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Study of *Curcuma* diversity from Central Java, Indonesia for sunscreen and antioxidant activity based on quantitative phytochemical analysis

HENNY NURHASNAWATI[1,♥], REKSI SUNDU[2], ANITA SUKMAWATI[3]

[1]Program of Diploma III Pharmacy, Sekolah Tinggi Ilmu Kesehatan Samarinda. Jl. Abdul Wahab Syahrani No. 226, Samarinda 75242, East Kalimantan, Indonesia. Tel.: +62-811-5576-817, ♥email: hennynurhasnawati@gmail.com

[2]Program of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Samarinda. Jl. Abdul Wahab Syahrani No. 226, Samarinda 75242, East Kalimantan, Indonesia

[3]Faculty of Pharmacy, Universitas Muhammadiyah Surakarta. Jl. Ahmad Yani, Sukoharjo 57169, Central Java, Indonesia

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Abstract. *Nurhasnawati H, Sundu R, Sukmawati A. 2023. Study of* Curcuma *diversity from Central Java, Indonesia for sunscreen and antioxidant activity based on quantitative phytochemical analysis. Biodiversitas 24: 6880-6887.* Indonesia is home to various plants, including those of the genus *Curcuma*. This study aimed to determine the effect of total phenolic and flavonoid content of *Curcuma* on antioxidant activity and SPF values. It is known that this rhizome contains secondary metabolites such as flavonoids and phenols, which are known to have antioxidant potential and are believed to act as natural ingredients for sun protection products. The samples used in this study were rhizomes of five *Curcuma* species from Central Java, namely *C. aeruginosa*, *C. longa*, *C. mango*, *C. xanthorrhiza*, and *C. zedoaria*. Alkaloids, flavonoids and tannins are present in all samples. UV-Vis spectrophotometry was used with the following results: the flavonoid total content ranged from 0.94-243.5 mg QE/g, while the total phenolic from 11.27-109.23 mg GAE/g. The DPPH and ABTS test for antioxidant activity showed that *C. longa* had the highest activity with an IC_{50} of 78.79 and 0.4273 ppm. The highest SPF values in *C. longa* were 31.55-36.97 (high protection). The results of the data analysis using the Spearman correlation test revealed a significant correlation between the antioxidant activity and SPF with the flavonoid and phenolic content of the *Curcuma* extracts.

Keywords: Antioxidant, *Curcuma,* flavonoid, SPF, total phenol

Abbreviations: QE: Quercetin Equivalent, GAE: Gallic Acid Equivalent, TFC: Total Flavonoid Content, TPC: Total Phenol Content

INTRODUCTION

Curcuma is a genus of rhizome plants consisting of more than 100 species, mostly native to Southeast Asia. *Curcuma* has a high economic and cultural value, because it is used as a spice, medicine, dye, and cosmetic ingredient. Some *Curcuma* species have high antioxidant and sun protection activity, which can be beneficial for skin health and cancer prevention (Subositi and Wahyono 2019). However, the biodiversity and bioactive potential of *Curcuma* species from Central Java, one of the centers of biodiversity in Indonesia have not been widely studied.

Being a tropical nation, Indonesia suffers a lot of sun exposure. Depending on a number of variables, including the length and frequency of exposure, the intensity of the sun, and personal sensitivity, the effects of sunlight on the human body can be either positive or negative (Maglio et al. 2016). The epidermis, the skin's outer layer, can become inadequately prepared to resist the negative effects of prolonged and excessive sun exposure, which can result in a range of skin issues, from moderate dermatitis to skin cancer (Merin et al. 2022). Sunlight, mostly UV-A and UV-B rays, penetrates the earth's surface and affects the skin (Guan et al. 2021). Hence, sunscreen is one way to reduce the negative effects of sun exposure.

The Sun Protection Factor (SPF) of sunscreen measures its effectiveness in protecting the skin from UV radiation. Sunscreens provide chemical protection by absorbing UV radiation and physical protection by diffusing and reflecting UV light. In general, sunscreen protects skin cells from UV radiation damage. Sunscreens with higher SPF ratings offer more protection (Guan et al. 2021). Ingredients in sunscreen might be man-made or derived from the environment. Natural sunscreens have the advantage of using natural base components, in addition to being less expensive. Consequently, it is crucial to carry out research to find natural substances that might act as powerful sunscreen agents (Sharma and Sharma 2023).

Quantitative analysis of phytochemicals is performed using UV-Vis spectrophotometry methods. Flavonoid levels are determined using an aluminum chloride reagent ($AlCl_3$) that can form yellow complexes with flavonoids. Total phenol levels are determined using Folin-ciocalteu reagents that can react with phenolic compounds to form blue complexes (Musdalipah et al. 2021). Determination of total flavonoid and phenol levels in plant extracts aims to determine the content of phytochemical compounds that have bioactivity, including sunscreen and antioxidant activity (Lefahal et al. 2018). In addition, the determination of total flavonoid and phenol levels can also be used as quality parameters and standardization of herbal medicinal raw materials (Alok et al. 2014).

Alkaloids, saponins, flavonoids, polyphenols, and essential oils are among the substances known to be present in the rhizomes of the *Genus Curcuma* (Nurjannah et al. 2023). One class of phenolic chemical called flavonoids is thought

to have antioxidant qualities as well as other advantages. These flavonoids are thought to be the sunscreen's active components because of their potent antioxidant properties. They can also chelate metal ions, which may reduce skin damage or at least help shield the epidermis from UV radiation's damaging effects (Nunes et al. 2018).

Results from earlier research demonstrated a significant correlation between the antioxidant activity of extracts from the rhizomes of many Indonesian medicinal plants and their flavonoid and phenol levels (Manuhara et al. 2022). According to previous research on Iranian medicinal herbs, there is no relationship between SPF and flavonoid content (r = 0.30) or antioxidant activity, however there is a strong association between SPF content and total phenol (r = 0.55) (Ebrahimzadeh et al. 2014). So far, there has never been any evidence linking the amount of flavonoids and total phenol in the genus *Curcuma* to SPF ratings. It is necessary to conduct a more thorough investigation into the connections between flavonoid and phenol variables, antioxidant activity, and SPF value in order to understand the properties of plant extracts as bioactive constituents to be turned into sunscreens. Therefore, this study aims to explore the biodiversity of five Curcuma species from Central Java, namely *C. aeruginosa, C. longa, C. mango, C. xanthorrhiza*, and *C. zedoaria* through studies of antioxidant activity and SPF values associated with total flavonoid and phenol content, and looking at the correlation between these parameters.

MATERIALS AND METHODS

Material gathering and simplisia preparation

Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) Tawangmangu, Karanganyar, Central Java, through assisted farmers provided the sample, which was composed of 5 (five) different types of rhizomes from *Genus Curcuma*. While plant determination is carried out in B2P2TOOT. Beginning with the collection of raw materials, wet sorting, washing, slicing, sun-drying while covered in black fabric, dry sorting, and packaging, simplisia is made. The size of each simplisia is then mashed to create simplisia powder.

Chemical reagents

The chemicals used are Merck products namely acetic anhydride, aluminum chloride, amyl alcohol, aquades, 70% ethanol, Folin-Ciocalteau, gallic acid, hydrochloric acid, iron(III) chloride, magnesium powder, n-hexane, potassium acetate, quercetin, Bouchard reagent, Dragendorf reagent, Mayer reagent, sodium hydroxide, sodium nitrite, sulfuric acid.

Extraction process

One thousand g of simplisia were used to extract each rhizome from the *Genus Curcuma* using the maceration method with 70% ethanol as the solvent in a ratio of 1:10. The same amount of solvent is used during re-maceration in order to produce a liquid extract. To create a thick extract, it is then concentrated using a rotary evaporator and a water bath at 60°C. Yield is calculated using the formula:

$$\%\ \text{yield} = \frac{\text{weight of viscous extract (g)}}{\text{weight of simplisia (g)}} \times 100\%$$

Moisture content in simplisia and extract measurement

A gravimetric analysis was done to determine the moisture content of the simplisia and extract sample. Two g of the sample were heated in an oven at 105°C for 1 hour to determine its moisture content. After around 10 minutes in the desiccator, the sample was weighed to determine its moisture content. Until a steady sample weight is attained, the test is repeated. The following formula is used to calculate the extract's moisture content, which is expressed as:

$$\text{Moisture content} = \frac{a - (c - b)}{a \times 100\%}$$

Where:
a : Weight of the extract (g)
b : Weight of the cup when empty
c : Weight of the cup plus the sample after heating (g)

Qualitative examination of phytochemicals

Following the testing technique for alkaloids, flavonoids, tannins, saponins, and steroids/terpenoids, the analysis was carried out qualitatively using phytochemical screening for the identification of secondary metabolite chemicals (Rajkumar et al. 2015).

Alkaloids

A sample of 0.5 g were mixed with 1 mL of 2 N hydrochloric acid and 9 mL of distilled water using this method. The mixture is heated for two minutes in a water bath, then chilled and filtered. Three drops of filtrate were combined with two drops of each of the following reagents: Mayer, Bouchardat, and Dragendorf to investigate the filtrate further. The filtrate produces a red brick, dark brown, and white/yellow precipitate, respectively. The extract is deemed alkaloid-positive if the results of two of these three tests are positive.

Flavonoids

In order to determine the presence of flavonoids, 0.5 g of samples were extracted with 10 mL of distilled water, filtered, and 5 mL of the filtrate was mixed with 0.1 g of magnesium powder, 1 mL of strong hydrochloric acid, and 2 mL of amyl alcohol. After mixing, the mixture was allowed to settle. Flavonoids are in the extract if the amyl alcohol layer is red, yellow, or orange.

Tannins

To determine the presence of tannins, 10 mL of aquades were used to extract 0.5 g of sample. The resulting extract is filtered, and the filtrate is colorless after being diluted in aquades. This solution is diluted to two milliliters, and one or two drops of 1% $FeCl_3$ were then added. When tannins are present, a color change to blue or a blackish-green hue occurs.

Saponins

For the saponin test, 10 mL of aquades and 0.5 g of sample were combined in a test tube. After cooling, the mixture was shaken violently for 10 seconds. Saponins were found to be present when foam with a height of at least 1 to 10 cm was formed and did not vanish when 2 N HCl was added.

Steroids/terpenoids

A sample of 0.5 g macerated in 20 mL of n-hexane for two hours in order to identify steroids or terpenoids, and then it is filtered. After the filtrate has evaporated in a water bath, two drops of anhydrous acetic acid and one drop of sulfuric acid were added. When steroids are present, terpenoids cause purple or red to develop, which later turns blue-green.

Analysis of Total Flavonoid Content (TFC)

Quercetin was used as a comparison to help determine the extract's flavonoid concentration using the UV-Vis spectrophotometry method, referring to previous research by Nurhasnawati et al. (2019) which is known as the aluminum chloride method.

Finding the maximum wavelength

A total of 1 mL of a standard solution of quercetin with a concentration of 6 ppm was inserted into a test tube. Then, in that sequence, 1.5 mL of 70% ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of potassium acetate 1 M, and 2.1 mL of aquades were added. The mixture was beaten until it is homogeneous. The absorbance was then measured using a UV-Vis spectrophotometer with a wavelength range of 350-550 nm, obtained λ max = 437 nm.

Making a calibration curve

A solution of 1,000 ppm quercetin (25 mg of quercetin in 25 mL of ethanol) was diluted into a series of solutions with concentrations of 12.5, 25, 50, 100, and 200 ppm with a volume of 10 mL each to create the calibration curve. One milliliter of each solution in the quercetin series was taken, and similar with the previous step, a reagent was added. A linear regression equation (y = bx + a) was then constructed by measuring the absorbance of each solution at a wavelength of 437 nm while using blanks (solutions containing reagents and solvents but without samples).

Determination of total flavonoid

A sample solution with a 1000 mg/L concentration was created then pipetted 0.75 mL inserted test tube and add reagents like the previous work. The absorbance value of the sample was used as the y-value in the linear equation to determine the concentration of flavonoids, allowing the x-value to be determined. The total flavonoid levels were calculated using the formula:

$$TFC = \frac{C \times V}{m}$$

Where:

FC : Flavonoid content (mgQE/g extract)

C : Sample concentration established by the calibration curve (mg/L)

V : Sample solution volume (L)

m : Weight of the ethanolic plant extract (g)

Milligrams of quercetin equivalent per gram of extract (mg QE/g) is the flavonoid content.

Analysis of Total Phenol Content (TPC)

Gallic acid was used as a comparison to help determine the extract's phenol concentration using the UV-Vis spectrophotometry method following earlier research with a few adjustments, the determination of total phenolic levels was carried out using Folin-Ciocalteau reagent (Nurhasnawati et al. 2019).

Finding the maximum wavelength

Pipettes of 0.75 mL of 50 ppm gallic acid solution were mixed with 3.75 mL of diluted Folin-Ciocalteau reagent (1:10 v/v). After this mixture has stood for five minutes, 3 mL of 7.5% Na_2CO_3 was added. The solution was then incubated in the dark for 30 minutes before being measured using a UV-Vis spectrophotometer between 600 and 800 nm, obtained λ max = 750 nm.

Making a calibration curve

To create serial solutions with concentrations of 12.5, 25, 50, 100, and 200 ppm, each with a volume of 10 mL, gallic acid solutions with a concentration of 1,000 ppm (25 mg gallic acid in 25 mL aquades) was employed. Additionally, the previous method was followed while adding chemicals. Using blanks, the absorbance of each series solution was calculated at a wavelength of 750 nm. Based on these measurements, a curve of concentration and absorbance relationship is obtained so that a linear regression equation y = bx + a is produced.

Determination of total phenol

A sample solution with a 1000 mg/L concentration was created then pipetted 0.75 mL inserted test tube and add reagents like the previous work. Next, absorbance was measured at a wavelength of 750 nm. The work is repeated three times. The sample's absorbance value was utilized as the y-value in the linear equation to calculate the concentration of total phenol (the x-value). The calculation uses the following formula:

$$TPC = \frac{C \times V}{m}$$

Where:

TPC : Total Phenol Content (mg GAE/g extract)

C : Sample concentration determined by the calibration curve (mg/L)

V : Sample solution volume (L)

m : Weight of the ethanolic plant extract (g)

The total phenol content is measured in mg of gallic acid equivalent per gram of extract (mg GAE/g).

Table 1. Constant Value EE x I

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Measuring antioxidant activity with the DPPH method

The method used in determining antioxidant activity is DPPH (1,1-diphenyl-2-picrylhydrazyl) by referring to previous research by Nurhasnawati et al. (2019). In order to determine the maximum wavelength value, absorbance in the 400-600 nm range of the DPPH 40 ppm solution was measured. Additionally, each sample series solution containing up to 1 mL of material at concentrations between 12.5 and 200 ppm, together with 2 mL of DPPH solution at a concentration of 40 ppm, were incubated for 30 minutes before the absorbance at a preset maximum wavelength was recorded. The calculation of percent inhibition uses the following formula:

$$\% \text{ Inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

Furthermore, by entering the concentration value as the x-axis and percent inhibition as the y-axis, the linear regression equation y = bx + a is obtained. The calculation of IC_{50} is done by substituting the value of y to 50, then the value of x can be known. The value of x is IC_{50}.

Measuring antioxidant activity with the ABTS method

The first step, ABTS solution (2.2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) 7.4 mM (0.1014 g/50 mL aquades) and $K_2S_2O_8$ solution 2.6 mM (0.0175 g/50 mL aquades) are made, both solutions are mixed and allowed to stand for 12-16 hours in a dark place, obtained ABTS stock solution. Pipetted 1 mL of ABTS stock solution and diluted with 70% ethanol in a 10 mL measuring flask. Then the maximum wavelength of the ABTS solution is determined by measuring the absorbance in the wavelength range of 600-800 nm. Next, a sample parent solution of 5,000 ppm and a series solution with a concentration of 0.1-0.5 ppm were made, measured at a wavelength of 744 nm. The calculation of IC_{50} is the same as the DPPH method.

Determining the sun protection factor value

The SPF value is calculated in vitro based on absorption using UV-Vis spectroscopy. The technique is based on research by Khan (2018). Absorbance was determined for each concentration of the extract solution against 70% ethanol at wavelengths ranging from 290 to 320 nm with wavelength intervals of 5 nm. The three distinct concentration variations used in the investigation were 500, 1000, and 1500 ppm for each sample solution. Three replicates of the test were run. The formula used to calculate the SPF, known as Mansur's equation, namely:

$$\text{SPF} = \text{CF} \times \sum_{\lambda=290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda)$$

Where:

CF : Correction Factor with value (10)

EE x I : Erythemal Effect times UV Intensity (in the Table 1)

Abs : Extract Absorbance Value

Data analysis

Both qualitative and quantitative data are included in the data collected. A narrative approach was used to provide descriptive descriptions of qualitative data, such as phytochemical screening. With the help of Spearman non-parametric correlation, quantitative data such as flavonoid concentrations, total phenols, antioxidant activity, and SPF values were examined. These were expressed as mean SD (n = 3). The ultimate correlation value ranges from -1 to +1. A correlation number close to 1 indicates a strong relationship between the two variables, whereas a correlation value close to 0 indicates a poor relationship. For correlation analysis, Principal Component Analysis was frequently utilized.

RESULTS AND DISCUSSION

Research has been conducted on the study of sunscreen and antioxidant activity based on quantitative phytochemical analysis against five types of rhizomes of the genus *Curcuma*. In order to identify the plants for the study, five distinct types of rhizomes were collected and identified by plant determination. The normal procedures for converting samples into simplisia include wet sorting, washing, knitting, natural sunshine drying under a black cloth cover, pollination, and packaging. To acquire extracts that match the requirements and are of a high caliber, the stage of creating simplisia must be carried out properly. As instructed in the Indonesian Herbal Pharmacopoeia, the simplisia powder was then extracted using the maceration process with a solvent ratio of 1:10 of 70% ethanol. Table 2 shows general parameter information about the yield and moisture content of simplisia and extract.

Determining sample yield is crucial since it has an impact on the choice of the best extraction technique for subsequent study. Additionally, the amount of active chemicals in the sample is correlated with the yield, therefore as yield increases, more active compounds are present in the sample (Hasnaeni et al. 2019). These numbers show that the average yield for all extracts has a good value, which is greater than 10%.

Qualitative examination of phytochemicals

Alkaloids, flavonoids, tannins, saponins, and steroids/ terpenoids were among the secondary metabolites screened as part of a qualitative phytochemical examination. Table 3 shows the test results.

According to an earlier study the genus *Curcuma* contains active chemicals from the alkaloids, flavonoids, and terpenoids groups (Rahaman et al. 2021). Thus, these results are in line with those findings.

Analysis of antioxidant activity, total phenol, flavonoid content, and SPF value

UV-Vis spectrophotometry was used to perform in vitro testing on antioxidant activity using the DPPH and ABTS techniques. Utilizing UV-Vis spectrophotometry techniques, total flavonoid and phenolic levels were calculated for quantitative phytochemical investigation. The flavonoid content is expressed in quercetin equivalence as a comparison, while the total phenolic content is expressed in gallic acid equivalence as a comparison (Nurhasnawati et al. 2019). Meanwhile, sunscreen activity was tested in vitro using UV-Vis spectrophotometry method, by measuring the absorbance of the sample solution in the wavelength range of 290-320 nm at intervals of 5 nm (Guan et al. 2021). Variable values are produced by the test, as indicated in Table 4.

Discussion

The outcomes of the test for antioxidant activity indicated that *Curcuma longa* extract in comparison to other samples showed the lowest IC_{50} value and the strongest antioxidant effects. After data for each variable were collected, a correlation between the flavonoid and phenolic content and SPF values of the five rhizome species of the genus *Curcuma* can be found. According to Panche et al. (2016), antioxidant substances like flavonoids and phenols can help shield body cells from harm caused by free radicals. UV-Vis spectrophotometry was selected for flavonoid analysis since it is a widely used technique for both quantitative and qualitative flavonoid analysis. A conjugated aromatic system found in flavonoids exhibits high absorption bands in the visible and ultraviolet spectrums. Since quercetin is one of the most prevalent flavonoid group chemicals, it was chosen as a comparison. According to Nurcholis et al. (2016), quercetin is a member of the flavonol group and, like flavones and flavonols, has a nearby hydroxyl group on the C-3 or C-5 atom and a keto group on the C-4.

Utilizing UV-Vis spectrophotometry equipment, a colorimetric approach was used to determine the total flavonoid concentration. Reagents used in this procedure include $AlCl_3$ and CH_3COOK. Complexes involving paired hydroxyl groups, ketones, or near hydroxyl groups are created when $AlCl_3$ reacts with chemicals that include flavonoid groups. Additionally, 7-hydroxyl groups can be found using CH_3COOK (Pękal and Pyrzynska 2014).

The total phenol content in vitro was calculated using the UV-Vis spectrophotometry technique. The Folin reagents, which can produce solutions with phenolic compounds and enable absorbance measurement, were used in this method, which was chosen for its simplicity in comparison to other devices. The Folin-Ciocalteau method's fundamental idea revolves around the formation of intricate blue compounds that may be detected at a wavelength of 750 nm. Only in an alkaline environment do phenolic compounds interact with Folin-Ciocalteau reagents, resulting in the breakdown of phenolic compounds' protons into phenolic ions, 7.5% Na_2CO_3 was utilized to create an alkaline environment. When the phenolic compound's hydroxyl group reacts with the Folin-Ciocalteau reagent, a blue tungsten-molybdenum complex was created that may be seen using a spectrophotometer. The amount of phenolic ions available to reduce hetero-polyacid (phosphomolybdate-phosphotungstic) to generate molybdenum-tungsten complexes is directly impacted by the concentration of phenolic compounds (Blainski et al. 2013).

Table 2. Percentage yield, moisture content of simplisia and plant extracts of genus *Curcuma*

Scientific name	Local name	Yield (%)	Water content (%)	
			Simplisia	Extract
Curcuma aeruginosa Roxb.	*Temu ireng*	12.40	8.00	12.15
Curcuma longa L.	*Kunyit*	13.52	5.28	13.50
Curcuma mangga Valeton & Zijp	*Temu mangga*	24.20	6.60	14.95
Curcuma xanthorrhiza Roxb.	*Temulawak*	12.43	8.00	12.40
Curcuma zedoaria (Christm.) Roscoe	*Temu putih*	12.02	5.90	16.63

Table 3. Phytochemical screening results of plants of genus *Curcuma*

Scientific name	Alkaloid	Flavonoid	Tannin	Saponin	Terpenoid
Curcuma aeruginosa	+	+	+	-	-
Curcuma longa	+	+	+	+	+
Curcuma mangga	+	+	+	+	-
Curcuma xanthorrhiza	+	+	+	+	+
Curcuma zedoaria	+	+	+	+	-

Table 4. Results of flavonoids, total phenols, SPF and IC_{50} of genus *Curcuma*

Scientific name	Flavonoid content (mg QE/g)	Total phenolic content (mg GAE/g)	SPF			IC_{50} (ppm)	
			500 ppm	1000 ppm	1500 ppm	DPPH	ABTS
Curcuma aeruginosa	5.37	20.19	11.64	23.25	34.52	143.28	0.4835
Curcuma longa	243.50	93.44	31.55	37.46	36.97	78.79	0.4273
Curcuma mangga	0.94	11.27	1.82	3.68	5.55	146.21	0.4851
Curcuma xanthorrhiza	119.08	69.40	13.76	28.06	36.30	98.31	0.4396
Curcuma zedoaria	7.31	109.23	14.93	29.16	37.38	109.67	0.4626
Ascorbic acid (positive control)						7.26	0.3300

When compared to the findings of Khumaida et al. (2019) found that variability in the total phenolic and total flavonoid contents of *C. aeruginosa* ranged from 29.08-46.92 mg GAE/g, and 21.31-33.81 mg QE/g. According to Suryani et al. (2022) indicated that the phenolic content and antioxidant in *C. xanthorrhiza* and *C. aeruginosa* plants is influenced by differences in growing location that are influenced by altitude, rainfall, and temperature conditions of cultivated area locations. As compared to the findings of Alafiatayo et al. (2014) on the determination for phenolic compounds of *C. longa* and *C. xanthorrhiza*, lower yields were obtained namely 39.38 mg/GAE/g DW and 38.01 mg/GAE/g DW. Due to variations in instrument sensitivity and lab testing settings, different outcomes are suspected.

Studies have demonstrated a correlation between the total phenol and flavonoid levels and the curcuminoid content of *Curcuma* rhizomes. The primary active ingredients found in *Curcuma* rhizomes are curcuminoids, which include curcumin, dimethoxy curcumin, and bis-dimethoxy curcumin. According to numerous research, *Curcuma* rhizomes with high concentrations of total phenols and total flavonoids are also likely to have higher curcuminoid contents. For instance, earlier research by Alafiatayo et al. (2014) on rhizomes from ten different species of Zingiberaceae revealed total phenolics and polyphenols with comparable activity. The highest total phenol levels were found in *C. longa* and *C. xanthorrhiza* at 42.71 mg/GAE/g DW and 22.03 mg/GAE/g DW, respectively, while total polyphenol levels were 39.38 mg/GAE/g DW and 38.01 mg/GAE/g DW. According to the findings of earlier research by Asyhar et al. (2023) antioxidant activity was substantially connected with polyphenol content of *C. xanthorrhiza.*

Curcuminoid content is higher in rhizomes with higher total phenol and total flavonoid content. The curcuminoid concentration of *Curcuma* rhizomes can also be affected by genetic variables, plant growing conditions, and the extraction and processing techniques utilized in the study, it should be mentioned. As a result, the findings of this study give a broad notion of the association between total phenol, total flavonoid, and curcuminoid concentration, but more research is required to confirm these results and clarify the underlying mechanisms.

Sunscreen activity testing based on SPF values in vitro, as previously reported, was performed on ethanol extract of *C. aeruginosa* rhizomes as secondary metabolite substances that are believed to function as natural sunscreen agents including phytochemical compounds, particularly phenolics and flavonoids. Due to their iron-binding characteristics, which have the potential to damage lipids and proteins in cell membranes, flavonoids exhibit photoprotective properties. Inhibiting xanthine oxidase, which is known to be a generator of Reactive Oxygen Species (ROS) that contribute to oxidative stress, is one of the signaling pathways they regulate. They also control other signaling pathways. Naringenin and apigenin are common edible plant-derived flavonoids with the potential to reduce inflammation and increase skin antioxidants (Li et al. 2023). Many phenolic compounds have been identified as promising antioxidants for treating various skin disorders, including those caused by UV radiation (Tungmunnithum et al. 2018).

Sunscreen activity testing based on SPF values in vitro, as previously reported by Budiati et al. (2021), shows the SPF value of *C. xanthorrhiza* extract at a concentration of 0.012% is 14.14, including the maximum protection category. Research related to the SPF value of rhizome extract of the genus *Curcuma* has not been widely reported so far. Various SPF values are obtained, possibly affected by differences in growing locations and various test conditions.

Antioxidant activity is the ability of a compound to capture or neutralize free radicals that can cause cell and tissue damage. Antioxidant activity test methods that are often used are DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). Both methods are based on decreasing the absorbance of free radical solutions after adding them with antioxidant compounds. The working principle of the DPPH method is to measure the ability of antioxidant compounds to donate hydrogen atoms or electrons to the purple DPPH radical. The working principle of the ABTS method is to measure the ability of antioxidant compounds to reduce blue ABTS cation radicals. The value of antioxidant activity can be expressed in terms of percentage decrease in absorbance or IC_{50} value (concentration of antioxidant compounds needed to lower absorbance by 50%). The results of antioxidant activity testing in this study have similarities with previous study by Budiati et al. (2021), namely in *C. xanthorrhiza* extract showed an IC_{50} value of 78.30 µg/mL, showing strong antioxidants.

According to the data presented, the Spearman correlation test was used to conduct statistical analysis on all variables. Because not every data has a normal distribution, this method was chosen. Table 5 displays the findings of the full correlation coefficient value study.

Table 5. The value of the Spearman correlation coefficient between variables

Variable	Correlation coefficient (r)					
	DPPH ($1/IC_{50}$)	ABTS ($1/IC_{50}$)	Flavonoid	Phenol	SPF 500 ppm	SPF 1000 ppm
ABTS	1.000					
Flavonoid	0.900	0.900				
Fenol	0.600	0.600	0.700			
SPF 500 ppm	0.900	0.900	1.000	0.700		
SPF 1000 ppm	0.900	0.900	1.000	0.700	1.000	
SPF 1500 ppm	1.000	1.000	0.900	0.600	0.900	0.900

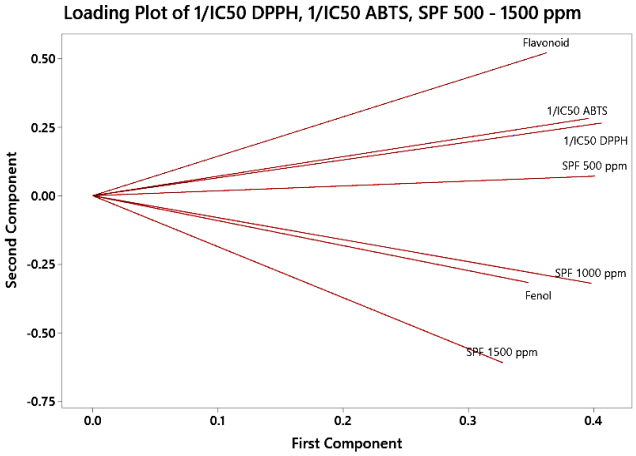

Figure 1. Loading plot curve between variables

Researchers can analyze the correlation between variables more flexibly thanks to the Spearman correlation coefficient. The value of the correlation coefficient is interpreted as perfect (r = ±1), a very strong (0.8-0.9), moderate (0.6-0.7), fair (0.3-0.5), poor (0.1-0.2) and none if r = 0 (Akoglu 2018). The results of the Spearman correlation test on the data in Table 5 indicate that there is a significant link between the antioxidant activity measured by the DPPH and ABTS techniques and the flavonoid and phenol content of total extracts of the genus *Curcuma*. Additionally, there is a significant correlation between SPF values and the level of flavonoids and total phenol. These results are consistent with previous research by Asyhar et al. (2023), positive connection ($p<0.001$, r value 0.90) was found between TPC and TFC. TPC was positively and strongly linked with DPPH antioxidant capacity and negatively correlated with ABTS antioxidant capacity with correlation coefficients of 0.62 and -0.96, respectively. Meanwhile, TFC positively and significantly correlates with DPPH antioxidant capability. In contrast, it negatively correlates with ABTS antioxidant capacity (0.60 and -0.91).

We further explored the sunscreen potential of the extracts based on their SPF values. The observed positive correlation between the phenolic and flavonoid contents and the SPF values suggests that these compounds may contribute to the sun-protective effects of *Curcuma* extracts. Flavonoids, due to their iron-binding characteristics, are known to exhibit photoprotective properties. Phenolic compounds, on the other hand, have been identified as promising antioxidants for treating skin disorders caused by UV radiation. However, further research is needed to elucidate the specific mechanisms by which these compounds confer sunscreen activity.

The variability in the total phenolic and flavonoid contents among the different *Curcuma* species highlights their potential as natural sources of antioxidants. The higher levels of phenolic and flavonoid compounds in certain species, such as *C. longa*, may explain their superior antioxidant activity compared to other species. Furthermore, the positive correlation between the phenolic and flavonoid contents and the SPF values suggests a potential relationship between these compounds and the sun-protective effects of *Curcuma* extracts. These findings support the hypothesis that the antioxidant properties of *Curcuma* extracts contribute to their potential as sunscreens.

According to Widodo et al. (2019), using the loading plot in Principal Component Analysis is an additional method of observing the relationship between the variables. The loading plot visualizes each variable as a vector to show how strongly it influences the other variables. If the vectors form an angle of less than 90°, the two variables are positively associated. The two variables are not likely to be connected if the angle is closer to 90°. The two variables display a negative connection when the angle is wider (greater than 90°) or close to 180°. Angles smaller than 90° are present in every data point in Figure 1, demonstrating a strong-to-very strong link between the variables. Therefore, it can be said that the total flavonoid and phenol content of rhizome extracts of the genus *Curcuma* greatly influences their antioxidant activity and sunscreen activity.

While the results provide valuable insights into the antioxidant and sunscreen potential of *Curcuma* species, it is important to note the limitations of this study. The investigation focused on a limited number of *Curcuma* species, and the specific mechanisms underlying the sunscreen activity were not elucidated in detail. Further research is necessary to validate these findings and explore the efficacy of *Curcuma* extracts as sunscreen agents in vivo.

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