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Effect of varieties of *Curcuma xanthorrhiza* and extraction solvent on total phenolic, total flavonoid content, and antioxidant capacity

GARDA YODI[1], I MADE ARTIKA[1], WARAS NURCHOLIS[1,2,♥]

[1]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Tanjung, Biochemistry Building, Dramaga IPB Campus, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8423267, ♥email: wnurcholis@apps.ipb.ac.id
[2]Tropical Biopharmaceutical Research Center, Institut Pertanian Bogor. Jl. Taman Kencana No. 3, Bogor 16128, West Java, Indonesia

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Abstract. *Yodi G, Artika IM, Nurcholis W. 2023. Effect of varieties of* Curcuma xanthorrhiza *and extraction solvent on total phenolic, total flavonoid content, and antioxidant capacity. Biodiversitas 24: 6425-6431.* Temulawak (*Curcuma xanthorrhiza* Roxb.) is widely recognized for its high phytochemical content and antioxidant capacity. Therefore, this study aimed to measure yields, total phenols, total flavonoids, and antioxidant capacity of acetone and ethyl acetate extracts from three varieties of Temulawak, namely Cursina 1, Cursina 2, and Cursina 3. The samples were extracted using the maceration method and evaporated with a rotary vacuum evaporator. In this study, total phenolic and flavonoid contents were analyzed using a colorimetric method, while antioxidant capacity was assessed through DPPH, ABTS, and FRAP assays. The highest acetone and ethyl acetate extract yields were obtained from Cursina 2, i.e., 5.8% and 4.3%, respectively. The highest phenol in the acetone and ethyl acetate extract was from Cursina 2, consisting of 9.55±0.37 mg GAE/g DW and 4.60±0.12 GAE/g DW. The highest flavonoid content in the acetone and ethyl acetate extract was from Cursina 2, i.e., 20.48±0.45 QE/g and 8.59±0.14 mg QE/g DW, respectively. The DPPH method showed that acetone and ethyl acetate extracts of Cursina 2 had the highest antioxidant activities, i.e., 3.28±0.03 µmol TE/g DW and 2.11±0.04 µmol TE/g DW, respectively. The highest antioxidant capacity based on the ABTS method was found in the acetone extract of Cursina 2 (148.91±6.10 µmol TE/g DW) and ethyl acetate extract of Cursina 3 (84.30±5.10 µmol TE/g DW). The FRAP method showed the highest antioxidant capacity was Cursina 2 acetone extract (115.23±2.30 µmol TE/g DW) and ethyl acetate extract of Cursina 3 (71.88±1.48 µmol TE/g DW). These results suggested that acetone is a more suitable solvent for the Cursina 2 variety, while ethyl acetate is more suitable for the Cursina 3 variety.

Keywords: Acetone, antioxidant, ethyl acetate, temulawak, varieties

INTRODUCTION

Free radicals are atoms or molecules with an unpaired electron in one of their orbitals, capable of existing independently. Moreover, free radicals are highly reactive and unstable (Lobo et al. 2010), initiating a detrimental chain reaction in the body. This reaction can damage cell membranes, hinder enzyme activity, disrupt essential cellular processes required for the proper functioning of the body, impede normal cell division, destroy DNA, and obstruct energy generation (Sharifi-Rad et al. 2020). In response to the constant threat of reactive oxygen species, the human body produces natural antioxidants called endogenous or intracellular antioxidants, which defend against the damaging effect. Furthermore, exogenous antioxidants such as vitamins C and E and flavonoids can be obtained from various sources, including fruits, tea, leaves, vegetables, and spices (Nurcholis et al. 2022). These antioxidants are secondary metabolites produced by plants to compete successfully in their natural environment by exerting a wide range of effects on the plant and other living organisms (Teoh 2015). In plant tissues, flavonoids are coloring compounds that support health and determine food quality, distributed throughout the plant body. Parts of plants that contain flavonoids are generally purple, red, blue, and yellow, with different activities (Xu et al. 2015). One of the natural ingredients that contains high antioxidant compounds such as flavonoids is Java turmeric (Elfin et al. 2021).

Curcuma xanthorriza is a native Indonesian plant locally known as temulawak or Java turmeric. This plant is mainly cultivated in Indonesia and other Southeast Asian countries such as Malaysia, Thailand, Vietnam, and the Philippines. *C. xanthorriza* has several local names in Indonesia, such as *koneng gede* (Sundanese), *temu labak* (Madurese), *tommo* (Bali), *tommon* (South Sulawesi), and *karbanga* (Ternate), grows in the lowlands up to an altitude of 2500 meters above sea level. This plant is widespread and cultivated in Indonesia on almost all major islands, including Java, Sumatra, Kalimantan, Sulawesi, and Maluku. Chemical studies of *C. xanthorriza* have yielded valuable insights, resulting in the identification of several secondary metabolites, such as curcuminoids and terpenoids, possessing essential biological properties. According to a previous study, xanthorrhizol is the most abundant secondary metabolite (Rahmat et al. 2021). The essential oil of the rhizomes of *C. xanthorrhiza* rhizomes contains a unique compound called xanthorrhizol (XNT), which differentiates from other species as the most potent and prevalent compound (Oon et al. 2015). Generally, herbs and natural ingredients were extracted to obtain chemical compounds such as flavonoids and phenolics (Hikmawanti et al. 2021).

The most common methods for extracting flavonoid and phenolic compounds from spices are ultrasonication, maceration, supercritical fluid extraction, and Soxhlet extraction. The method selection and the duration of extraction are the primary factors influencing the quantity of extracted antioxidants. Other factors, such as solvent polarity, temperature, and the sample-to-solvent ratio, affect the compound yields, which can be expressed as total flavonoid and phenolic content (Nurcholis et al. 2022). Maceration is a direct extraction method by soaking the fine plant material or powder form in a selected solvent at room temperature for a minimum of three days, with intermittent agitation. The selection of an appropriate solvent is essential in the maceration because the solvent determines the phytochemicals extracted from the samples and enables the extraction of thermolabile phytochemicals (Bitwell et al. 2023). The selection and amount of solvent, temperature, duration of extraction, and external energy such as ultrasonic, microwave, and supercritical generally affect the extraction process (Simon et al. 2022). Distinctive varieties of natural ingredients also affect the extracted content due to the different abundance of compounds. Research by Mapoung et al. (2023) on 8 varieties of black rice showed variations in the phytochemical content, particularly phenolic and flavonoid. Therefore, this study aimed to determine the yield, total phenols, flavonoid, and antioxidant capacity of acetone and ethyl acetate extracts from three varieties of temulawak rhizome.

MATERIALS AND METHODS

Study area

This study was conducted from September to November 2022 at the Research Laboratory, Department of Biochemistry, Institut Pertanian Bogor, Bogor, West Java, Indonesia. The primary materials used were three varieties of *C. xanthorriza*, including Cursina 1, Cursina 2, and Cursina 3, from Tropical Biopharmaca Research Center, Institut Pertanian Bogor at 6E32’25.47" N and 106E42’53.22" E.

Preparation and extraction

The temulawak rhizome was cleaned, peeled, and sliced before being dried in an oven at 45°C for 6-8 hours. After drying, the sample was ground into powder, and the simplisia was filtered using an 80-mesh sieve to obtain softer temulawak powder, which was extracted using the maceration method. For the extraction process, 25 grams of temulawak powder was extracted using two different solvents, ethyl acetate, and acetone, with a ratio of 1:5 for 24 hours. The macerate was filtered using Whatman filter paper. The residue was added to 125 mL of methanol and macerated for 24 hours. The extraction process was repeated three times at room temperature with a rotation of 240 rpm. Subsequently, the extracted solution was separated from the solvent using a rotary evaporator at 45°C and 80 cmHg pressure to obtain a concentrated extract. The extract yield was calculated and stored in a vial bottle at -20°C to 4°C for measuring phenolic and flavonoid content and antioxidant capacity.

Phytochemical analysis

Phenolic content measurement

Total phenolic content was determined using the method described by Nurcholis et al. (2022), with minor modifications. A total of 120 µL of 10% Folin-Ciocalteu reagent, diluted with 160 µL of water, was added to 20 µL of temulawak extract in a microplate and left to stand for 5 minutes. Subsequently, 80 µL of 7.5% Na_2CO_3 (w/v in water) was added and incubated for 30 minutes in a dark room. The concentration of the solution was determined using a microplate reader at a wavelength of 750 nm. A standard curve was prepared using gallic acid with concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm, and 200 ppm. The final unit of analysis was expressed as mg GAE (gallic acid equivalent)/g DW (dry weight).

Flavonoid content measurement

Total flavonoid content was determined following the method of Khumaida et al. (2019). In this process, 10 mg of quercetin was dissolved in methanol (1000 ppm) and was diluted to 25 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm to prepare the standard curve. Subsequently, 20 µL of the sample, 10 µL of $AlCl_3$, 10 µL of CH_3COOK, 120 µL of water, and 60 µL of methanol were added to a microplate and left to stand for 30 minutes in a dark room. The solution was measured using an ELISA reader at a wavelength of 415 nm. Flavonoid content was obtained by entering the absorbance into the equation obtained from the quercetin standard curve. The results were converted to mg QE (quercetin equivalent)/g dry weight.

Antioxidant assays

DPPH assay

The DPPH antioxidant capacity was measured according to Nurcholis et al. (2022). 100 µL of extract was added to 100 µL of 125 µmol DPPH in a microplate, followed by incubation for 30 minutes. Before the incubation, 50 mg of Trolox was dissolved in pro-analysis methanol to obtain a concentration of 1000 ppm. The solution was incubated for 30 minutes, and the absorbance was measured using a nano-spectrophotometer (SPECTROstarNano BMG LABTECH) at a wavelength of 515 nm. Free radical scavenging capacity was expressed in µmol TE/g DW, with Trolox standards at concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, 30 ppm, and 35 ppm.

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

Antioxidant capacity was also assessed using the ABTS method by Nurcholis et al. (2022) with slight modifications. A 7.7 mM ABTS stock solution was made by adding 90 mg of ABTS to water in a volumetric flask until it reached 25 mL. The solution was transferred into a dark bottle, wrapped in aluminum foil, and stored in the refrigerator. Potassium persulfate was prepared: 66.289 mg

of K2S2O8 was added to aquabidest up to 100 mL, resulting in a 2.4 mM solution. The mixture was collected in a dark bottle, wrapped in aluminum foil, and stored in the refrigerator. The ABTS reagent was also prepared by mixing 7 mM ABTS and 2.4 mM K2S2O8 in a ratio of 2:1. Subsequently, various amounts of aquabidest were added, and the absorbance was measured with a nano spectrophotometer at 734 nm to obtain A=0.7±0,02. Approximately 20 µL of the sample was added with 180 µL of ABTS reagent and incubated for 6 minutes in the dark at room temperature. The absorbance was measured using a nano spectrophotometer at 734 nm. This test was expressed in µmol TE/g dry weight, with the Trolox standard of 0-500 ppm. Each test was carried out in triplicate.

FRAP assay

The FRAP antioxidant capacity assay was conducted using the procedures of Nurcholis et al. (2022). Approximately 1000 ppm concentration of Trolox was prepared by dissolving 50 mg of Trolox in pro-analysis methanol. The FRAP solution was prepared by mixing acetate buffer with a pH of 3.6, 10 µmol of TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 40 mM HCl and 20 mM of FeCl3 at 10:1:1 and was protected from light. The assay was performed by adding 300 µL of the FRAP solution to 10 µL of the sample and allowing the mixture to incubate for 5 minutes. The absorbance was recorded at 593 nm using a nano-spectrophotometer (SPECTROstarNano BMG LABTECH). The results were expressed as µmol TE/g DW. The standards were Trolox at concentrations of 100 ppm, 300 ppm, 400 ppm, 600 ppm, and 700 ppm.

Data analysis

Scottt-Knot's test performed statistical analysis to identify significant differences between variances of *C. xanthorriza* ($p<0.05$).

RESULTS AND DISCUSSION

Yield and phytochemical content of three varieties of *C. xanthorrhiza*

Table 1 shows that the highest yield of acetone extract of temulawak rhizome was Cursina 2 (C2) acetone extract (6.0492%). This yield was significantly higher than Cursina 1 (C1) (3.3796%) but not insignificantly different from Cursina 3 (C3), 0.2268% lower than that of C2. The highest yield of ethyl acetate was C2 ethyl acetate extract (4.2568%). The lowest yield was found in the extract of Cursina 1 (3.5188%), while Cursina 3 produced a yield of 3.8932%. Acetone extract had a higher yield than ethyl acetate for the C2 and C3 varieties but resulted in a lower yield for the C1 varieties. Sari et al. (2013) showed that the acetone extract of the temulawak plant produced a higher yield (7.77%) compared to ethanol (5.96%) and ethyl acetate (7.06%) extracts. Table 1 shows that the C2 sample seems to be more easily extracted than other varieties.

The TPC analysis showed that the acetone extract of C2 temulawak rhizome had the highest phenolic content (9.55±0.37 mg GAE/g dry weight), which was significantly different from other varieties ($p<0.05$). The lowest total phenolic content of acetone extract was obtained from C1 temulawak rhizome (3.49±0.11 mg GAE/g dry weight). The highest total phenolic content of ethyl acetate extract was from C2 ethyl acetate extract (4.60±0.12 mg GAE/g DW) but was not significantly different from C3. Moreover, the highest total flavonoid content (TFC) was obtained from the C2 acetone extract (20.48±0.45 mg GAE/g dry weight). TFC of C@ acetone extract was significantly different ($p<0.05$) compared to the C1 extract (6.60±0.19 mg QE/g DW) and C3 extract (10.03±0.57 mg QE/g dry weight). Acetone extracts of three varieties were significantly different from one another. The total flavonoid content of the C2 and C3 ethyl acetate extracts was significantly different ($p<0.05$) compared to the C1 extract, but the TFC of the C2 and C3 extracts was not significantly different. The C2 varieties showed the highest total flavonoid content of ethyl acetate extract was C2 (8.59±0.14 mg QE/g DW). Extraction using different solvents on three temulawak varieties produced different yields in the C2 and C3 but not in the C1 varieties, with acetone extract showing a higher flavonoid content.

Antioxidant capacity

The total antioxidant capacity of acetone and ethyl acetate extracts of three varieties of *C. xanthorrhiza* rhizome was determined using the DPPH, ABTS, and FRAP methods. The Scott-Knott test showed significant results with $p<0.05$, as presented in Table 2.

The highest DPPH antioxidant capacity of acetone extract was obtained from C2 (3.28±0.03 µmol TE/g DW), while the lowest was obtained in C1 (1.56±0.04 µmol TE/g DW). This result is significantly different ($p<0.05$) from one another. Extraction using ethyl acetate solvent showed that The DPPH antioxidant capacity of the C2 ethyl acetate extract differed significantly ($p<0.05$) compared to that of C1. The C2 ethyl acetate extract showed the highest antioxidant capacity (2.11±0.04 µmolol TE/g DW), while the C1 ethyl acetate extract had the lowest antioxidant capacity (1.59±0.05 µmolol TE/g DW). Different solvents on these three varieties resulted in significantly varied antioxidant capacity in the C2 and C3 but not in the C1.

Table 1. Yields, TPC, and TFC of acetone and ethyl acetate extract of three varieties of *C. xanthorrhiza*

Varieties	Yield (%)		Total phenolic (mg GAE/g dry weight)		Total flavonoid (mg QE/g dry weight)	
	Acetone	Ethyl acetate	Acetone	Ethyl acetate	Acetone	Ethyl acetate
Cursina 1	3.3796	3.5188	3.49±0.11cA	3.73±0.02bA	6.60±0.19cA	6.56±0.08bA
Cursina 2	6.0492	4.2568	9.55±0.37aA	4.60±0.12aB	20.48±0.45aA	8.59±0.14aB

Cursina 3	5.8224	3.8932	6.13±0.20bA	4.51±0.21aB	10.03±0.57bA	8.51±0.23aB

Notes: TPC: total phenolic content, TFC: total flavonoid content. C1: Cursina 1, C2: Cursina 2, C3: Cursina 3, DW: Dry Weight. Different lowercase letters in the same column is significantly different ($p<0.05$); Capital letter in the same row is significantly different ($p<0.05$)

Table 2. Antioxidant capacity of acetone and ethyl acetate extracts of three varieties of *C. xanthorrhiza* rhizome

Varieties	DPPH		ABTS		FRAP	
	Acetone	Ethyl acetate	Acetone	Ethyl acetate	Acetone	Ethyl acetate
Cursina 1	1.56±0.04cA	1.59±0.05bA	66.82±4.22cA	57.02±4.30cB	51.24±1.32cA	51.76±1.25cA
Cursina 2	3.28±0.03aA	2.11±0.04aB	148.91±6.10aA	84.30±5.10bB	115.23±2.30aA	65.41±2.11bB
Cursina 3	2.44±0.09bA	2.04±0.13aB	92.86±3.10bA	90.38±6.19aA	82.68±1.50bA	71.88±1.48aB

Notes: Different lowercase letters in the same column is significantly different ($p<0.05$); Different capital letter in the same row is significantly different ($p<0.05$)

The highest ABTS antioxidant capacity among the three varieties of acetone extract of temulawak rhizome was found in C2 (148.91±6.10 µmol TE/g DW), and the lowest was in C1 (66.82±4.22 µmolol TE/g DW). The ABTS antioxidant capacity was significantly different ($p<0.05$) among the three varieties. The antioxidant activities of acetone and ethyl acetate extract of C1 and C2 have substantial variation, but not for C3. The highest antioxidant capacity of ethyl acetate extract was obtained in C2 (90.38±6.19 µmolol TE/g DW), while C1 had the lowest (57.02±4.30 µmolol TE/g DW).

The highest FRAP antioxidant capacity of acetone extract was C2 (115.23±2.3 µmol TE/g DW), while the lowest was obtained from C1 (51.24±1.32 µmol TE/g DW). Notably, this difference was significant only when comparing the C2 and C3 varieties, highlighting the impact of utilizing distinct solvents. The highest antioxidant capacity of ethyl acetate extract was obtained in the C3 (71.88±1.48 µmol TE/g DW). C1 had the lowest antioxidant capacity (51.76±1.25 µmol TE/g DW), significantly differing from C3 and C2. As presented in Table 2, using different solvents on these three varieties showed significantly different results in the antioxidant capacity of ABTS and FRAP in C1 and C2 compared to C3.

Discussion

The extract yield is the weight ratio of the extract to the weight of the sample. Generally, yield reflects the effectiveness of solvent in extracting a substance without showing the capacity level (Vieito et al. 2018). The effectiveness of the solvent in the extraction process depends on several factors, such as solvent concentration, temperature, time, pH, etc. (Pham et al. 2019), and the plant varieties. Aboul-Maaty and Oraby (2019) noted variation in polyphenols, and other secondary metabolites among different plant species. The observed disparity in yield between the two solvents can be attributed to the similarity in polarity between the solvents and the compounds present in the sample. Based on the concept of like dissolves like, polar compounds will dissolve in a polar solvent, and nonpolar compounds will dissolve in a nonpolar solvent (Montes et al. 2003). Acetone and ethyl acetate are both polar aprotic solvents (Jakob et al. 2021), with polar indices of 5.1 (Zarrinmehr et al. 2022) and 4.4 (Barbosa et al. 2022), respectively. A higher polarity index indicates a more polar solvent. The results of this study show that the chemical compounds of temulawak (*C. xanthorrhiza*) rhizome tend to have polarity like that of acetone solvent compared to ethyl acetate. It shows that acetone extracts secondary metabolites from the temulawak rhizome more effectively.

Phenolic compounds are secondary metabolites characterized by one or more phenol rings, which are hydroxyl groups attached to an aromatic ring and easily oxidize by donating a hydrogen atom to free radicals. (Shahidi et al. 2015). The ability to form stable phenoxy radicals during oxidation reactions makes phenolic compounds highly potent antioxidants (Ali et al. 2013). In this study, TPC in the extracts of three varieties of temulawak rhizomes was measured using the Folin-Ciocalteu method, with gallic acid as a standard. This method used the oxidation reaction of phenolic compounds in an alkaline environment by the Folin-Ciocalteu reagent, resulting in a blue-colored complex and a strong absorption at a wavelength of 760 nm, allowing the measurement of TPC in the sample. The blue color in the solution was caused by the reduction of molybdenum (Mo(VI)) in the complex of the reagent into Mo(V) in the presence of an electron donor from an antioxidant compound (Munteanu et al. 2021). The intensity of the blue color in the sample was directly proportional to the amount of phenolic compounds (Blainski et al. 2013). Polyphenols' solubility and separation properties can vary depending on their structural differences. For instance, a compound's structure significantly impacts its polarity level, conjugation, and interaction with the sample matrix (Alara et al. 2021). According to Rachmawaty et al. (2018), acetone is a polar solvent with a ketone functional group (C=O). The O atom within the ketone group of acetone can form hydrogen bonds with the H atom of the hydroxyl group of phenolic compounds.

The high content of phenolic in temulawak rhizome extract can be attributed to the presence of curcuminoids such as curcumin, demethoxycurcumin, bisdemethoxycurcumin, and diarylheptanoids phenolic compounds (Nurcholis and Bintang 2017). It is in line with Sari et al. (2013), who found that acetone produced the highest curcuminoid content in temulawak extract compared to methanol and ethyl acetate. Curcuminoids are phenolic compounds

derived from diferuloylmethane, characterized by a yellow color and a slightly bitter taste (Susilowati et al. 2014). Moreover, acetone can prevent protein-polyphenol binding, a complex that is not readily soluble. Acetone can also inhibit the formation of protein-polyphenol complexes during extraction or break the interaction between the polyphenol functional group (-OH) and the protein carboxyl group, resulting in more efficient extraction of phenolic compounds (Sepahpour et al. 2018).

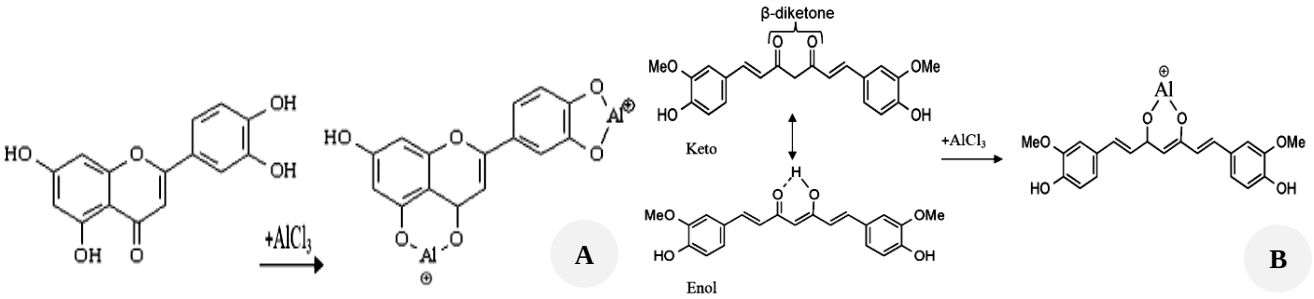

Figure 1. A. The reaction between $AlCl_3$ and flavonoid, B. The reaction between $AlCl_3$ and curcumin

Flavonoids are secondary metabolites belonging to the polyphenol group commonly found in plants. These secondary metabolites have pigments ranging from yellow, red, orange, and blue to purple and are soluble in water. Flavonoids are a group of low molecular weight compounds with two phenol-hormone nuclei resulting from the biosynthesis of acetic acid/phenylalanine derivatives through the shikimate acid pathway (Rehan 2021). Furthermore, flavonoids consist of an A aromatic ring, a B aromatic ring, and a central heterocyclic ring containing oxygen, which divides the compound into subgroups. The various types of flavonoids include flavones, flavanones, flavonols, catechins, flavanols, chalcones, and anthocyanins. In this study, the TFC measurement of the three samples showed a similar trend to total phenolic content because flavonoids are a phenolic compound (polyphenol) (Panche et al. 2016). Based on the results, higher total phenolic content has higher total flavonoid content, showing the close relationship between both compounds.

A high TFC does not always accurately represent the actual total content. This is because elevated flavonoid content can be caused by compounds measured using the colorimetric method with $AlCl_3$ (Figure 1A). These include compounds containing ortho-hydroxyl carbonyl groups such as curcuminoids, which are not flavonoids but have similar properties and react with $AlCl_3$ reagent, as shown in Figure 1B (Sepahpour et al. 2018.). Therefore, the increased total flavonoid content of temulawak can be associated with the high amount of curcuminoids contained in the extract. Revathy et al. (2011) found that the percentage of curcuminoid content in acetone extract (43.5%) was higher than in ethyl acetate extract (35.5%) in the turmeric plant (*Curcuma longa* L.). The standard for TFC determination is quercetin, a flavonol compound with a keto group on the C-4 atom, hydroxyl groups on the C-3, and adjacent C-5 atoms (Zheng et al. 2017).

Antioxidants are chemical compounds capable of reducing oxidative stress-induced carcinogenesis by directly scavenging ROS and inhibiting cell proliferation secondary to protein phosphorylation (Lobo et al. 2010). These compounds donate their electrons to free radical molecules, leading to stabilization, stopping chain reactions, and preventing damage to lipids, proteins, and DNA (Sharifi-Rad). The antioxidant capacity of a plant extract is significantly influenced by total phenolic and flavonoid, with higher content resulting in greater antioxidant capacity (Nur et al. 2019). Therefore, the high total phenolic and flavonoid content in the acetone extract of C2 produced the highest antioxidant capacity compared to other varieties using ethyl acetate solvent. Meanwhile, the lowest total phenolic and flavonoid content in C1 yielded the lowest DPPH, ABTS, and FRAP antioxidant capacity.

The DPPH Hantioxidant capacity method was selected for its rapid simplicity, cost-effectiveness, and wide usage (Kedare and Singh 2011). DPPH antioxidant capacity was determined using a nano spectrophotometer by changing from purple to yellow or colorless, showing the conversion of the stable free radical DPPH into a stable diamagnetic molecule. In the DPPH radical-scavenging assay, antioxidants react with DPPH and convert it to yellow-colored diphenylpicrylhydrazine. The extent of color fading indirectly indicates the radical-scavenging capacity of antioxidants (Fatiha and Abdelkader 2019). DPPH radical is an unstable organic compound containing nitrogen with strong absorbance at a wavelength of 517 nm with a dark purple color (Souhoka et al. 2019). Generally, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is an assay standard used as a comparative antioxidant compound due to its exceptional antioxidant properties, surpassing alpha-tocopherol, propyl gallate, and ascorbyl palmitate.

The ABTS method's antioxidant capacity is based on forming a blue/green ABTS suitable for both water-soluble and fat-soluble antioxidant systems (Floegel et al. 2011). The more faded the green color, the higher the ABTS antioxidant capacity. It occurs due to interactions between

antioxidants reducing the sample's ABTS radical compound. Stable ABTS radicals are formed from ABTS with potassium persulfate that can stand for 12-16 hours until it creates a blue-green color (Anh-Dao et al. 2022). The ABTS and DPPH methods have a similar reaction mechanism, where the radicals receive electrons from the antioxidant compound and reduce it to a non-radical form (Munteanu et al. 2021). However, antioxidant capacity results by the ABTS and DPPH methods are not always linear, showing that there may be other mechanisms besides electron transfer. In the study by Floegel et al. (2011), the ABTS method produced higher antioxidant capacity, which was a better method than the DPPH. The ABTS method reacts quickly, provides specific absorbance, and can be used at various pH levels (Shalaby et al. 2013), while in the DPPH method, an overly acidic solution pH interferes with stability (Dawidowicz and Olzowy 2012). Sadeer et al. (2020) explained that the redox potential of ABTS is higher than that of the DPPH compound, making it easier and more susceptible to being reduced by antioxidant compounds.

FRAP is a simple, fast, and inexpensive method commonly used to test antioxidants in plants without requiring specialized equipment. (Munteanu et al. 2021). The principle of the FRAP method is based on the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+} ions (Priya et al. 2021). The antioxidant strength of a compound is analogized with its ability to reduce these ions (Halvorsen et al. 2002). The reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ is indicated by a color change from yellow to intense blue in an acidic environment (Irshad et al. 2012). Compared to DPPH and ABTS, the FRAP method shows a slightly different antioxidant capacity, attributed to the mechanism characteristics. Additionally, the FRAP method is less specific due to its capability to detect non-antioxidant compounds (Sukweenadhi et al. 2020). The high reduction potential of Fe^{3+} ions (0.70 V) makes the Fe^{3+}-TPTZ complex easily reduced to form Fe^{2+}-TPTZ by any compound with a lower reduction potential (Sadeer et al. 2020), affecting the accuracy of the FRAP method. In this study, antioxidant capacity measurement using the FRAP method produced the same pattern of capacity value sequence as the DPPH and ABTS methods, with acetone extract of the C2 having the highest value.

In conclusion, the acetone extract of the three turmeric varieties showed higher results than the ethyl acetate extract. It showed that the active compound of C2 rhizome was most effectively extracted using acetone because it produced the highest total phenolic, total flavonoid, and antioxidant capacity compared to other varieties of ethyl acetate extract. Different solvents, i.e., acetone and ethyl acetate, resulted in significant differences in yield, total phenolic and flavonoid content, and antioxidant capacity of C2 and C3 extracts but had no significant effect on C1 extract. The total phenolic and flavonoid content of acetone extract differed significantly among varieties. The phenolic, flavonoid, and DPPH antioxidant capacity of the C2 and C3 ethyl acetate extract did not vary significantly. The ABTS and FRAP antioxidant capacity of ethyl acetate extract of the C3 varieties is the highest compared to other varieties. Based on the results, the C2 variety contained abundant secondary metabolites with the best antioxidant capacity.

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REFERENCES

Aboul-Maaty NAF, Oraby HAS. 2019. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Cent 43 (25): 1-10. DOI: 10.1186/s42269-019-0066-1.

Alara OR, Abdurahman NH, Ukaegbu CI. 2021. Extraction of phenolic compounds: A review. Curr Res Food Sci 2 (4): 200-214. DOI: 10.1016/j.crfs.2021.03.011.

Ali HM, Abo-Shady A, Sharaf Eldeen HA. 2013. Structural features, kinetics and SAR study of radical scavenging and antioxidant activities of phenolic and anilinic compounds. Chem Cent J 7: 53. DOI: 10.1186/1752-153X-7-53.

Anh-Dao LT, Nhon-Duc L, Cong-Hau N, Thanh-Nho N. 2022. Variability of total polyphenol contents in ground coffee products and their antioxidant capacities through different reaction mechanisms. Biointerface Res Appl Chem 12: 4857-4870. DOI: 10.33263/BRIAC124.48574870.

Barbosa FG, Marcelino PRF, Lacerda TM, Philippini RR, Giancaterino ET, Mancebo MC, dos Santos JC, da Silva SS. 2022. Production, Physicochemical and structural characterization of a bioemulsifier produced in a culture medium composed of sugarcane bagasse hemicellulosic hydrolysate and soybean oil in the context of biorefineries. Fermentation 8: 618. DOI: 10.3390/ fermentation8110618.

Bitwell C, Indra S, Luke C, Kakoma MK. 2023. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Sci Afr 19: e01585. DOI: 10.1016/j.sciaf.2023.e01585.

Blainski A, Lopes GC, De Mello JCP. 2013. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules 18 (6): 6852-6865. DOI: 10.3390/molecules18066852.

Fatiha M, Abdelkader T. 2019. Study of antioxidant activity of pyrimidinium betaines by DPPH radical scavenging method. J Anal Pharm Res 8 (2): 33-36. DOI: 10.15406/japlr.2019.08.00308.

Dawidowicz A, Olszowy M. 2012. Mechanism change in estimating of antioxidant activity of phenolic compounds. Talanta 97 (1): 312-317. DOI: 10.1016/j.talanta.2012.04.036.

Elfin, Fachrial E, Lister I Nyoman E, Ginting CN. 2021. The potential of temulawak (*Curcuma xanthorrhiza* Roxb.) as a rejuvenating agent. IOSR J Pharm 11 (2): 29-34.

Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compost Anal 24 (7): 1043-1048. DOI: 10.1016/j.jfca.2011.01.008.

Halvorsen BL, Holte K, Myhrstad MCW, Barikmo I, Erlend H, Fagertun RS, Anne-Brit W, Karin H, Halvard B, Frost AL, Jan A, David J, Rune B. 2002. A systematic screening of total antioxidant in dietary plants. J Nutr 132 (3): 461-471. DOI: 10.1093/jn/132.3.461.

Hikmawanti NPE, Fatmawati S, Asri AW. 2021. The Effect of ethanol concentrations as the extraction solvent on antioxidant activity of katuk (*Sauropus androgynus* L. Merr.) leaves extracts. IOP Conf Ser Earth Environ Sci 755: 012060. DOI: 10.1088/1755-1315/755/1/012060.

Irshad M, Zafaryab M, Singh M, Rizvi MM. 2012. Comparative analysis of the antioxidant activity of *Cassia fistula* extracts. Intl J Med Chem 2012: 157125. DOI: 10.1155/2012/157125.

Jakob A, Grilc M, Teržan J, Likozar B. 2021. Solubility temperature dependence of bio-based levulinic acid, furfural, and hydroxymethylfurfural in water, nonpolar, polar aprotic and protic solvents. Processes 9 (6): 924. DOI: 10.3390/pr9060924.

Kedare SB, Singh RP. 2011. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48 (4): 412-422. DOI: 10.1007/s13197-011-0251-1.

Khumaida N, Syukur M, Bintang M, Nurcholis W. 2019. Phenolic and flavonoid content in ethanol extract and agromorphological diversity of *Curcuma aeruginosa* accessions growing in West Java, Indonesia. Biodiversitas 20 (3): 656-663. DOI: 10.13057/biodiv/d200306.

Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4 (8): 118-126. DOI: 10.4103/0973-7847.70902.

Mapoung S, Semmarath W, Arjsri P, Thippraphan P, Srisawad K, Umsumarng S, Phromnoi K, Jamjod S, Prom-U-Thai C, Dejkriengkraikul P. 2023. Comparative analysis of bioactive-phytochemical characteristics, antioxidants activities, and anti-inflammatory properties of selected black rice germ and bran (*Oryza sativa* L.) varieties. Eur Food Res Technol 249 (2): 451-464. DOI: 10.1007/s00217-022-04129-1.

Munteanu IG, Apetrei C. 2021. Analytical methods used in determining antioxidant activity: A review. Intl J Mol Sci 22 (7): 3380. DOI: 10.3390/ijms22073380.

Montes I, Lai C, Sanabria D. 2003. Like dissolves like: A guided inquiry experiment for organic chemistry. J Chem Edu 80 (4): 447. DOI 10.1021/ed080p447.

Nurcholis W, Alfadzrin, R, Izzati N, Arianti, R, Vinnai BÁ, Sabri F, Kristóf E, Artika IM. 2022. Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of Java cardamom (*Amomum compactum* Soland Ex Maton) fruit. Plants 11: 2221. DOI: 10.3390/ plants11172221.

Nurcholis W, Bintang M. 2017. Comparison of antioxidant activity and phenolic content of *Curcuma xanthorrhiza* and *Curcuma aeruginosa*. Jurnal Jamu Indonesia 2 (1): 25-29. DOI: 10.29244/jji.v2i1.27. [Indonesian]

Nur S, Sami FJ, Wilda R, Awaluddin A, Afsari MIA. 2019. Correlation between total flavonoid and phenolic content of *Gmelia arborea* Roxb. leaf extract and fraction and its antioxidant activity. J Galenika Pharm 5 (1): 33-42. DOI: 10.22487/j24428744.2019.v5.i1.12034.

Oon SF, Nallappan M, Tee TT, Shohaimi S, Kassim NK, Sa'ariwijaya MS, Cheah YH. 2015. Xanthorrhizol: A review of its pharmacological activities and anticancer properties. Cancer Cell Intl 15: 100. DOI: 10.1186/s12935-015-0255-4.

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: An overview. J Nutr Sci 5: e47. DOI: 10.1017/jns.2016.41.

Pham TN, Lam T, Nguyen M, Le T, Vo Dai-Viet, Quoc TT, Sang VT. 2019. Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam. IOP Conf Ser Materials Sci Eng 544: 012013. DOI: 10.1088/1757-899X/544/1/012013.

Priya RR, Bhaduhsha N, Manivannan V, Gunasekaran T. 2021. Evaluation of in-vitro antioxidant activity of hydroalcoholic and methanolic extract of *Senna alata*. Ann Romanian Soc Cell Biol 25 (4): 5291-5303.

Rachmawaty A, Munisa, Hasri H, Pagarra, Hartati, and Maulana Z. 2018. Extraction of active compounds from cocoa and its potential as a fungicide. J Phys 1028 (1): 12-13.

Rahmat E, Lee J, Kang Y. 2021. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evid Based Complement Alternat Med 2021: 9960813. DOI: 10.1155/2021/9960813.

Rehan M. 2021. Biosynthesis of diverse class flavonoids via shikimate and phenylpropanoid pathway. Bioactive Compounds - Biosynthesis, Characterization and Applications. DOI: 10.5772/intechopen.96512.

Revathy S, Elumalai S, Merina Benny and Benny Antony. 2011. Evaluation of curcuminoids in turmeric rhizome (*Curcuma longa* L.) collected from different places in India. Biosci Biotechnol Res Asia 8 (1): 259-264. DOI: 10.13005/bbra/853.

Sadeer NB, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. 2020. The versatility of antioxidant assays in food science and safety: Chemistry, applications, strengths, and limitations. Antioxidants 9 (8): 709-738. DOI: 10.3390/antiox9080709.

Sari DLN, Cahyono B, Kumoro AC. 2013. The effect of solvent type on the extraction of curcuminoids from temulawak (*Curcuma xanthorrhiza* Roxb.) rhizomes. Chem Info 1 (1): 101-107.

Sepahpour S, Selamat J, Abdul Manap MY, Khatib A, Abdull Razis AF. 2018. Comparative analysis of chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. Molecules 23 (2): 402-419. DOI: 10.3390/molecules23020402.

Shahidi F, Ambigaipalan P. 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review, J Funct Food 18: 820-897. DOI: 10.1016/j.jff.2015.06.018.

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC and Sharifi-Rad J. 2020. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol 11: 694. DOI: 10.3389/fphys.2020.00694.

Simon S, Sedhulakshmi K, Joseph J, George D. 2022. Optimization of extraction parameters of bioactive components from *Moringa oleifera* leaves using Taguchi method. Biomass Convers Biorefin 13 (11): 1-10. DOI: 10.1007/s13399-021-02276-1.

Shalaby EA, Shanab SMM. 2013. Antioxidant compounds, assays of determination and mode of action. Afr J Phram Pharmacol 7 (10): 535-537. DOI: 10.5897/AJPP2013. 3474.

Souhoka FA, Hattu N, Huliselan M. 2019. Antioxidant activity test of methanol extract from keeling kesumba seeds (*Bixa orellana* L). Indonesian J Chem Res 7 (1): 25-31. DOI: 10.30598//ijcr.2019.7-fas.

Sukweenadhi J, Yunita O, Setiawan F, Kartini, Siagian MT, Danduru AP, Avanti C. 2020. Antioxidant activity screening of seven Indonesian herbal extracts. Biodiversitas 21 (5): 2062-2067. DOI: 10.13057/biodiv/d210532.

Susilowati T, Kawiji, Ariviani S. 2014. Kapasitas antioksidan dan kadar kurkuminoid ekstrak rimpang temulawak (*Curcuma xanthorrhiza*) menggunakan pelarut air dengan variasi proporsi pelarut dan metode pemanasan. Biofarmasi 12 (2): 83-89. DOI: 10.13057/biofar/f120205. [Indonesian]

Teoh ES. 2015. Secondary metabolites of plants. In: Medicinal Orchids of Asia. Springer, New York. DOI: 10.1007/978-3-319-24274-3_5.

Vieito C, Fernandes É, Vaz Velho M, Pires P. 2018. The effect of different solvents on extraction yield, total phenolic content and antioxidant activity of extracts from pine bark (*Pinus pinaster* subsp. *atlantica*). Chem Eng Trans 64: 127-132. DOI: 10.3303/CET1864022.

Xu DP, Li Y Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. 2017. Natural antioxidants in foods and medicinal plants: Extraction, assessment, and resources. Intl J Mol Sci 18: 96. DOI: 10.3390/ijms18010096.

Zarrinmehr MJ, Daneshvar E, Nigam S, Gopinath KP, Biswas JK, Kwon EE, Wang H, Farhadian O, Amit Bhatnagar. The effect of solvents polarity and extraction conditions on the microalgal lipids yield, fatty acids profile, and biodiesel properties. Bioresour Technol 344: 126303. DOI: 10.1016/j.biortech.2021.126303.

Zheng YZ, Deng G, Liang Q, Chen DF, Guo R, Lai RC. 2017. Antioxidant activity of quercetin and its glucosides from propolis: A theoretical study. Sci Rep 7: 7543. DOI: 10.1038/s41598-017-08024-8.