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Total phenolic and flavonoid contents and their antioxidant capacity of *Curcuma xanthorrhiza* accessions from Jambi

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Abstract. *Asyhar R, Minarni M, Arista RA, Nurcholis W. 2023. Total phenolic and flavonoid contents and their antioxidant capacity of* Curcuma xanthorrhiza *accessions from Jambi. Biodiversitas 24: 5007-5014. Curcuma xanthorrhiza* Roxb., is an effective natural antioxidant and is widely used as a raw material for jamu in Indonesian traditional medicine. This study aims to evaluate the biochemical characteristics and antioxidant capacity of *C. xanthorrhiza* rhizome accessions. Ten accessions of *C. xanthorrhiza* rhizome from 10 districts in Jambi province were evaluated for total phenolic (TPC) and total flavonoid (TFC) content and antioxidant capacity using four methods: 2,2-diphenyl picrylhydrazyl (DPPH), 2,2 azinobis (3-ethyl benzothiazoline) -6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant power (CUPRAC). The content of total phenolic (TPC) and total flavonoids (TFC) varied from 1.74±0.085 to 18.72±1.47 mg GAE/g DW and 1.74±0.085 to 18.72±1.47 mg GAE/g DW, respectively. Antioxidant capacities ranged from 0.13-1.11, 0.72-4.14, 7.71-81.48, 18.37-211.68 µmol TE/g DW, assayed by DPPH, ABTS, FRAP, and CUPRAC methods, respectively. Antioxidant activity was significantly correlated with the polyphenol content of *C. xanthorrhiza* rhizome accessions. Accession to *C. xanthorrhiza* rhizome with the code JSS, JBE, JG, and JK has the capacity for polyphenolic compounds (phenolics and flavonoids) and antioxidant capacity compared to other accessions, indicating that these accessions are promising for further breeding programs and commercial purposes.

Keywords: Antioxidant, *Curcuma xanthorrhiza* Roxb., flavonoid, polyphenol

INTRODUCTION

Indonesia is a biodiversity country with abundant plants, and almost 75% of the world's plants are in Indonesia, with around 90% of the total medicinal plants in Asia growing in Indonesia (Irwanta et al. 2015; Susanto et al. 2021). *Curcuma xanthorrhiza* Roxb., (Family: Zingiberaceae) is a native Indonesian plant with a high economic value known as *temulawak* or java turmeric. This plant is also cultivated in other Southeast Asian Regions such as Malaysia, Thailand, Vietnam, Sri Lanka, and the Philippines (Salleh et al. 2016; Rahmat et al. 2021). Meanwhile, turmeric in Indonesia is spread across 13 provinces in each of the islands, namely Sumatra, Java, Bali, Kalimantan, and Sulawesi (Purnomo et al. 2018). Traditionally *C. xanthorrhiza* it is used as a spice vegetable and traditional medicine a treat various ailments such as stomach ache, lack of appetite, liver disease, bloody diarrhea, dysentery, constipation, gastric disease, fever in children, hemorrhoids, arthritis, hypotriglyceridemia, vaginal discharge, rheumatism, skin eruptions (Hwang et al. 2000; Nurcholis et al. 2018). In addition, *C. xanthorrhiza* it is also used as an ingredient in jamu (Indonesian herbal supplements and medicines). Several studies reported the pharmacological activity of *C. xanthorrhiza* namely as anticancer (Indrayanti et al. 2021), antimicrobial (Akarchariya et al. 2017), anti-inflammatory (Devaraj et al. 2010), antitumor (Rahmat et al. 2021), antibacterial (Atun et al. 2020), antihypertensive (Campos et al. 2008; Oon et al. 2015), antihyperglycemic (Kim et al. 2014), nephroprotective (Kim et al. 2005), and antioxidant activities (Rosidi et al. 2016). The active compounds influence this pharmacological effect in the rhizome of *Curcuma xanthorrhiza* Roxb, namely curcuminoid, which also gives a yellowish-orange color to the rhizome of *C. xanthorrhiza* (Erpina et al. 2017) and xanthorrhizol which belongs to the sesquiterpenoid class (Susanto et al. 2021).

The quantity of chemical markers can be used as an indicator of the quality of medicinal plants (Li et al. 2008). The composition and bioactive content of medicinal plants can be influenced by various factors, both internal and external, in the form of genotype factors (Batubara et al. 2020) and environmental factors (Mahajan et al. 2020). In addition, the content of polyphenolic and curcuminoid compounds is also affected by geographic location (Nurcholis et al. 2016a, 2016b). Ramdani et al. (2016) reported that the rhizome of *C. xanthorrhiza* contained the main components of starch (48.18-59.64%), volatile oils (3-12%) (phellandrene, camphor, tumerol, cineol, and borneol), xanthorrhizol (1.48-1.63%), sesquiterpenes (β-curcumene, ar-curcumene, bisabolene, lactone germacone), flavonoids (catechin, epicatechin, quercetin, myricetin, kaemferol, apigenin, luteolin, naringenin), and curcuminoids. The yellow or orange color in *temulawak* rhizomes is due to the compound curcuminoids, which accumulates in *temulawak* rhizomes, the most dominant bioactive ingredient (Rafi et al. 2011). Akinola et al. (2014) reported

3 out of 10 plant species from the Zingiberaceae family (*Curcuma longa* L., *Zingiber officinale* Roscoe, and *C. xanthorrhiza*) had higher antioxidant activity than other species. Rosidi et al. (2016) also reported that the rhizome of *C. xanthorrhiza* is an excellent natural antioxidant with an antioxidant capacity (IC_{50}). Research on *Curcuma aeruginosa* rhizomes harvested from various places in Indonesia showed fluctuations in curcuminoid content and cytotoxic activity (Nurcholis et al. 2016a), high total phenolic and flavonoid content (Nucrholis et al. 2016b). Based on previous research, it was stated that variations in the bioactive content and biological activity of a plant could be influenced by several factors, such as genetic and geographical variations; this was reported by Widyastuti et al. (2021) *C. xanthorrhiza* rhizome sample which derived from Sambi Market, Kediri, and East Java contained the highest TPC and TFC values and antioxidant activity compared to other *C. xanthorrhiza* rhizome accessions. Therefore, this study aims to evaluate the total phenolic and flavonoid content and antioxidant activity based on four methods of ten accessions of *C. xanthorrhiza* rhizome originating from 10 districts in Jambi province, so the results will reflect differences in geographical location between the accessions studied in terms of total phenolic content, flavonoids, and their activity as antioxidants.

MATERIALS AND METHODS

Plant material and sample extraction

Ten rhizome accessions of *C. xanthorrhiza* were used in this study. Rhizome accession of *Curcuma xanthorrhiza* Roxb. collected in July 2022 from local farmers in Jambi province, consisting of Jambi Tembesi (JT), Jambi Geragai (JG), Jambi Bangko Barat (JBB), Jambi Bangko (JB), Jambi Kerinci (JK), Jambi Mendahara (JM), Jambi Berbak (JBE), Jambi Jaluko (JJ), Jambi Pasar Bangko (JPB), and Jambi Sarolangun Singkut (JSS). The rhizome of the Zingiberaceae family is the central part used as a raw material for medicine (Nurcholis and Bintang 2017). In addition, several studies have stated that each organ of an herbal plant has different metabolite content, which results in differences in its pharmacological effects (Silalahi 2017). *C. xanthorrhiza* rhizomes from each accession were dried and ground to a size of 100 mesh. Samples were extracted separately using pro-analyzed ethanol solvent (Merck KGaA., Germany) by sonication and maceration methods. Briefly, four grams of rhizome samples from each accession were dissolved in 40 mL of solvent. In the dark, the mixture was homogenized and sonicated (Decon Ultrasorics Ltd., England) for 30 minutes. Then it was macerated at 30°C for 180 minutes using a water bath shaker (Memmert., Germany). The filtrate was filtered using filter paper and prepared at a concentration of 0.2 g/mL using a rotary vacuum evaporator (Hahnvapor HS-2005V., Korea) at 45°C. Concentrated filtrate (0.2 g/mL) from each accession was used as an extract to determine the total phenolic and flavonoid content and antioxidant capacity. The information on the geographic locations of each accession of *C. xanthorrhiza* is summarized in Table 1.

Total phenolic and flavonoid content analysis

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method based on modifications by Nisar et al. (2015). Briefly, 20 µL of ethanol extract of *C. xanthorrhiza* rhizome accession was added with 120 µL Folin-Ciocalteu (10%) into a 96-well microplate and incubated for 5 minutes in the dark. Then added with 80 µL of Na_2CO_3 solution (10%) and incubated again for 30 minutes in a dark room and at room temperature. The absorbance of each rhizome accession of *C. xanthorrhiza* was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) at a wavelength of 750 nm. The total phenolic content was expressed as mg gallic acid equivalent per g of extract (mg GAE/g DW).

Total Flavonoid Content (TFC) was determined by the calorimetry method using aluminum chloride ($AlCl_3$) reagent based on Calvindi et al. (2020) modified. Briefly, 10 µL of ethanol extract of *C. xanthorrhiza* rhizome accession. 50 µL of pro-analyzed ethanol, 10 µL of 10% aluminum chloride ($AlCl_3$), 10 µL of glacial acetic acid (CH_3COOH) and 120 µL of distilled water were added to a 96-well microplate. Then homogenized and incubated for 30 minutes in the darkroom and at room temperature. Absorbance of each rhizome accession of *C. xanthorrhiza* was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) at a wavelength of 415 nm. The total content of flavonoids is expressed as mg quercetin equivalent per g of extract (mg QE/g DW).

Table 1. Description of the location of each accession of *Curcuma xanthorrhiza* Robx.

Accession code	Location	Geographic coordinates		Altitude (m asl.)
		Latitude (S)	Longitude (E)	
JT	Jambi Tembesi	1º40'53"	103º05'31"	156
JG	Jambi Geragai	1º13'03"	103º40'20"	156
JBB	Jambi Bangko Barat	2º10'25"	102º14'16"	78.2
JB	Jambi Bangko	2º05'30"	102º18'23"	78.2
JK	Jambi Kerinci	2º05'38"	101º29'15"	816
JM	Jambi Mendahara	0º59'55"	103º35'48"	78.1
JBE	Jambi Berbak	1º13'22"	104º10'34"	78.1
JJ	Jambi Jaluko	1º35'33"	103º27'43"	2.53
JPB	Jambi Pasar Bangko	2º04'36"	102º16'11"	6.19
JSS	Jambi Sarolangun Singkut	2º32'21"	102º43'01"	78.1

Antioxidant capacity

Four in vitro tests were performed to evaluate the antioxidant capacity in the ethanol extract of *C. xanthorrhiza* rhizome accessions. The 2,2-diphenyl picrylhydrazyl (DPPH) and 2,2 azinobis (3-ethyl benzothiazoline)-6-sulfonate acid (ABTS) methods aim to evaluate the free radical scavenging activity of each sample extract. In contrast, the Cupric Reducing Antioxidant Power (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP) methods aim to evaluate the antioxidant properties of the reducing power of each sample extract.

The antioxidant activity of 2,2-diphenyl picrylhydrazyl (DPPH) radicals was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) based on Nurcholis et al. (2016a) with modifications. Briefly, 100 µL of ethanol extract of *C. xanthorrhiza* rhizome accession. 100 µL of 125 µM DPPH solution (in pro-analytical ethanol) was added to a 96-well microplate (Biologix Europe GmbH). Then homogenized and incubated for 30 minutes in the dark and at room temperature. The absorbance of each rhizome accession of *C. xanthorrhiza*. was measured using a nano-spectrophotometer at 515 nm. The final unit is expressed in µmol TE (Trolox equivalent)/g DW.

The radical antioxidant activity of 2,2 azinobis (3-ethyl benzothiazoline)-6-sulphonic acid (ABTS) was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) based on Nurcholis et al. (2022) with modifications. Briefly, 20 µL of ethanol extract from each rhizome accession of *C. xanthorrhiza*. 180 µL of ABTS reagent (prepared by mixing ABTS 7 mM (in distilled water) and $K_2S_2O_8$ 2.4 mM (in distilled water) was added to the microplate-96 well (Biologix Europe GmbH). Then homogenized and incubated for 6 minutes in the dark and at room temperature. The absorbance of each rhizome accession of *C. xanthorrhiza* was measured using a nano-spectrophotometer at 734 nm. The final unit is expressed in µmol TE (Trolox equivalent)/g DW.

Ferric Reducing Antioxidant Power (FRAP) activity was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) according to Arista et al. (2023) with modifications. Briefly, 10 µL of each ethanol extract of *C. xanthorrhiza* rhizome accession, then added with 300 µL of FRAP reagent (prepared by mixing acetate buffer pH 3.6 with 10 µM TPTZ solution (in 40 µM HCl) and 20 µM $FeCl_3$ (in distilled water) with a v/v/v ratio of 10:1:1) was put into a microplate-96 well (Biologix Europe GmbH). Then homogenized and incubated for 30 minutes in the dark and at room temperature. The absorbance of each rhizome accession of *C. xanthorrhiza* was measured using a nano-spectrophotometer at 593 nm. The final unit is expressed in µmol TE (Trolox equivalent)/g DW.

Cupric Reducing Antioxidant Power (CUPRAC) activity was measured using a nano-spectrophotometer (SPECTROstar^{Nano} BMG LABTECH) based on modifications by Tunnisa et al. (2022). Briefly, the ethanol extract (50 µL) of each rhizome accession of *C. xanthorrhiza* 50 µL of 0.01 M $CuCl_2$ solution, 50 µL of 0.0075 M neocuproine solution, and 50 µL of ammonium acetate buffer pH 7 were added to a 96-well microplate (Biologix Europe GmbH). Then homogenized and incubated for 30 minutes in the dark and at room temperature. The absorbance of each rhizome accession of *C. xanthorrhiza* was measured using a nano-spectrophotometer at 450 nm. The final unit is expressed in µmol TE (trolox equivalent)/g DW.

Data analysis

Statistical analysis in the form of quantitative data was analyzed using ANOVA-Oneway analysis at a significant level of $\alpha = 5\%$ with Tukey's follow-up test using the SPSS Version 25 program (IBM, Armonk, NY, USA), which was used to determine the effect of *C. xanthorrhiza* rhizome accession to the test parameters. Number graph generated using GraphPad Prism 8 for Windows (GraphPad Software Inc., San Diego, California, USA) Version 8.0.1. Data are shown as mean ± standard deviation. Correlation analysis uses the performance analytics package available in R software.

RESULTS AND DISCUSSION

Total phenolic content (TPC) dan total flavonoid content (TFC)

Total phenolic content (TPC) was evaluated using the Folin-Ciocalteu method with gallic acid as the standard phenolic compound. While the total flavonoid content (TFC) was evaluated using the calorimetry method with $AlCl_3$ reagent and quercetin as a standard for flavonoid compounds. The main plant constituents categorized as phenolic acids, flavonoids, tannins, curcuminoids, lignins, and quinone are phenolic compounds (Ismail et al. 2017). The ten accessions used in this study came from 10 regions in Jambi province symbolized by accession codes consisting of JT, JG, JBB, JB, JK, JM, JBE, JJ, JPB, and JSS (details of which are given in Table 1). The ten accessions were selected based on the color of their rhizomes, which can be grouped into four groups, namely: (i) yellow with almost no orange tint; (ii) bright orange, that is, the dominant bright orange color; (iii) dark orange; and (iv) has a deep dark orange color resembling a dark brown color. Characteristics of the rhizome of *C. xanthorrhiza* it was marked with a yellow or orange color indicating the curcuminoids' presence (Kustina et al. 2020). In addition, there are differences in the geographical location of each rhizome accession of *C. xanthorrhiza* it also affected the quantity and quality of secondary metabolites, which were significantly responsible for the pharmacological activity of each *C. xanthorrhiza* rhizome accession (Table 1).

The total phenolic and flavonoid content of the ethanol extract of the rhizome accession of *C. xanthorrhiza* can be seen in (Figures 1A and 1B). Polyphenols are antioxidant biochemical compounds (Khumaida et al. 2019; Kalisz et al. 2020; Calvindi et al. 2020). The total phenolic content in the ethanol extract depended on the accession of *C. xanthorrhiza* used for extraction. Among the ten accessions investigated, the total phenolic content ranged from 1.74±0.085 to 18.72±1.47 mg GAE/g DW. The total phenolic

content of the ethanol extract of the rhizome accession of *C. xanthorrhiza* was the lowest and highest, respectively, obtained in the accession with JK and JSS codes. Meanwhile, the total flavonoid content in the ethanol extract of the rhizome accession ranged from 3.47±0.40 to 39.54±4.08 mg QE/g DW with the total flavonoid content of the ethanol extract of the rhizome accession of *C. xanthorrhiza* the lowest and highest were obtained with the JK and JSS accession codes, respectively. The content of total phenolics and flavonoids had significant differences ($p<0.05$) in each ethanol extract of the rhizome accession studied.

Based on these data, the ethanol extract of the total phenolic and flavonoid contents of the rhizome accession of *C. xanthorrhiza* had the lowest and highest content, respectively, in accession codes JK and JSS, compared to other accessions. The accumulation of bioactive compounds contained in plants can be influenced by environmental factors such as light, pH, altitude, temperature, humidity, harvest time, nutrients and soil properties, including plant physiological conditions, which will affect the level of secondary metabolite production in plants (Zhang et al. 2021). The complexity of the biosynthesis of polyphenolic compounds (Yudha et al. 2022) and plant species are internal factors that are known to participate in influencing the production of phenolic and flavonoid amounts in plants (Oliveira et al. 2013; Moghaddam and Mehzadeh 2015; Moghaddam and Pirbalouti 2017; Nurcholis et al. 2018; Khumaida et al. 2019). This research was conducted by random sampling according to the location of the accession area of *C. xanthorrhiza* rhizome so that the possible reason for the variation in total phenolic and flavonoid content was environmental factors, namely differences in geographical location, rainfall, soil moisture, and soil nutrient content, can affect the content of secondary metabolites. Widyastuti et al. (2021) reported that the methanol extract of *C. xanthorrhiza* with code TL02 (Pasar Sambi, Kediri, East Java) had the highest total phenolic and flavonoid content compared to other samples, namely 170.44±7.68 mg GAE/g DW and 392.39±16.14 mg RE/g DW, respectively.

Suryani et al. (2022) reported that the average total phenolic content of the ethanol extract of 80% of the rhizome of *C. xanthorrhiza* cultivated in Bogor (3.872 mg GAE/g DW) was higher than that cultivated in Cianjur (3.728 mg GAE/g DW) and Sukabumi (3.651 mg GAE/g DW). The total phenolic and flavonoid content results in this study were lower when compared to the results reported by Widyastuti et al. (2021). However, in this study, the ethanol extract of *C. xanthorrhiza* rhizome originating from the Jambi Sarolangun Singkut (JSS) area had a higher total phenolic content when compared to the 80% ethanol extract of *C. xanthorrhiza* cultivated in the Bogor area (Suryani et al. al. 2022). In addition, Khumaida et al. (2019) also reported that the ethanol extract of *C. aeruginosa* rhizome with code PK (46.92 mg GAE/g) originating from the Pakem, Yogyakarta had the highest total phenolic content, while the highest total flavonoid content was obtained in accession code GK originating from Gunung Kidul, Yogyakarta, Indonesia. Nurcholis et al. (2016a) also reported differences in the total phenolic and flavonoid content levels of 20 accessions of 70% ethanol extract of *C. aeruginosa*. These differences are due to several factors, namely the age of the plant, growing environmental factors, the extraction method, and the solvent used that will affect the metabolite extract, which acts as an antioxidant in the sample plants. Nisar et al. (2015) also reported that differences in extraction methods and solvents significantly affected the extraction results and their antioxidant potential.

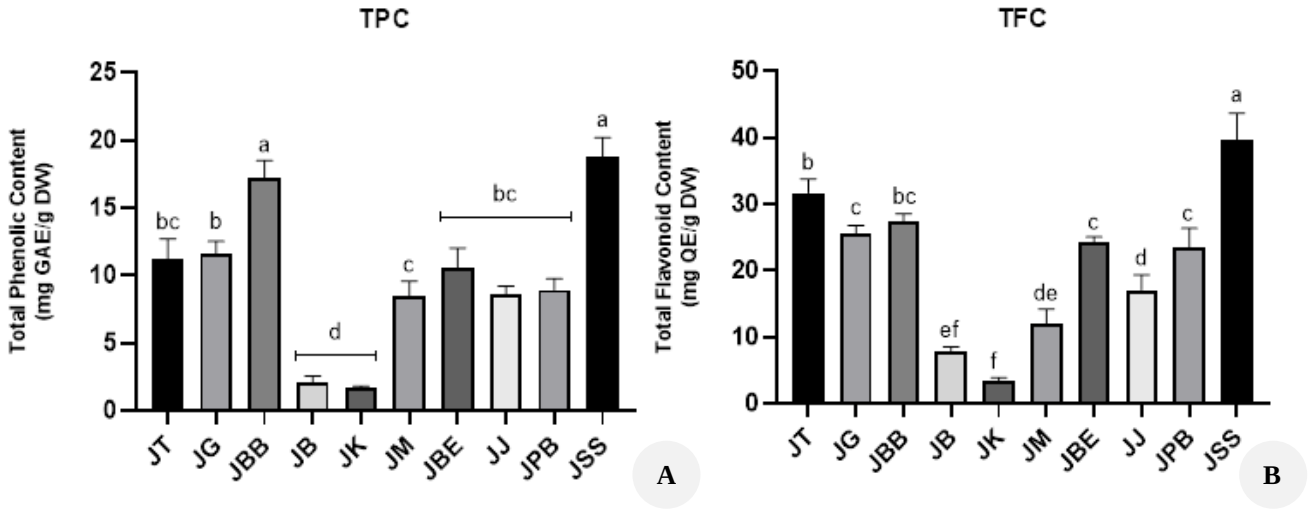

Figure 1. The total polyphenol content of each ethanol extract of *C. xanthorrhiza* rhizome accessions: A. Total phenolic content (TPC) and; B. Total flavonoid content (TFC). Values show the mean ± SD for n=3. The mean value in each column marked with different letters differs significantly at $p<0.05$ and Tukey test results 5%. GAE: Gallic acid equivalent; QE: Quercetin equivalent

Antioxidant capacity

The antioxidant capacity test aims to measure the total antioxidant capacity of each rhizome accession of *C. xanthorrhiza*. Antioxidant capacity among ten rhizome accessions of *C. xanthorrhiza* was evaluated using four different methods, DPPH, ABTS, FRAP, and CUPRAC, with Trolox as a standard antioxidant compound because it is the result of synthesis as a derivative of Vitamin E and antioxidants that can dissolve in the air (Setiawan et al. 2018), in addition to testing antioxidant was carried out using method four because the antioxidant activity of plants depends on the mechanism of metabolites in the extract (Mercado-Mercado et al. 2020) so that different tests can determine the mechanism of antioxidant capacity in the plant extracts studied. Free radical scavenging capacity was evaluated using the DPPH and ABTS methods (Suwardi and Ranggaini 2022), while the measurement of antioxidant reduction power was evaluated using the FRAP and CUPRAC methods (Rahmat et al. 2021). The antioxidant capacity of the ten accessions of the DPPH and ABTS methods ranged from 0.13±0.02 to 1.11±0.02 µmol TE/g dry weight and 0.72±0.18 to 4.14±0.06 µmol TE/g DW with the lowest and highest antioxidants respectively obtained for the JM and JBE accession codes for the DPPH method, while for the ABTS method obtained for the JBB and JK accession codes (Figure 2). Meanwhile, the antioxidant capacity of each ethanol extract of the rhizome accession of *C. xanthorrhiza* for the FRAP and CUPRAC methods, which ranged from 7.71±1.29 to 81.48±4.43 µmol TE/g DW and 18.37±4.30 to 211.68±1.53 µmol TE/g DW respectively with the lowest and highest antioxidant capacities obtained in the JK and JG for the FRAP method, while for the CUPRAC method obtained on the JK and JSS accession codes. The antioxidant capacity of the four methods (DPPH, ABTS, FRAP, and CUPRAC) had significant differences ($p<0.05$) in each ethanol extract of *C. xanthorrhiza* rhizome accession.

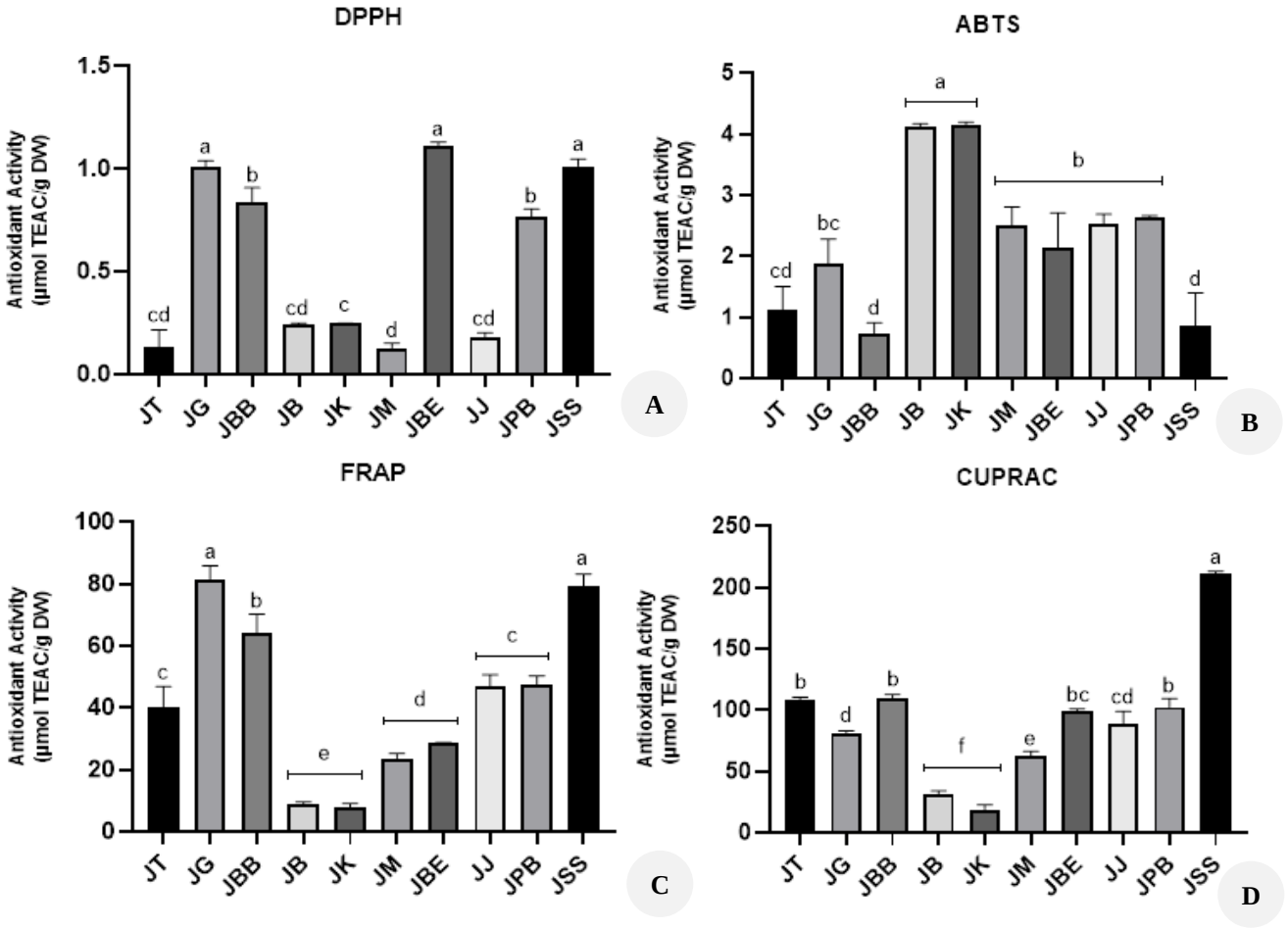

Figure 2. Antioxidant activity of each ethanol extract of *C. xanthorrhiza* rhizome accessions: A. DPPH; B. ABTS; C. FRAP; D. CUPRAC. Values show the mean ± SD for n=3. The mean value in each column marked with different letters differs significantly at $p<0.05$ and Tukey test results 5%. TEAC: Trolox equivalent antioxidant capacity

Based on these data, the ethanol extract of the rhizome accession of *C. xanthorrhiza* the four methods showed different antioxidant activity. Accession codes JBE (1.11±0.02 µmol TE/g dry weight) and JK (4.14±0.06 µmol TE/g dry weight) had the highest antioxidant activity, respectively, in the DPPH and ABTS methods, while accession codes JG (81.48±4.43 µmol TE /g dry weight) and JSS (211.68±1.53 µmol TE/g dry weight) had the highest antioxidant activity in the FRAP and CUPRAC methods, respectively. These results indicate that the ten rhizome accessions of *C. xanthorrhiza* have higher activity in terms of reduced capacity than free radical scavenging activity; this is related to the antioxidant mechanism of the ten rhizome accessions of *C. xanthorrhiza* which is more dominant using a single electron transfer mechanism (SET) compared to hydrogen atom transfer (HAT) (Arista et al. 2023). The results of the antioxidant activity of the four methods showed that the ethanol extract of *C. xanthorrhiza* had higher CUPRAC antioxidant capacity followed by FRAP, ABTS, and DPPH. This is in line with research conducted by Nurcholis et al. (2021), namely, the antioxidant potential of cardamom fruit accession extracts originating from Bogor and Sukabumi using the CUPRAC method is higher than the DPPH method. This DPPH value is also consistent with research by Arista et al. (2023).

The results of this study indicate that each region has a different level of antioxidant capacity from the four antioxidant methods for *C. xanthorrhiza* rhizomes. This is similar to research conducted by Widyastuti et al. (2021), who reported that where plants grow affects the level of antioxidant activity, namely one in ten rhizome accessions of *C. xanthorrhiza* which came from Sentul Market, Yogyakarta City, DIY had the lowest DPPH antioxidant activity when compared to the other nine accessions. In contrast, Nurcholis et al. (2012) reported that the ethanol extract of *C. xanthorrhiza* from Sukabumi had lower DPPH antioxidant activity when compared to *C. domestica* from the same area. Suryani et al. (2022) also reported that the DPPH antioxidant activity of the 80% ethanol extract of *C. xanthorrhiza* rhizome cultivated in Bogor (14.960 µmol TE/g DW) had higher antioxidant activity when compared to the Sukabumi area (8,533 µmol TE/g DW) and Cianjur. (11.729 µmol TE/g DW). In addition, Rosidi et al. (2016) reported that *C. xanthorrhiza* rhizome extract from Purworejo, Central Java has antioxidant activity DPPH method, which is classified as an active antioxidant and is an excellent natural antioxidant. The results showed that the antioxidant activity of DPPH ethanol extract of *C. xanthorrhiza* in this study was lower when compared to other studies; this is probably due to differences in where it grows and the age of the rhizomes when harvested, which will affect the content of metabolites that act as antioxidants. Kusuma (2012) stated that if the curcuminoid levels in older *C. xanthorrhiza* rhizome was higher, it would affect its antioxidant capacity.

Correlation between phenolics, flavonoids, and antioxidant capacities of *C. xanthorrhiza* accessions

Polyphenolic compounds, such as phenolic compounds and flavonoids, are secondary metabolites related to the antioxidant capacity of plant extracts (Zhang et al. 2018; Lim et al. 2019). Phenolic compounds and flavonoids are secondary metabolites produced by plants in response to conditions of cold, heat, drought, salinity, UV radiation, and pathogens (Idris et al. 2018). The results of the correlation analysis between polyphenol content (TPC and TFC) and antioxidant activity (DPPH, FRAP, ABTS, and CUPRAC) of ten accessions of the ethanol extract of *C. xanthorrhiza* rhizome (Figure 3).

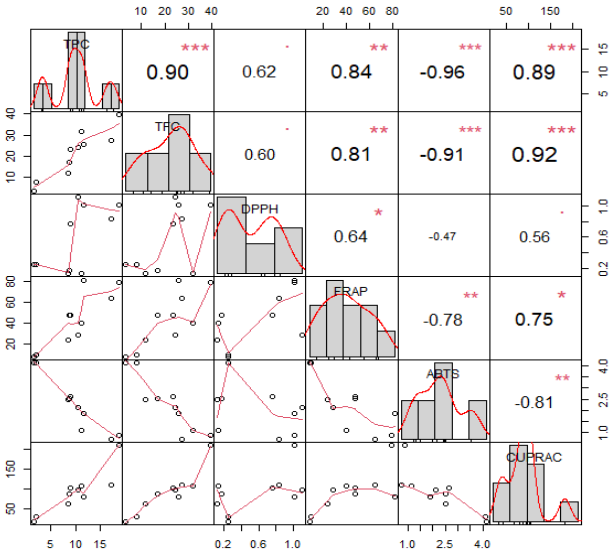

Figure 3. The correlation for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties (DPPH, 22-diphenyl picrylhydrazyl; FRAP, ferric reducing antioxidant power; ABTS, 2,2 azinobis (3-etilbenzotiazolin)-6-asam sulfonate; CUPRAC, cupric reducing antioxidant power). ***, **, *, . showed significant level with p-values of 0.001, 0.01, 0.05, and 0.1, respectively. The figure showed the variable on the diagonal, the bivariate scatter plots with a fitted line on the bottom of the diagonal, and the value of the correlation with the significance level on the top of the diagonal

Determining the correlation value aims to determine the relationship between several characters, especially in a series of plant breeding programs that are useful for selection purposes (Yudha et al. 2022). A positive correlation ($p<0.001$, r value 0.90) was obtained between TPC and TFC. In this study, TPC was positively and significantly correlated with the antioxidant capacities of DPPH, FRAP, and CUPRAC, while negatively correlated with the antioxidant capacity of ABTS with correlation coefficients of 0.62, 0.84, 0.89, and -0.96, respectively. Meanwhile, TFC has a positive and significant correlation with the antioxidant capacity of DPPH, FRAP, and CUPRAC. In contrast, it negatively correlates with the antioxidant capacity of ABTS with correlation coefficients of 0.60, 0.81, 0.92, and -0.91. In this study, the correlation coefficient between TPC and FRAP was higher than between TFC and FRAP. Still, the correlation coefficient between TPC and CUPCAC was lower than TFC and CUPRAC, even though both had a positive correlation with FRAP and CUPRAC. This shows that the antioxidant capacity of the rhizome extract of *C. xanthorrhiza* is more strongly influenced by TPC, indicating that the phenolic compounds in the rhizome of *C. xanthorrhiza* act as an antioxidant which is more dominant in reducing power mechanisms. Zeb (2020) reported that phenolic is the leading secondary metabolite effective as an antioxidant. The same thing was also reported by Yudha et al. (2022). Based on the four methods used to evaluate antioxidant capacity, the correlation coefficients between DPPH/FRAP, DPPH/ABTS, and DPPH/CUPRAC were 0.64, -0.47, and 0.56, respectively. The correlation coefficients between FRAP/ABTS and FRAP/CUPRAC are -0.78 and 0.75, respectively. In contrast, the correlation coefficient between ABTS/CUPRAC is -0.81. Based on the data listed shows that the antioxidant activity of the rhizome of *C. xanthorrhiza* was more dominantly measured using the FRAP and CUPRAC methods compared to the DPPH and ABTS methods; a similar thing was also reported by Calvindi et al. (2020). Based on the evaluation of the data in this study, it shows that the antioxidant activity and biochemical parameters of the rhizome of *C. xanthorrhiza* depend on each accession. Accession to *C. xanthorrhiza* rhizome with the code JSS, JBE, JG, and JK have the capacity of polyphenolic compounds (phenolics and flavonoids) and antioxidant capacity that is superior to other accessions. Therefore, based on the total phenolic and flavonoid content as well as the antioxidant activity of DPPH and FRAP in this study, shows that the rhizome of *C. xanthorrhiza* with JSS, JBE, JG, and JK accession codes is recommended for plant breeding and commercial consumption programs.

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