Piper cubeba</i> L., <i>Cinnamomum zeylanicum</i> , <i>Curcuma aeruginosa</i> Roxb., <i>Melaleuca fructus</i> , <i>Baeckea frutescens</i> , <i>Sonchus arvensis</i>

Table 2. The yield percentages of the samples

Sample	Weight (g)	Extract	
		Weight (g)	Yield (%)
A	72.60	6.25	8.61
B	144.70	12.94	8.94
C	156.03	20.29	13.01
D	224.78	18.52	8.24
E	71.70	8.88	12.38
F	211.09	18.65	8.84
G	180.50	13.86	7.68
H	153.09	14.35	9.37

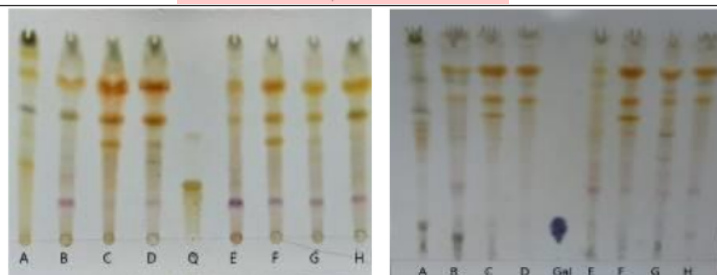


Figure 1. Thin-layer chromatography of samples

A–H (sample codes); Q (quercetin); Gal (gallic acid); 1 (stationary phase: silica F₂₅₄; mobile phase: CHCl₃:MeOH:H₂O (80:12:2); spray reagent: ammonia). 2 (stationary phase: silica F₂₅₄; mobile phase: CHCl₃:MeOH:Formic acid (80:15:10); spray reagent: FeCl₃)

Further, each sample was analyzed using DPPH³ Phosphomolybdenum, and FRAP assays for the qualitative analysis of the antioxidant activity. The results are listed in Table 3.

Table 3. Qualitative analysis of flavonoid and phenolic contents

Sample	Flavonoid	Phenolic	DPPH	Phosphomolybdenum	FRAP
33	+	+	+	+	+
B	+	-	+	+	+
C	+	-	+	+	+
D	+	-	+	+	+
E	+	-	+	+	+
F	+	+	+	+	+
G	+	+	+	+	+
H	+	+	+	+	+

Note: + (positive result); - (negative result)

The tube test analysis results showed the presence of flavonoid and phenolic contents along with their ability to react with 3 different types of oxidative reagents. However, samples B, C, D, and E did not show any specific color indicating the presence of phenolic content; they exhibited a dark green color instead. These results correlated with the TLC assay, which showed an intensive color indicator of flavonoid content and a weak color indicator of phenolic content. Quantification of antioxidant ability was then performed based on these qualitative assays, the results of which indicated that the contents were capable of antioxidant activity.⁴

Table 4 presents the total flavonoid and phenolic contents of each *jamu* sample. The total flavonoid content was lower than the total phenolic content (2.42 ± 0.01 to 5.98 ± 0.01 mgQE/g extract vs 13.42 ± 0.05 to 22.66 ± 0.15 mgGAE/g extract). The difference in flavonoid and phenolic contents between the samples was caused by the difference in composition between the *jamu* products.⁵

The first antioxidant assay was determined with scavenging method using the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). When a chemical constituent donates a hydrogen electron to DPPH, the DPPH will react and transform into DPPH-H (1,1-diphenyl-2-picrylhydrazine), indicated by an intensive yellow color (Sukweenadhi et al., 2020). This method is commonly used in antioxidant assays. Antioxidant activity is categorized under the DPPH scavenging activity method according to (Molyneux, 2004) into very strong (IC₅₀ value < 50 ppm), strong (IC₅₀ value = 50–100 ppm), moderate (IC₅₀ value = 101–150 ppm), and weak (IC₅₀ value > 150 ppm). As shown in Table 5, all of the samples demonstrated weak antioxidant activity under this method.

Table 4. Quantitative analysis of total flavonoid and phenolic content

Sample	Flavonoid (mgQE/g) extract*	Phenolic (mgGAE/g) extract**
A	4.61 ± 0.07	19.36 ± 0.30
B	2.40 ± 0.01	13.42 ± 0.05
C	5.98 ± 0.01	13.58 ± 0.19
D	5.25 ± 0.07	13.77 ± 0.18
E	2.89 ± 0.02	22.66 ± 0.15
F	8.66 ± 0.11	15.10 ± 0.20
G	2.42 ± 0.01	13.55 ± 0.04
H	4.31 ± 0.01	15.03 ± 0.02

Note: *QE = quercetin equivalent; regression equation of standard curve of quercetin: $y = 0.042x + 0.028$ with $r^2 = 0.9941$; **GAE = gallic acid equivalent; regression equation of standard curve of gallic acid: $y = 0.165x + 0.019$ with $r^2 = 0.9991$; ± SD (n = 3)

Phosphomolybdenum assay is based on the reduction of molybdenum (VI) to molybdenum (V), indicated by a green color (Prieto et al., 1999). The phosphomolybdenum reducing power of the samples was between 38.85 ± 0.17 and 48.58 ± 0.45 mgQE/g extract.

FRAP assay, meanwhile, has some advantages in its fast, easy, and simple administration. The FRAP reaction implicates the reduction of Fe^{3+} from tripyridyl-triazine Fe^{3+} (TPTZ) into Fe^{2+} (TPTZ), indicated by a blue color (Sukweenadhi et al., 2020). The FRAP reducing power of the samples was between 174.36 ± 0.45 and 226.98 ± 0.19 mgQE/g extract (Table 5).

Overall, 8.66 ± 0.11 mgQE/g of total flavonoid content and 22.66 ± 0.15 mgGAE/g of total phenolic content showed a weak DPPH scavenging ability (± 400 ppm), with phosphomolybdenum reducing the power of 48.58 ± 0.45 mgQE/g extract and FRAP of 226.98 ± 0.19 mgQE/g extract. The weak DPPH scavenging ability was mostly attributed to the storage quality of the products, given that the herbal constituents of the products are commonly known as antioxidant agents (Table 1).

Table 5. Quantitative analysis of the antioxidant activity of the samples

Sample	DPPH IC ₅₀ *	Phospho-molybdenum (mgQE/gextract)**	FRAP (mgQE/gextract)***
A	397.09 ± 0.17	43.70 ± 0.22	176.99 ± 0.39
B	393.60 ± 0.98	40.13 ± 0.33	174.36 ± 0.45
C	418.14 ± 0.54	38.85 ± 0.17	175.34 ± 0.24
D	398.94 ± 0.42	44.90 ± 0.32	180.56 ± 0.22
E	392.14 ± 30.77	48.58 ± 0.45	186.46 ± 0.22
F	374.89 ± 9.45	39.40 ± 0.18	176.32 ± 0.27
G	423.98 ± 0.31	40.70 ± 0.13	175.83 ± 0.21
H	391.69 ± 0.40	46.88 ± 0.11	226.98 ± 0.19
Q	9.227 ± 0.02	-	-

Note: Q = quercetin; *IC₅₀ = 50% inhibitory concentration with regression equation of quercetin: $y = 4.52x + 8.159$ and $r^2 = 0.9991$; **mgQE = quercetin equivalent antioxidant capacity of phosphomolybdenum with $y = 15.44x - 0.138$ and $r^2 = 0.9996$; ***mgQE = quercetin equivalent antioxidant capacity of FRAP with $y = 21.94x - 1.007$ and $r^2 = 0.9993$; Data are shown as mean ± SD (n = 5).

As described above, all of the samples were composed of herbal constituents mostly containing flavonoid and phenolic compounds. These phytochemical compounds were subjected to measurement for contents and antioxidant activity. Even though the potential of these *jamu* products as antioxidants

was categorized as low according to (Molyneux, 2004), this research supports related information about antioxidants and antidiabetes.

CONCLUSION

In conclusion, the highest total flavonoid content was 8.66 ± 0.11 mgQE/g, and the highest total phenolic content was 22.66 ± 0.15 mgGAE/g. All of the samples exhibited weak DPPH radical scavenging activity (± 400 ppm), and the highest phosphomolybdenum and FRAP reducing power were 48.58 ± 0.45 mgQE/g extract and (226.98 ± 0.19 mgQE/g extract), respectively. The samples were found to contain flavonoid and phenolic compounds with low potential as exogenous antioxidant sources. In relation to antidiabetes, further investigation is needed to discover the mechanisms that might be involved.

ACKNOWLEDGEMENTS

The research work was supported by Lembaga Penelitian dan Pengabdian Kepada Masyarakat UAD (Penelitian Dosen Pemula) [Institute of Research and Community Service of UAD (Lecturer Research Grant)] in 2021 with contract number: PDP-102/SP3/LPPM-UAD/VI/2021.

REFERENCES

- Agarwal, A., Prabakaran, S., & Allamaneni, S. (2006). *What an andrologist/ urologist should know about free radicals and why*. 2–8. <https://doi.org/10.1016/j.urology.2005.07.012>
- Alfian, R., & Susanti, H. (2012). *Determination of total phenolic content of Methanolic extracts Red Rosella (Hibiscus sabdariffa Linn) Calyx in Variation of Growing Area by Spectrophotometry*.
- Badarinath, A. V., Rao, K. M., Madhu, C., Chetty, S., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). A Review on In-vitro Antioxidant Methods: Comparisons, Correlations and Considerations. *International Journal of PharmTech Research*, 2(2), 1276–1285.
- Chandrasekara, A., & Shahidi, F. (2011). Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. *Journal of Functional Foods*, 3(3), 159–170. <https://doi.org/10.1016/j.jff.2011.03.008>
- Choung, M., Hwang, Y., Lee, M., Lee, J., Kang, S., & Jun, T. (2013). *Original article Comparison of extraction and isolation efficiency of catechins and caffeine from green tea leaves using different solvent systems*. 1–7. <https://doi.org/10.1111/ijfs.12454>
- Elfahmi, Woerdenbag, H. J., & Kayser, O. (2014). Jamu : Indonesian traditional herbal medicine towards rational phytopharmacological use. *Perspectives in Medicine*. <https://doi.org/10.1016/j.hermed.2014.01.002>
- FHI. (2017). *Farmakope Herbal Indonesia*.
- Hanggaresty, M. D., & Warditiani, N. K. (2021). Review of potential plants in Indonesia as an antidiabetic with hypoglycemic activities. *Journal of Pharmaceutical Science and Application*, 3(2), 57. <https://doi.org/10.24843/JPSA.2021.v03.i02.p01>
- Kothari, S., Thompson, A., Agarwal, A., & Plessis, S. S. (2010). *Free radicals : Their beneficial and detrimental effects on sperm function*. 48(May), 425–435.
- Kunwar, A., & Priyadarsini, K. . (2011). Free radicals, oxidative stress and importance of antioxidants in human health. *Journal of Medical and Allied Sciences*, 1(2), 53–60.
- Molyneux, P. (2004). *The use of the stable free radical diphenylpicryl- hydrazyl (DPPH) for estimating antioxidant activity*. 50(June 2003).
- Mulyani H, Widyastuti S.H, E. V. . (2016). *Herbs as traditional medicine Jamu of the Disease in serat Primbon Jampi Jawi Jilid I*. 73–91.
- Nugroho, A. E., Kusumaramdani, G., Widyaninggar, A., Anggoro, D. P., & Pramono, S. (2014). Antidiabetic effect of combinations of n-hexane insoluble fraction of ethanolic extracts of *Andrographis paniculata* with other traditional medicines. *International Food Research Journal*, 21(2), 785–789.
- Ofosu, F. K., Elahi, F., Daliri, E. B., Chelliah, R., Ham, H. J., Kim, J., Han, S., Hur, J. H., & Oh, D.

- (2020). *Potential exerted by millet grain varieties*. 1–14.
- Pan, S., Zhou, S., Gao, S., Yu, Z., Zhang, S., Tang, M., Sun, J., Ma, D., Han, Y., Fong, W., & Ko, K. (2013). *New perspectives on how to discover drugs from herbal medicines : CAM ' s outstanding contribution to modern therapeutics*. 2013. <https://doi.org/10.1155/2013/627375>
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric Quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2), 337–341. <https://doi.org/10.1006/abio.1999.4019>
- Rais, I. R. (2015). *Isolation and determination of flavonoid content of Andrographis paniculata (Burm.f.) Ness Ethanollic Herb extract*. 100–106.
- Rais, I. R. (2016). *Antioxidant activity of Andrographis paniculata (Burm.f.) Ness extract depends on two evaporation difference*. 95–100.
- Salamah, N., & Farahana, L. (2014). *Antioxidant activity assay of Ethanolic extract of Centella asiatica (L.) Urb Hern using Phosphomolybdate method*.
- Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *CURBIO*, 24(10), R453–R462. <https://doi.org/10.1016/j.cub.2014.03.034>
- Shahwar, D., & Raza, M. A. (2012). *Antioxidant potential of phenolic extracts of Mimusops elengi*. 2(7), 547–550. [https://doi.org/10.1016/S2221-1691\(12\)60094-X](https://doi.org/10.1016/S2221-1691(12)60094-X)
- Shi, P., Du, W., Wang, Y., Teng, X., Chen, X., & Ye, L. (2019). *Total phenolic , flavonoid content , and antioxidant activity of bulbs , leaves , and flowers made from Eleutherine bulbosa (Mill .) Urb. July 2018*, 148–154. <https://doi.org/10.1002/fsn3.834>
- Sukweenadhi, J., Yunita, O., Setiawan, F., Siagian, M. T., Danduru, A. P., & Avanti, C. (2020). *Antioxidant activity screening of seven Indonesian herbal extract*. 21(5), 2062–2067. <https://doi.org/10.13057/biodiv/d210532>
- Unuofin, J. O., & Lebelo, S. L. (2020). *Review article antioxidant effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes : An Updated Review*. 2020. <https://doi.org/10.1155/2020/1356893>
- Vinayagam, R., & Xu, B. (2015). Antidiabetic properties of dietary flavonoids : a cellular mechanism review. *Nutrition & Metabolism*, 1–20. <https://doi.org/10.1186/s12986-015-0057-7>
- Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products : a comprehensive review. *Chinese Medicine*, 1–26. <https://doi.org/10.1186/s13020-018-0177-x>
- Zulaikhah, S. T. (2017). *The role of antioxidant to prevent free radicals in the body*. 8(1), 39–45. <https://doi.org/10.26532/sainsmed.v8i1.1012>

The antioxidant activity of several antidiabetic herbal products

ORIGINALITY REPORT

14%

SIMILARITY INDEX

12%

INTERNET SOURCES

9%

PUBLICATIONS

5%

STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universitas Sebelas Maret Student Paper	1%
2	digilib.unimed.ac.id Internet Source	1%
3	www.researchgate.net Internet Source	1%
4	Elena Pichichero. "Characterisation of the phenolic and flavonoid fractions and antioxidant power of Italian honeys of different botanical origin", Journal of the Science of Food and Agriculture, 03/15/2009 Publication	1%
5	mdpi-res.com Internet Source	1%
6	www.mdpi.com Internet Source	1%
7	www.tandfonline.com Internet Source	1%
8	Submitted to Binus University International Student Paper	

<1 %

9

vital.seals.ac.za:8080

Internet Source

<1 %

10

www.ajol.info

Internet Source

<1 %

11

repository.uantwerpen.be

Internet Source

<1 %

12

Submitted to University College London

Student Paper

<1 %

13

journal.unimma.ac.id

Internet Source

<1 %

14

John Parry, Lan Su, Jeffrey Moore, Zhihong Cheng, Marla Luther, Jaladanki N. Rao, Jian-Ying Wang, Liangli Lucy Yu. "Chemical Compositions, Antioxidant Capacities, and Antiproliferative Activities of Selected Fruit Seed Flours", Journal of Agricultural and Food Chemistry, 2006

Publication

<1 %

15

aocs.onlinelibrary.wiley.com

Internet Source

<1 %

16

Submitted to Higher Education Commission
Pakistan

Student Paper

<1 %

17	dspace.bracu.ac.bd:8080 Internet Source	<1 %
18	ir.mu.ac.ke:8080 Internet Source	<1 %
19	repository.uhamka.ac.id Internet Source	<1 %
20	Sehajveer Kaur, Navjot Kaur, Poonam Aggarwal, Kiran Grover. " Bioactive Compounds, Antioxidant Activity and Color Retention of Beetroot (powder: Effect of Steam blanching with refrigeration and storage ", Journal of Food Processing and Preservation, 2021 Publication	<1 %
21	Submitted to University of Nottingham Student Paper	<1 %
22	www.clinicaltrials.gov Internet Source	<1 %
23	Salwa Salwa. "Antioxidant Activity of Sunflower (Helianthus Annuus L.) Ethanolic Extract with DPPH Method and Determination of Total Phenolic and Flavonoid Levels", Journal of Nutraceuticals and Herbal Medicine, 2021 Publication	<1 %

24	Sukardi, M H Pulungan, I Purwaningsih, P F Sita. " Extraction of phenolic compounds from basil (L.) leaves with pretreatment using pulsed electric field (PEF) ", IOP Conference Series: Earth and Environmental Science, 2020 Publication	<1 %
25	Submitted to University of Northumbria at Newcastle Student Paper	<1 %
26	al-kindipublisher.com Internet Source	<1 %
27	file.scirp.org Internet Source	<1 %
28	iptek.its.ac.id Internet Source	<1 %
29	www.dovepress.com Internet Source	<1 %
30	e-sciencecentral.org Internet Source	<1 %
31	ogma.newcastle.edu.au Internet Source	<1 %
32	Tran Thanh Men, Huynh Hong Phien, Tran Thi Tu Ai, Nguyen Van Ay, Nguyen Thi Kim Hue, Do Tan Khang, Tran Duy Binh. "The insecticidal capacity of ethanol extract from	<1 %

Cascabela peruviana (L.) Lippold against fruit fly", Heliyon, 2022

Publication

33

epawebapp.epa.ie

Internet Source

<1 %

34

seer.ufrgs.br

Internet Source

<1 %

35

tuceel.isc.tuc.gr

Internet Source

<1 %

36

www.ifrj.upm.edu.my

Internet Source

<1 %

37

www.recentscientific.com

Internet Source

<1 %

38

www.scielo.br

Internet Source

<1 %

Exclude quotes On

Exclude matches Off

Exclude bibliography On