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Short Communication: Analysis of rhizome color and phytochemical content of 10 accessions of *Curcuma zanthorrhiza* Roxb. in Jambi, Indonesia

MINARNI MINARNI[1], RAYANDRA ASYHAR[1], DARA JULIANA[2], YOSHUA SHANDY YUDHA[3], WARAS NURCHOLIS[2,4,♥]

[1]Department of Chemistry, Faculty of Education, Universitas Jambi. Jl. Jambi - Muara Bulian Km. 15, Mendalo Darat, Jambi 36361, Indonesia
[2]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Meranti, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8625481, ♥email: wnurcholis@apps.ipb.ac.id
[3]Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor. Bogor 16680, West Java, Indonesia
[4]Tropical Biopharmaca Research Center, Institute of Research and Community Services, Institut Pertanian Bogor. Jl. Taman Kencana, Bogor 16128, West Java, Indonesia

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Abstract. *Minarni M, Asyhar R, Juliana D, Yudha YS, Nurcholis W. 2023. Short Communication: Analysis of rhizome color and phytochemical content of 10 accessions of* Curcuma zanthorrhiza *Roxb. in Jambi, Indonesia. Biodiversitas 24: 149-155.* Plants are a source of secondary metabolites that have various biological functions and are beneficial for humans, particularly in the health sector. One such beneficial herbal plant, temulawak (*Curcuma zanthorrhiza* Roxb.), a member of the genus *Curcuma* L. in the Zingiberaceae family, is widely known as Javanese turmeric. *C. zanthorrhiza* is a potential source of drugs in the field of pharmacology. One part of temulawak that has an important role is the rhizome, commonly used in the formulation of traditional herbal medicines. There are many pharmacologically active components contained in the *C. zanthorrhiza* rhizome. Thus, this plant, found in many parts of Indonesia, such as Jambi and Sumatra, is worthy of detailed pharmacological investigation. The study reported in this paper aimed to determine the potential content of secondary metabolites of alkaloids, flavonoids, saponins, tannins/phenolics, and triterpenoids/steroids in 10 accessions of temulawak rhizomes in Jambi. The phytochemical content analysis in temulawak was carried out by qualitative phytochemical screening using dye reagents. Multivariate cluster analysis was carried out to determine the relationships between the 10 accessions based on similarities in the color and phytochemical content of their rhizomes. Generally, the screening results showed that the rhizomes of the 10 *C. zanthorrhiza* accessions contained the same intensities of alkaloids (dragendof, mayer and wagner), flavonoids, tannins/phenolics (+++) and steroid compounds (++). On the other hand, saponin and triterpenoid were not detected in the tests of rhizomes of the 10 accessions (-). The clustering analysis of the results divided the 10 local temulawak accessions into three clusters. Cluster-1 consisted of two accessions (JM and JBB), cluster-2 consisted of three accessions (JT, JPB, and JBS), and cluster-3 consisted of five accessions (JSS, JJ, JB, JBE, and JG).

Keywords: Accession, *Curcuma zanthorrhiza*, phytochemical screening, qualitative analysis, secondary metabolites

INTRODUCTION

'Temulawak', with the scientific name *Curcuma zanthorrhiza* Roxb., is a medicinal plant with a variety of phytochemicals of potential benefit to human health. *C. zanthorrhiza* is widely used in traditional medicine to treat ulcers, gastric disorders, constipation, diarrhea, dysentery, hemorrhoids, arthritis, hypotriglyceridemia, rheumatism, skin eruptions, and vaginal discharge (Rahmat et al. 2021). *C. zanthorrhiza* is one of the most widely used and cultivated herbal plants, especially in Southeast Asian countries such as Indonesia, Malaysia, the Philippines, Vietnam, and Thailand (Salleh et al. 2016). The *C. zanthorrhiza* plant usually lives and grows in tropical forests at an altitude of 1500 meters. The plant part of *C. zanthorrhiza* that is most widely studied and used as the main ingredient for herbal medicines is the rhizome. *C. zanthorrhiza* rhizome grows and thrives in friable soil. The rhizome has properties that make it valuable as a natural medicine suitable for the body's metabolism. It contains secondary metabolite compounds with promising potentials, such as essential oils, curcumin, flavonoids, alkaloids, phenolics, and steroids (Kustina et al. 2020). Based on many studies, it is known that two compounds, terpenoids and curcuminoids have a particularly important role because they are the most abundant essential phytochemicals in the rhizomes. They result in the rhizome having the potential to treat various diseases because of their pharmacological properties, such as antioxidant, antibacterial, antitumor, neuroprotective, and hepatoprotective activities (Rahmat et al. 2021).

Curcuma zanthorrhiza is one of the plants native to Indonesia that have pharmacological properties useful in herbal medicines. Its rhizome is an especially valuable part. Most *C. zanthorrhiza* rhizomes contain various sesquiterpene derivatives, including alpha and beta turmerone derived from the essential oil. Various studies have shown that the essential oil content of the rhizomes is in the range of 7.3-29.5% on a dry weight basis (Musfiroh et al. 2020). In addition to terpenoids and curcuminoids, other compounds that are also known to be abundant in *C. zanthorrhiza* rhizomes are the sesquiterpene phenol xanthorrhizol compounds (32%); beta-curcumin (17%), zingiberene (13%) and beta bisabolol (3.5%). Xanthorrhizol is the dominant compound from the sesquiterpene group of *C. zanthorrhiza* that has pharmacological activity against cancer cell metastatic activity and for inhibition of tumor cells (Musfiroh et al. 2020).

The bioactive compounds contained in *C. zanthorrhiza* rhizomes have been identified in pharmacological studies to determine the potential of these compounds for medicinal use. Various methods are used to detect and identify secondary metabolites contained in plant materials, one of which is phytochemical screening, based on either quantitative procedures such as analysis of absorbance using a spectrophotometer or qualitative procedures using a color change test. This phytochemical screening is one of the commercial ways used to determine the content of the dominant phytochemical compounds for medicinal and industrial purposes (Muhammad et al. 2019).

Previous research has shown that the levels of secondary metabolites in *C. zanthorrhiza* plant material are influenced by genetics, environmental, and management factors such as plant harvesting age, soil nutrient content, altitude, cultivation methods and plant protection. Environmental factors such as rainfall, soil moisture, and soil types play essential roles in the accumulation of secondary metabolites such as curcuminoids. Curcuminoid compounds in *C. zanthorrhiza* have better pharmacological activity than *C. aeruginosa* plants (Rosidi 2020). Curcuminoid compounds belong to phenolic compounds with high antioxidant properties (Awin et al. 2019).

The study reported in this paper was conducted to analyze by qualitative methods the presence of alkaloids, flavonoids, saponins, tannins, phenolics, triterpenoids and steroids in the rhizomes of *C. zanthorrhiza* obtained from several locations in Jambi. The similarity between the rhizome samples was assessed statistically based on the color of the rhizomes and the results of the qualitative detection of these secondary metabolites.

MATERIALS AND METHODS

Study area and plant material

The present study was conducted in June 2022. Ten accessions of local *C. zanthorrhiza* collected from several locations in Jambi province, Indonesia, were used as samples for biochemical analysis. The information on the geographic locations of each accession of *C. zanthorrhiza* is summarized in Table 1.

Sample extraction

The procedure of sample extraction from the rhizome of *C. zanthorrhiza* was based on Khumaida et al. (2019) with modifications. The fresh rhizomes of *C. zanthorrhiza* were cleaned, and then cut into small pieces and dried in the sun for five days. The drying process aimed to remove the water content of the rhizome. The longitudinal and transverse sections of *C. zanthorrhiza* rhizomes were characterized by the presence of a yellow or orange color (Rosidi et al. 2016). The dried rhizomes of *C. zanthorrhiza* became small after being cut, and the rhizomes were mashed using a blender until they were powdered. 5 g of each rhizome sample was extracted by maceration using 50 mL 97% ethanol for 3 x 24 hours with two replications. The samples were stirred every 24 hours. After that, they were filtered to obtain the *C. zanthorrhiza* rhizome extracts.

Biochemical analysis

The alkaloid analysis was performed by adding 1 mL of the sample dissolved in a few drops of 2N sulfuric acid. Afterward, the mixed solution was tested with Dragendorff's reagent, Mayer's reagent and Wagner's reagent to determine the presence of alkaloids in the sample. A positive test result for Drafendorff's reagent is a red-to-orange precipitate. A yellowish-white precipitate is obtained for a positive test result with Mayer's reagent, while a brown precipitate indicates a positive using Wagner's reagent. Flavonoid testing was carried out by adding a few drops of concentrated HCl and Mg powder to the sample. If there is a color change to orange and foam is formed, the sample is positive for flavonoid compounds. Then, saponin phytochemical compounds were tested by dissolving the sample in 2 mL of hot water. If a stable foam is formed for 30 minutes, the sample is positive for saponin compounds. The phenolic/tannin content test was carried out by mixing the sample with $FeCl_3$. If a blackish-purple color is formed, the sample contains tannin compounds. Lastly, qualitative analysis of triterpenoid/steroid compounds was carried out by dripping Lieberman-Burchard reagent into the sample. A positive result for triterpenoids is indicated by a purple-red color reaction, whereas a positive result for steroids was indicated by a blue-green color reaction.

Data analysis

The results of the biochemical analysis of *C. zanthorrhiza* were presented using a qualitative method. The variations in the colors of the *C. zanthorrhiza* rhizomes were shown photographically (Figure 1). The Cluster packages in R studio (version 4.2.2) were used to cluster the 10 accessions of *C. zanthorrhiza* based on the results of the phytochemical testing and the rhizome colors. Cluster analysis consists of two steps: first, estimating the dissimilarity matrix of 10 accessions of *C. zanthorrhiza* using the gower dissimilarity method; second, the visualization of the dissimilarity matrix in the form of clusters was performed using the complete linkage method.

Table 1. Description of the location of each accession of *Curcuma zanthorrhiza*

Accession code	Location	Geographic coordinates		Altitude (m asl.)
		Latitude (S)	Longitude (E)	
JPB	Jambi, Pasar Bangko	2º04’36”	102º16’11”	6.19
JT	Jambi Tembesi	1º40’53”	103º05’31”	156
JJ	Jambi Jaluko	1º35’33”	103º27’43”	2.53
JM	Jambi Mendahara	0º59’55”	103º35’48”	78.1
JBE	Jambi Berbak	1º13’22”	104º10’34”	78.1
JG	Jambi Geragai	1º13’03”	103º40’20”	156
JB	Jambi Bangko	2º05’30”	102º18’23”	78.2
JBB	Jambi Bangko Barat	2º10’25”	102º14’16”	78.2
JSS	Jambi Sarolangun Singkut	2º32’21”	102º43’01”	78.1
JBS	Jambi Berbak Simpang	1º15’34”	104º06’01”	9.76

RESULTS AND DISCUSSION

The accessions used in this study are symbolized by the letters JPB, JT, JJ, JM, JBE, JG, JB, JBB, JSS, and JBS, which refer to the geographic locations from which the rhizome samples were obtained (details of which are given in Table 1). The screening for phytochemical compounds in the *C. zanthorrhiza* rhizomes employed a qualitative method based on color change tests (dye reagent) described in the methods section above. The results of screening phytochemical compounds in *C. zanthorrhiza* rhizomes from the 10 locations in the Jambi regions are presented in Table 2.

Several research results indicate that qualitative biochemical analysis is an important first step in determining the presence and intensity of a plant's biochemical compounds of potential pharmacological value. Aisyah et al. (2019) obtained information on the biochemical content of putative mutants of *Celosia cristata* through color reactions analysis. Muhallilin et al. (2019) performed a qualitative biochemical study to understand the biochemical composition of *Celosia cristat*a after the irradiation mutation treatment.

In this study, the phytochemicals screened were alkaloids, flavonoids, saponins, tannins/phenolics, triterpenoids, and steroids. These metabolites are of potential significance because they are considered to reduce the effects of various diseases, are non-toxic, and have helpful physiological activity for treatment; thus, they have a broad pharmacological scope (Alhassan et al. 2018).

The rhizomes from the 10 *C. zanthorrhiza* accessions scattered in Jambi were classified into four groups based on their color (Figure 1), namely: (i) having a yellow color with almost no orange color; (ii) having a bright orange color, i.e. the bright orange color is dominant; (iii) having a deep orange color; and (iv) having an intense dark orange color resembling a dark brown color. The presence of orange color is a distinctive color feature of *C. zanthorrhiza* rhizomes, which is different from the color of the rhizomes in other *Curcuma* species, such as *C. aeruginosa*, which has a distinctive blue color. The presence of yellow or orange color in the *C. zanthorrhiza* rhizome is due to the presence of curcuminoid compounds (Kustina et al. 2020). Differences in the geographic location of *C. zanthorrhiza* accessions are pivotal factors influencing the quantity and quality of secondary metabolites directly responsible for the pharmacological activity of *C. zanthorrhiza* accessions. The *C. zanthorrhiza* plant is an annual with false stems and grows in clusters of 3-9 saplings.

Screening of phytochemical compounds to identify the content of alkaloid compounds in *C. zanthorrhiza* rhizomes was carried out using the Dragendorf, Mayer, and Wagner reagent tests. From these three reagent tests, positive alkaloid results were obtained (Table 2), which was indicated by the formation of the brownish-orange color of the residues. A dark brown precipitate was obtained for the test with Mayer's reagent, while a brownish-orange precipitate was obtained with Wagner's reagent. The results of the Mayer test showed various reaction responses, which indicated qualitatively a range in the alkaloid content of the accessions studied. These results conform with the research reported by Muharrami et al. (2020), which stated that for the alkaloid tests using Dragendorf and Wagner reagents, a yellow to brown precipitate was obtained, indicating a positive result for the content of alkaloid phytochemical compounds in the *C. zanthorrhiza* rhizome. Alkaloid compounds have bioactivity properties such as antitumor and anticancer, antispasmodic and antibacterial effects. These alkaloid compounds are also used pharmacologically as local anesthetics, analgesics, and antimalarial drugs (Muhammad et al. 2019).

Positive results for flavonoid compounds in the *C. zanthorrhiza* rhizomes were indicated by the formation of a brownish-yellow color in the reaction response of the rhizomes extracted with 97% ethanol. This result is in harmony with the research results of Muharrami et al. (2020), which stated that using ethanol as a solvent, extracts of *Parameria laevigata*, *Kaempferia galanga* L, *Curcuma domestica*, *Curcuma xanthorrhiza*, and *Punica granatum* showed positive results for the content of flavonoids as indicated by the formation of thick yellow color. The *C. zanthorrhiza* test results in this and other studies indicate the presence of abundant flavonoid compounds. Flavonoids are one of the polyphenol group compounds with rings A and B connected by ring C with the molecular formula (C6-C3-C6) (Wang et al. 2018). Based on the literature, it is said that almost all green plants contain flavonoid compounds. Flavonoid compounds have structural derivatives of 2-phenylchromone, most commonly found in plants with specific bioactivities such as preventing cell aging, helping the body's resistance to bacterial infections, and strengthening the body's immune system (Zhang et al. 2021). This flavonoid activity correlates with Glutathione S-Transferase (GST) activity in human platelets, rat liver, and rat kidney tissue. The mechanism of this biosynthetic pathway of flavonoid compounds plays an important pharmacological role in their antioxidant and antibacterial activity (Song et al. 2021).

Table 2. Qualitative phytochemical screening of *Curcuma* rhizomes (*Curcuma zanthorrhiza*) from 10 locations in the vicinity of Jambi, Indonesia

Secondary metabolites tested	Accessions									
	JPB	JT	JJ	JM	JBE	JG	JB	JBB	JSS	JBS
Alkaloid										
- Dragendorff	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
- Mayer	+++	+	+	+++	+++	+++	+	+++	+	+++
- Wagner	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoid	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Saponin	-	-	-	-	-	-	-	-	-	-
Taninns/phenolics	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Triterpenoid	-	-	-	-	-	-	-	-	-	-
Steroid	++	++	++	++	++	++	++	++	++	++

Note: Color reaction (+++) = strong, (++) = medium, (+) = low, (-) = not detected. For details of the accession codes for the rhizome samples, see Table 1

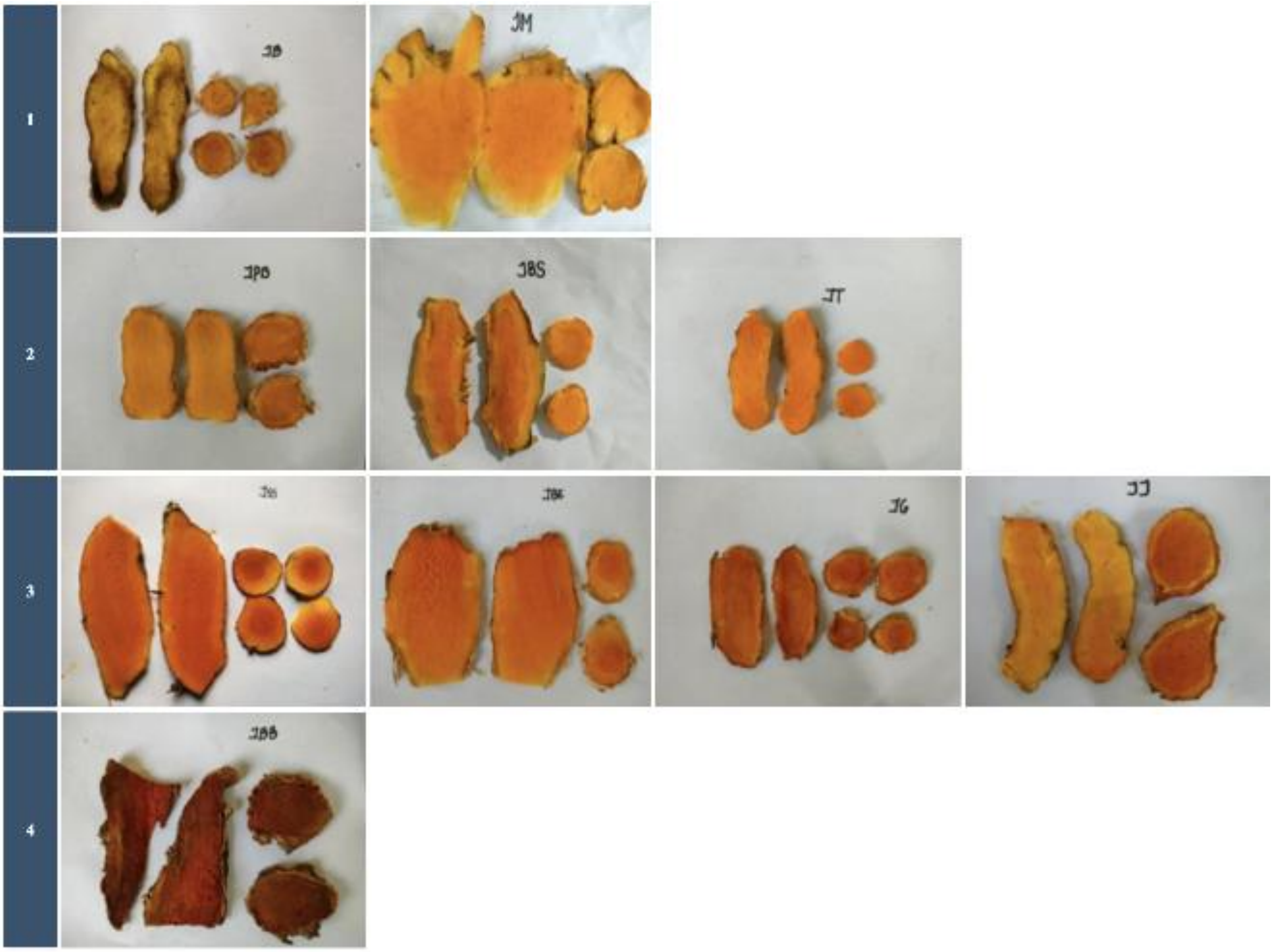

Figure 1. The rhizome color of 10 accessions of *C. zanthorrhiza* was obtained from the Jambi Region. (1) JB and JM have a yellow color with almost no orange (2) JPB, JBS, and JT have a bright orange color, (3) JSS, JBE, JG, JJ have a dense orange color, and (4) JBB has a very dark orange color approaching a dark brown color. For details on the Accession codes, see Table 1

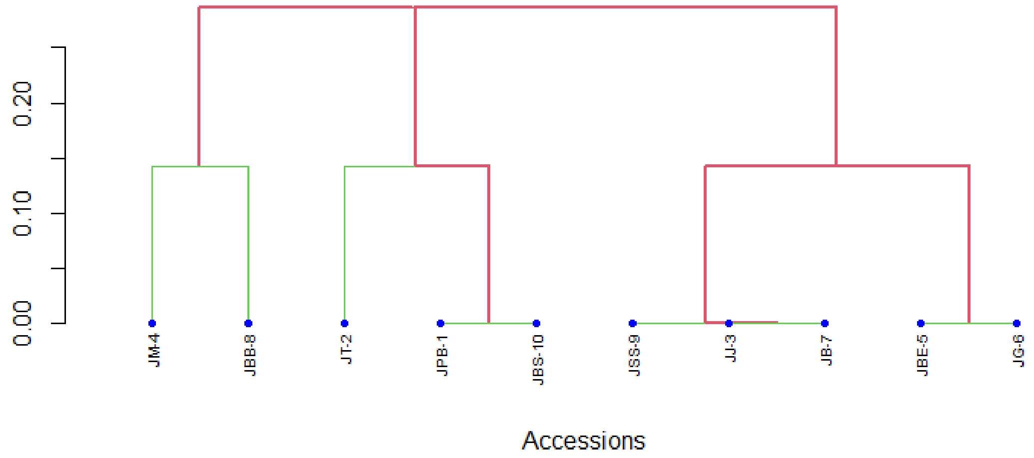

Figure 2. Clustering analysis of 10 accessions of *Curcuma zanthorrhiza* rhizomes from Jambi, Indonesia

Sapononins are another group of secondary metabolites that play a role in the pharmacological activity of certain plants. Tests for saponin compounds in the *C. zanthorrhiza* rhizomes originating from 10 localities spread across the Jambi region showed negative results, indicating the absence of saponin compounds. The results obtained can be seen in Table 2. However, this study's results differ from those of Muharrami et al. (2020), which succeeded in identifying the presence of saponin compounds contained in the stems of *Parameria laevigata*, a rhizome of *Kaempferia galanga* L. (kencur), a rhizome of *C. zanthorrhiza* and extracts of *Punica granatum* (pomegranate). The standard saponin test indicates a positive result for the presence of saponins if a relatively stable foam is observed when the sample is dissolved in hot water (Ashour et al. 2019). However, in this study, the results obtained were extracts with a yellow residue that did not form a stable foam, indicating that there was no content of saponin compounds in the *C. zanthorrhiza* rhizomes. Saponin compounds have been found in the rhizome extracts of *Curcuma zedoaria* with ethanol solvent. Saponins have a polar structure with a relatively high molecular weight. They are derived from triterpene saponins, which contain one or more glucose moieties attached to oleanane or pentacyclic triterpenoid aglycones. Saponins have recognized pharmacological effects: overcoming and preventing inflammation, preventing oxidative stress, anticancer agents, preventing viral infections, and acting as immunoregulators (Li et al. 2020). They are known to have significant activity for the inhibition of inflammation in accordance with the carrageenan induction method related to dose and protein denaturation in vitro.

The qualitative tests conducted on the *C. zanthorrhiza* rhizomes for the presence or absence of phenolic compounds produced strong positive results in all 10 samples (Table 2), indicating significant content of tannin/phenolic compounds. But this was somewhat in contrast to the variation in color observed directly in the rhizome samples themselves (Figure 1): namely, yellow for the JB, JG, and JGB rhizomes, brown for the JT, JBB, and JPB rhizomes and dark brown for the JM, JS, JBE, and JGG rhizomes. Plants with lots of phenolic compounds can inhibit lipid peroxidation by stabilizing free radicals. Phenolic compounds, in acting as antioxidants, suppress the formation of reactive oxygen species (ROS) (Sabir et al. 2021). Phenolic compounds in *C. zanthorrhiza* rhizomes can be assumed to have pharmacological activity based on research on other plants from the family *Zingiberaceae*, such as *Curcuma longa* L. which contains phenolic compounds with pharmacological activity - anti-inflammatory or pro-inflammatory cytokines that can be used to treat the severity of arthritis. (Oliviero et al. 2019). Additionally, The phenolic compounds of *Curcuma longa* L. have medical uses as antioxidants. (Jyotirmayee and Mahalik 2022).

The qualitative test for triterpenoid compounds in the *C. zanthorrhiza* rhizomes produced the same results (Table 2) as for the identification of saponin compounds; namely, the test results for all 10 rhizome samples from the Jambi area were negative, indicating an absence of detectable triterpenoid compounds in the *C. zanthorrhiza* rhizomes. The color of the test reaction with the extract precipitate was brownish-yellow. Triterpenoid compounds belong to the terpenoid group, which can inhibit the activation of chemical carcinogens, inactivate genotoxic activation and inhibit tumor development by inhibiting the tumor transduction pathway (Aqeela et al. 2013). Triterpenoid compounds in other species of the genus *Curcuma* play a role as antibacterial agents, significantly inhibiting *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* bacteria (Kasta 2020). One particular compound, a pentacyclic triterpene compound with five cyclic rings in its molecular structure, has been reported as having bioactive properties related to pharmacological effects, namely acting as an antitumor agent and having immunomodulatory, anti-inflammatory, antiviral, and antimicrobial effects (Agatonovic-Kustrin et al. 2022).

The final qualitative test carried out on the rhizome samples was to detect steroid compounds based on the color of the reaction precipitate formed with Liebermann-Burchard reagent. All 10 rhizomes from the Jambi region produced a strong positive response to the presence of steroids (Table 2). The observed color of the reaction precipitates with the Liebermann-Burchard reagent was orange, light brown to dark brown, in agreement with the results of Muharrami et al. (2020). Plants containing steroid compounds have pharmacological benefits as anti-inflammatory and anticancer agents. Steroid compounds in plants of *C. zanthorrhiza* can act as antibiotic agents to treat dermatological diseases (Ogidi et al. 2021). The results of a qualitative biochemical analysis conducted by Bargah (2015) reveal that a reddish-orange color indicates the presence of steroid compounds. Ullah et al. (2014) stated that steroids and saponins have similar pharmacological activity as anti-inflammatory agents.

The 10 accessions of *C. zanthorrhiza* from Jambi were divided into three clusters (Figure 2) with different characteristics between the clusters. Cluster-1 consisted of two accessions: JM and JBB. The characteristics of cluster-1 were the strong intensity of the alkaloids and the overall yellow and dark brown color of the rhizomes. Cluster-2 consisted of three accessions: JT, JPB, and JBS, with a medium-high alkaloid intensity and bright orange rhizome color. Cluster 3 comprised five accessions: JSS, JJ, JB, JBE, and JG. Cluster-3 was characterized by the medium-strong intensity of alkaloids and a yellow and dense orange rhizome color.

The grouping of accessions in Figure 2 shows that geographical location affects the content of secondary metabolites obtained. Variations in the origin of a plant can cause differences in the secondary metabolites of the phenotype (Mishra et al. 2021). Pharmacological properties are influenced by the content of secondary metabolites contained in a plant (Karchesy et al. 2018). The findings of this study are in line with Klau et al. (2023), who state that the pharmacological attributes of the secondary metabolites of *C. zanthorrhiza*, are influenced by many variables, one of which is the location of growth or the geographical location of the plant. The genetic diversity of plants in the same geographical area occurs because of continuous adaptation, causing physiological and biochemical changes. The genetic diversity of a plant also causes the content of secondary metabolites to be different.

To sum up, analysis of the similarity of the rhizomes from the 10 *C. zanthorrhiza* accessions, based on rhizome color and content of phytochemical compounds, identified three clusters: the first cluster consisting of two accessions, namely JM and JBB; the second consisting of three accessions namely JT, JPB, and JBS, and the third cluster consisting of five accessions, namely JSS, JJ, JB, JBE, and JG. Identification of phytochemical compounds from the ethanol extract of the *C. zanthorrhiza* rhizomes identified four positive compounds in the rhizomes. The four compounds contained in *C. zanthorrhiza* rhizomes possess different pharmacological effects. Based on this research, the intensity of alkaloids, flavonoids, and tannins/phenolic compounds was abundant, the steroid compounds were less abundant, and saponins and triterpenoids were not identified in the rhizomes of the 10 accessions of *C. zanthorrhiza* obtained from the Jambi region.

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