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Original Article

ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT FROM SURIAN LEAVES (*TOONA SINENSIS*)

UCE LESTARI[1,2], MUHAIMIN MUHAIMIN[3,4*], ANIS YOHANA CHAERUNISAA[4], WAWAN SUJARWO[5]

[1]Doctoral Study Program, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang-45363, West Java, Indonesia. [2]Department of Pharmacy, Faculty of Medicine and Health Sciences, University of Jambi, Jambi, Indonesia. [3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Padjadjaran, Jawa Barat. [4]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang-45363, West Java, Indonesia. [5]Ethnobotany Research Group, Research Center for Ecology and Ethnobiology, National Research and Innovation Agency (BRIN), Jakarta
[*]Corresponding author: Muhaimin Muhaimin; [*]Email: muhaimin@unpad.ac.id

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ABSTRACT

Objective: The objective of the present study is to determine antioxidant activities and phytochemical screening of ethanol extract from surian leaves (*Toona sinensis*).

Methods: This study evaluated the antioxidant activity and conducted phytochemical screening of ethanol extracts obtained from surian leaves. Phytochemical screening was performed using qualitative Thin-Layer Chromatography (TLC), and antioxidant activity was assessed using the DPPH method.

Results: The results revealed the presence of polyphenolic compounds (153.10±0.310 mg/g), tannins, flavonoids (33.19±0.890 mg/g), monoterpenoids, quinones, and saponins with Rf values of 0.607±0.019 (flavonoids) and 0.958±0.019 (terpenoids) 0.513±0.038 (steroids) 0.418±0.019 (phenolics). The antioxidant activity test of the ethanol extract from Surian leaves yielded an IC_{50} value of 12.351±0.092 ppm, which closely matches the IC_{50} value of the reference vitamin C (7.805±0.686 ppm).

Conclusion: In conclusion, based on research methods, the ethanol extract of surian leaves contains flavonoid and phenolic compounds which show strong antioxidant activity.

Keywords: Antioxidants, Surian leaves, IC_{50}

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INTRODUCTION

Skincare is one way to shield the skin from external or environmental factors, including the influence of ultraviolet radiation that leads to aging, skin darkening and burning, the development of freckles, and hyperpigmentation. This is due to oxidative stress within the body triggered by free radicals [1]. Skin damage caused by free radicals occurs in biomolecules, such as lipids, proteins (enzymes), or nucleic acids-a result of *Reactive Oxygen Species* (ROS) acting as catalysts for the aging process, skin darkening and burning, freckles, and hyperpigmentation [1, 2]. ROS results in telomere shortening, autophagy, and depletion of stem cells [3]. Oxidative stress significantly influences one's age and physiological state, potentially leading to degenerative diseases, such as atherosclerosis, insulin resistance, cardiometabolic and neurodegenerative diseases, and premature aging [1, 3]. Consequently, to mitigate oxidative stress levels, it is beneficial to employ antioxidants capable of neutralizing free radicals and thwarting premature aging, skin darkening and burning, freckles, and hyperpigmentation. Essentially, the body can produce antioxidants to inhibit free radicals through cellular oxidation reactions. However, the body predominantly relies on antioxidants that come from external sources [4].

Antioxidants are compounds that can hinder the oxidation rate of other molecules or neutralize free radicals. They can be obtained either naturally or through synthesis [5]. Commonly used synthetic antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tetrabutyl hydroxyquinone (TBHQ). However, prolonged use of these synthetic antioxidants has side effects as they are considered carcinogenic [6]. Therefore, there is a need for natural antioxidants, abundantly available in nature. Natural antioxidants operate systemically and have the potential to prevent aging at both cellular and tissue levels [7]. Natural antioxidants commonly found in plants include phenolic or polyphenolic compounds belonging to the flavonoid group, derivatives of cinnamic acid, coumarin, tocopherols, and polyfunctional organic acids [8].

Surian leaves (*Toona sinensis*) are among the plants frequently found in Indonesia and possess the potential to serve as a source of natural antioxidants. This plant is widely utilized in traditional medicine for various purposes, such as treating skin conditions, deworming, dysentery, expectorants, tonics, blood sugar reduction, and syphilis treatment [9]. Secondary metabolites present in this plant encompass limonoids, phytol, flavonoids, essential oils, triterpenoids, phenols, and catechins [10, 11]. These compounds are capable of countering various pathological conditions in the body that are mediated by oxidative stress, which is related to the aging and inflammation processes [12].

Fig. 1: Surian leaves (*Toona sinensis*) (Self documentation)

Based on the aforementioned background, a study was undertaken to investigate the antioxidant activity of ethanol extract from surian

leaves (*Toona sinensis*) *in vitro* using the DPPH method, as well as to assess the potential of surian leaves in neutralizing free radicals. Consequently, both qualitative and quantitative phytochemical screening of the ethanol extract of surian leaves is deemed necessary [11]. The phytochemical screening process of the ethanol extract of surian leaves provides a comprehensive overview of the active compound content with antioxidant properties. Specific standardization tests for the ethanol extract of surian leaves are essential to establish quality parameters for the extract. It is anticipated that the ethanol extract of surian leaves can be further developed into an anti-aging cosmetic product or for facial care. As for the novelty of this research, no test has been found to determine the antioxidant activity of the ethanol extract of surian leaves (*Toona sinensis*). Therefore, through this research, the antioxidant activity of the ethanol extract of surian leaves was tested using the DPPH method.

MATERIALS AND METHODS

Materials

The materials used consist of surian leaves obtained from Rantau Suli Village, East Jangkat, Merangin Regency, Jambi Province. The chemicals include distilled water (Brataco), 70% ethanol (Brataco), pro-analysis 96% ethanol (Brataco), chloroform (Brataco), methanol (Brataco), HCl (Brataco), H_2SO_4 (Brataco), $FeCl_3$ (Brataco), Mayer's reagent, Dragendorff's reagent, and powdered Mg (Brataco).

Collection and determination of materials

The utilized surian leaves (*Toona sinensis)* were procured from Rantau Suli Village, East Jangkat, Merangin Regency, Jambi Province. Subsequently, these collected surian leaves underwent determination at the Directorate of Scientific Collection Management of the National Research and Innovation Agency in Central Jakarta.

Preparation of simplicia of surian leaves

The srian leaves, once collected, were placed in a storage container. The process began with wet sorting, followed by washing with flowing clean water to remove impurities and then weighing. The wet-sorted leaves were then finely chopped and dried in an oven at a temperature of 50-60 °C. The dried leaves were subjected to dry sorting to separate any remaining impurities. Weighing was done daily until a constant weight was obtained. Once the weight stabilized, the drying process was stopped, and the dried leaves were ground using a grinder and stored in a clean, dry container before being weighed. The yield was calculated using the formula proposed [12].

Extraction of surian leaves

The preparation of surian leaf extract was carried out using the maceration method. The solvent used was 70% ethanol. The process involved soaking one part of the simplicia powder in ten parts of the solvent for 24 h in a dark bottle with occasional stirring. Subsequently, two additional macerations were performed. The resulting macerate had its solvent evaporated using a rotary vacuum evaporator to obtain a concentrated extract, and the yield was calculated using the formula proposed [12].

Specific characteristics testing of simplicia and ethanol extract of surian leaves

Phytochemical screening

Phytochemical screening is conducted to provide an overview of the groups of compounds present in a plant. This examination was performed to determine secondary metabolite compounds in simplicia, and it included the following tests [13].

Phenolic test

1 ml of the extract was added to 10 drops of 1% $FeCl_3$ solution. A positive result indicated the presence of phenols if the solution turned green, red, purple, blue, or dark black [13].

Flavonoid test

1 ml of the extract was mixed with a few drops of 10% NaOH, and color changes were observed. A positive result for flavonoids was indicated by the appearance of red, yellow, green, or brown color. Additionally, the test was performed with concentrated HCl, Mg powder, and 10% NaOH. The sample was first treated with a few drops of concentrated HCl, followed by the addition of Mg powder. A positive result was indicated by the formation of foam and a color change to orange [13].

Alkaloid test

1 ml of the extract was dissolved in a few drops of 2N H_2SO_4 and was then tested with three alkaloid reagents: Mayer's reagent, Dragendorff's reagent, and Wagner's reagent. A positive reaction with Mayer's reagent was characterized by the formation of a yellowish-white precipitate. A positive reaction with Dragendorff's reagent resulted in a red-to-orange precipitate, while a positive reaction with Wagner's reagent produced a brown precipitate [13].

Tannin test

1 ml of the extract was mixed with a few drops of 1% $FeCl_3$ solution, and the mixture was homogenized. A positive reaction was indicated by the formation of a red color [13].

Terpenoid and steroid test

1 ml of the extract was mixed with 2 drops of acetic anhydride and stirred. Then, 1-2 drops of concentrated H_2SO_4 (Lieberman-Bouchardat's reagent) were added, and the resulting color change was observed. The formation of purple or red color indicated the presence of terpenoids, while blue or green color indicated the presence of steroids [13].

Saponin test

The extract was placed in a test tube, followed by the addition of 10 ml of hot water. It was then cooled and vigorously shaken for 10 seconds. A positive result for saponins was indicated by the formation of foam reaching a height of 1-10 cm, lasting for at least 10 min, and disappearing upon the addition of 1 drop of 2N HCl [13].

Chromatogram pattern using thin-layer chromatography (TLC)

Phytochemical screening was conducted qualitatively using TLC. The stationary phase employed silica gel F 254 plates, and the mobile phase consisted of an eluent (chloroform: methanol = 9:1) [14].

Determination of total flavonoid content in ethanol extract of surian leaves

The determination of flavonoid content was carried out using the colorimetric method with $AlCl_3$. The total flavonoid compound content was calculated based on the aluminum chloride method and expressed as quercetin equivalent (mg/g of extract) using the regression equation from the calibration curve. The total flavonoid content was determined using the aluminum chloride colorimetric assay method [15].

Determination of total phenolic content in ethanol extract of surian leaves

The preparation of the gallic acid stock solution was conducted following Lima *et al.* (2023). Approximately 1 mg of gallic acid was weighed and dissolved in 1 ml of MeOH (1000 µg/ml). The gallic acid stock solution was serially diluted to obtain final concentrations of 5, 10, 20, 30, and 40 µg/ml [16].

Determination of moisture content

The moisture content was determined using a moisture balance instrument. The method employed the principles of gravimetric or thermogravimetric moisture measurement or loss on drying (LOD) [17].

Microbial and mold contamination tests

One gram of the extract was dissolved in 10 ml of diluent, which was a NaCl solution, and shaken until homogenized to obtain a 10-1 dilution. Three tubes were prepared, and 9 ml of diluent was added to each tube. A 1 ml aliquot from the 10-1 dilution was transferred into the first tube and shaken until homogenized to obtain a 10-2 dilution. Subsequently, dilutions of 10-3 and 10-4 were prepared [18]. Each dilution was pipetted as 1 ml into separate Petri dishes

containing 15 ml of NA (Nutrient Agar) medium for bacterial contamination and PDA (Potato Dextrose Agar) medium for mold contamination. The process was replicated three times, and blank tests were conducted. According to BPOM RI (2014) requirements, bacterial and fungal contamination should be ≤ 10,000 colonies/g [18].

Total ash content examination

Two grams of each simplicia and extract were weighed and placed into pre-fired porcelain crucibles, which were then tared. They were slowly heated in a furnace, and the temperature was gradually increased to 600 °C [17].

Determination of acid-insoluble ash content

The ash obtained during the determination of total ash content was boiled with 25 ml of dilute HCl for 5 min. The part that remained insoluble in acid was collected and filtered through ashless filter paper, and the acid-insoluble ash content was calculated relative to the weight of the test material, expressed as % w/w [17].

Heavy metal contamination test for Pb, Cu, Cd, and Zn

Quantitative testing for the presence of metals Pb, Cd, Cu, and Zn in the ethanol extract of surian leaves was carried out using the atomic absorption spectrophotometry (AAS) method. The AAS method has high sensitivity with a detection limit of less than 1 mg/l, an easy working process, and requires relatively small sample sizes. The principle of this method is based on the ability of neutral atoms to absorb energy from light, typically visible or ultraviolet light [19].

In the analysis of Pb, Cd, Cu, and Zn using the AAS method, it can work optimally at a concentration of 0.01 mg/l. This requirement is stated in the instrument's specifications. Therefore, to ensure that the metal content of Pb, Cd, Cu, and Zn can be read optimally on the AAS instrument, it can be achieved by adding the volume of the destruction sample or by concentrating the sample [20].

Antioxidant activity testing of the ethanol extract of surian leaves

The antioxidant testing was conducted following the method developed by Taslim and Pratama (2020), as follows:

Preparation of DPPH solution (1,1-diphenyl-2-picrylhydrazyl)

A total of 10 mg of DPPH was weighed and then dissolved in 100 ml of 96% ethanol to create a solution with a concentration of 1000 ppm. Then, 3.5 ml of this solution was pipetted and diluted with 96% ethanol to achieve a final concentration of 35 ppm [12, 21].

Determination of the antioxidant activity of the samples

Preparation of sample stock solution

The ethanol extract of Surian leaves was prepared by weighing 0.1 g of the ethanol extract and dissolving it in ethanol up to the 100 ml mark, resulting in a 1000 ppm solution, which was then further diluted to 100 ppm [21].

Preparation of test solutions with various concentrations

Test solutions with different concentrations were prepared from the sample stock solution. From the 100-ppm stock solution, volumes of 0.5, 1, 1.5, and 2 ml were pipetted and diluted with ethanol to a total volume of 20 ml, resulting in concentrations of 2.5, 5, 7.5, and 10 ppm. 1 ml was taken from each test solution with different concentrations (2.5, 5, 7.5, and 10 ppm) and placed in test tubes wrapped with aluminum foil. To each test tube, 2 ml of DPPH solution at 35 ppm was added. The mixture was allowed to stand for 30 min, and the absorbance at the maximum wavelength was measured. The same procedure was performed for the reference, which was vitamin C [21].

RESULTS AND DISCUSSION

Collection and determination of materials

The srian leaves used in this study were obtained from Rantau Suli Village, East Jangkat, Merangin Regency, Jambi Province. The determination results showed that the surian leaves sample belonged to the red surian leaf variety (*Toona sinensis* (A. Juss) M. Roem) from the Meliaceae family (Number: B-4601/II.6.2/DI.05.07/12/2022).

Yield of simplicia of surian leaves

The purpose of creating simplicia was to ensure its long-term storage. Drying should not exceed a temperature of>70 °C to avoid damaging the antioxidant compounds present in Surian leaves [22]. The drying process was carried out to reduce the moisture content of the material for longer storage and to prevent mold growth [22]. Particle size reduction was performed to enhance contact between the solid material and the solvent in the extraction process, optimizing the amount of extract obtained. Finer simplicia powder leads to a more efficient extraction process as the surface area in contact with the extraction fluid increases. However, it is important not to make the particle size too small, as the resulting extract may contain impurities [17]. The purpose of calculating the yield of simplicia was to determine the success rate and to find the optimal concentration for the production of simplicia and extract. A good simplicia yield is typically above 20%. In this study, a simplicia yield of 54.364% was obtained, which exceeded the 20% benchmark. This indicates that the simplicia produced was more than 50% of the total fresh sample quantity.

Yield of ethanol extract from surian leaves

The purpose of extraction was to extract chemical components from natural materials. This extraction was based on the principle of mass transfer of components into a solvent, where the transfer began at the interfacial layer and then diffused into the solvent [13]. The extraction technique used in this research was the maceration method. Maceration is a simple extraction method without heating, specifically suitable for heat-sensitive active ingredients (cold extraction). Ethanol was used as the solvent because it is safe and non-toxic [24]. Ethanol is capable of extracting many compounds, such as flavonoids, saponins, tannins, anthraquinones, terpenoids, and alkaloids [13]. The yield is the ratio of the obtained extract to the initial simplicia [12, 17]. According to the Herbal Pharmacopoeia, the minimum extract yield should not be less than 7.2%. In this study, an extract yield of 33.278% was obtained, which exceeded the 7.2% requirement. The higher the yield value is, the greater the amount of concentrated extract produced will be.

Specific characteristics testing of simplicia and ethanol extract of surian leaves

The purpose of standardization was to ensure the quality and safety standards of herbal plant extracts. The determination of quality standards included specific and non-specific parameters.

Table 1: Phytochemical screening of simplicia and ethanol extract of surian leaves

Phytochemical screening	Simplicia	Ethanol extract
Alkaloids	(-)	(-)
Polyphenols	(+)	(+)
Tannins	(+)	(+)
Flavonoids	(+)	(+)
Monoterpenoids	(+)	(+)
Sesquiterpenoids	(-)	(-)
Quinones	(+)	(+)
Saponins	(+)	(+)

Note: (+) means there are compounds, (-) means there are not compounds

Phytochemical screening

Chemical content testing provided an initial overview of the chemical composition [17, 25]. Chemical content testing was carried out on simplicia and ethanol extract of surian leaves. From the results of phytochemical screening that simplicia and ethanol extract of surian leaves contain polyphenolic compounds, tannins, flavonoids, triterpenoids, steroids, quinones and saponin and does not contain alkoloid compounds.

Chromatogram pattern using TLC

Phytochemical screening was qualitatively performed using TLC. The separation of mixtures was based on the differential distribution of these mixture components between two phases: the stationary phase and the mobile phase. The stationary phase employed silica gel plate F 254. In thin-layer chromatography, the choice of solvent was based on the principle of "like dissolves like" [13, 14]. Activating the silica gel plate aimed to increase the absorption capacity of the stationary phase. Before the mobile phase testing, the mixture was saturated using eluent (chloroform: methanol = 9:1) so that the separation could be optimal and expedite the elution process [14].

Determination of total flavonoid and fenolic content

The determination of flavonoid content was carried out using the aluminum chloride colorimetric ($AlCl_3$) method. This method was also employed to calculate the total flavonoid compound content, which was expressed as quercetin equivalents (mg/g extract) based on the regression equation from the calibration curve. Specifically, the total flavonoid content was determined using the aluminum chloride colorimetric assay [26]. From the results above, it is stated that the total flavonoid content in ethanol extract of Surian leaves at a concentration of 1000 ppm was 33.19±0.89 mg/g. From the results above, it is stated that the total phenolic content in ethanol extract of Surian leaves at a concentration of 1000 ppm was 153.10±0.31 mg/g.

Table 2: Rentention factor of ethanol extract of surian leaves

Average *Rf* ± SD	Standard	Compound	Reference
0.216±0.0381	0.216	Quercetin	[13]
0.216±0.0381	0.216	Quercetin	[13]
0.513±0.0381	0.53	Steroid	[13]
0.704±0.0098	0.670	Terpenoid	[13]
0.607±0.0190	0.607	Flavonoid	[13]
0.418±0.0190	0.454	Phenol	[13]

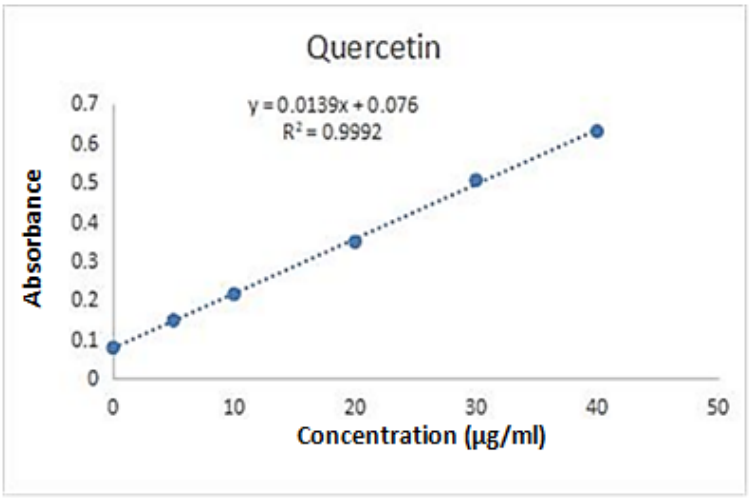

Fig. 2: Linear regression quercetin standard solution (n = 5)

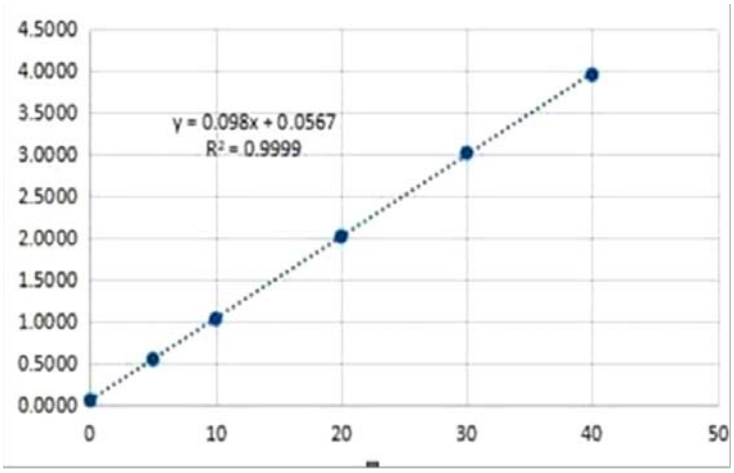

Fig. 3: Linear regression gallic acid standard solution (n = 5)

Determination of moisture content

Moisture content was a parameter used to determine the water residue after the drying process using a moisture balance instrument. Moisture content in the simplicia and ethanol extract of Surian leaves complied with quality standards, which is ≤ 10%. Concentrated extract has a moisture content between 5-30% [23]. The determination of moisture content is also related to the purity of the extract. Excessive moisture content (>10%) can lead to the growth of microbes, which can reduce the stability of the extract [23].

Table 3: Moisture content testing in the simplicia and ethanol extract of surian leaves

Examination	Surian leaf samples	Weight (g)	Total (%)	Moisture content (%)±SD	MMI standard, 1994
Moisture Content	Simplicia	0.505	92.080	7.920	≤ 10%
		0.505	91.660	8.340	Complies
		0.506	91.230	8.770	
				8.343±0.4250	
Moisture Content	Ethanol Extract	0.721	91.820	8.180	≤ 10% Complies

Microbial contamination test (bacteria and mold)

Testing for bacterial and mold contamination is one of the tests for examining the purity of the simplicia and ethanol extract [17]. The aim was to ensure that the simplicia and ethanol extract of Surian leaves did not contain microbial and fungal contaminants above the specified limits [29]. The results showed that bacterial and mold contamination in ethanol extract of Surian leaves were both<10 CFU/g from the observation results, the average number of colonies was 0 colonies This complies with the ALT parameter of SNI ISO 4833-2:2015 method, which specifies<10 CFU/g from the observation results, the average number of colonies was 0 colonies, while the mold and yeast parameter of SNI ISO 21527:2012 specifies<10 CFU/g [18]. Contamination could occur during the sample processing into an extract and also during the storage of the extract, which was likely to be contaminated by the air around the storage area [29].

Total ash content examination

The purpose of testing the ash content was to provide an overview of the internal and external mineral content originating from the initial processing to the formation of the extract [17]. Additionally, determining the total ash content aimed to assess the effectiveness of processing the simplicia and ethanol extract of Surian leaves. At this stage, the extract was heated until organic compounds and their derivatives were destroyed and vaporized, leaving only mineral and inorganic elements [17].

The total ash content in the simplicia was 5.333%, while in the extract, it was 2.643%. The ash content for both the simplicia and ethanol extract of Surian leaves was relatively low and complied with the established standards. According to MMI (1995), the maximum allowable total ash content is ≤ 10%. The resulting maximum value becomes the upper limit to indicate purity. A higher total ash content indicates a higher internal mineral content in Surian leaves. In this case, both the simplicia and the ethanol extract of Surian leaves had a very low mineral content [18].

Minerals required by humans include calcium, phosphorus, and magnesium for bone growth, sodium and chloride for bodily fluids, and iron for hemoglobin and red blood cell formation [30]. It is different for toxic minerals (heavy metals), such as mercury, lead, copper, cadmium, and strontium. Accumulation of heavy metals in the human body over a long period can disrupt the circulatory system, nerve endings, and kidney function [31].

Table 4: Total ash content in simplicia and ethanol extract of surian leaves

Surian leaf	I (%)	II (%)	III (%)	mean±SD (%)	MMI Standard, 1995
Simplicia	5.000	5.000	6.000	5.333±0.5773	≤ 10%
Ethanol Extract	2.830	2.242	2.857	2.643±0.348	≤ 10%

Determination of acid insoluble ash content

The determination of acid-insoluble ash content aimed to assess the presence of mineral or metal impurities that are insoluble in acid in a product. According to the MMI standard (1995), the limit for acid-insoluble ash content is≤ 2.60%. This serves as a n indicator of the presence of non-volatile metal contaminants when subjected to high-temperature heating [18]. The obtained acid-insoluble ash content in the simplicia was 1.1336%, while in the ethanol extract, it was 0.398%. A high level of acid-insoluble ash content indicates the presence of silica (originating from soil or sand) and metallic elements (e. g., silver, lead, and mercury) [31].

Table 5: Acid-insoluble ash content in simplicia and ethanol extract of surian leaves

Surian leaf	I (%)	II (%)	III (%)	mean±SD (%)	MMI Standard, 1995
Simplicia	1.1935	0.7208	1.4866	1.1336±0.3863	≤ 2.60%
Ethanol Extract	0.214	0.177	0.802	0.398±0.3510	≤ 2.60%

Heavy metal contamination test for Pb, Cu, Cd, and Zn

The atomic absorption spectrometry (AAS) is an analytical method based on the process of radiation energy absorption by different atoms at their ground energy levels. AAS serves as a tool used in analytical methods for the determination of metal and metalloid elements based on the absorption of light at a specific wavelength by free-state metal atoms. This test is suitable for metal analysis due to its high sensitivity (detection limit of less than 1 ppm). Its implementation is relatively straightforward, and it has minimal interference [32].

Zinc, in small amounts, is an essential element for metabolism because a deficiency of zinc in the body may lead to delayed child growth. Apart from that, copper is also an essential metal that, when present in low concentrations, can stimulate organism growth, but at high concentrations, it can be inhibitory. Based on the toxicity, copper and zinc have high toxicity [31].

Several factors can cause heavy metal contamination in the environment, including the geological conditions of the soil where plants are cultivated, the quality of water used for irrigation, specific heavy metal contamination from industries if the cultivation location is near industrial areas, and even unforeseen disasters. Contamination of heavy metals, such as copper and zinc, can occur during pre-harvest, including during planting and maintenance, and may also be due to the use of micronutrient fertilizers containing copper and zinc [32].

Table 6: Heavy metal contamination test for the ethanol extract of surian leaves

Heavy metal contamination test	Result (mg/kg)±SD	Requirements (BPOM, 2022)	Note
Pb (Lead)	0.2750±0.00	Not more than 20 mg/kg	Complies with requirements
Cu (Copper)	16.9500±0.00	0.1-150 mg/kg	Complies with requirements
Cd (Cadmium)	0.2250±0.00	Not more than 5 mg/kg	Complies with requirements
Zn (Zinc)	4.7500±0.00	2.0-100 mg/kg	Complies with requirements

Antioxidant activity testing of the ethanol extract of surian leaves

Antioxidant activity testing was conducted on the samples with vitamin C as a reference. The DPPH method was chosen in this research for its simplicity, ease, speed, sensitivity, and the requirement of only DPPH with a reference compound. The antioxidant activity value using DPPH was expressed as IC_{50}. It is a standard parameter that indicates the antioxidant's ability to absorb or counteract free radical compounds, represented in this case by the DPPH radical [12, 34]. The IC_{50} value is inversely related to the antioxidant activity level. The higher the IC50 value is, the weaker the antioxidant activity will be. Conversely, a smaller IC_{50} value indicates stronger antioxidant activity [21, 35].

Table 7: Levels of antioxidant strength by the DPPH method

Antioxidant activity	The IC_{50} value (ppm)
Very Strong	<50
Strong	50-100
Moderate	101-150
Weak	151-200

The use of DPPH as a blank requires prior equilibration (operating time). Operating time, or the operational duration, refers to the period during which the compound that absorbs light exhibits stable absorption. The measurement duration for the DPPH method varies, including 5, 10, 20, 30, and 60 min. In this study, a 30-minute operating time was chosen, following the research conducted by Su *et al.* (2020), which utilized a 30-minute operating time.

Both sample preparation and the creation of blank solutions, aimed at assessing the extent of absorption by non-analyte substances, involve dilution. This dilution serves to decrease the solution's concentration and, consequently, reduce molecular collision rates due to friction [12]. Antioxidant activity testing of the ethanol extract of Surian leaves was performed using a spectrophotometer with a wavelength of 517, which corresponds to the maximum wavelength of DPPH. This specific wavelength provides the highest level of absorption and sensitivity. According to table 7, the ethanol extract of surian leaves exhibited exceptionally strong antioxidant activity, approaching the IC_{50} value of vitamin C. Specifically, the IC_{50} value of the ethanol extract of Surian leaves was less than 50 ppm, at 12.351±0.1229 ppm, indicating its potential for further development into various anti-aging pharmaceutical products, such as sunscreen creams, serums, peel-off gel masks, lotions, hydrogels, and other facial skincare treatments.

This is also the same thing that happened in research by Yang, Li *et al.*, 2024 is Hosta plantaginea (Lam.) Aschers flowers (HPF) are well-known for their high flavonoid content and the IC50 of TF to DPPH were 2.02 mg/ml in the very strong category [36].

Table 8: The IC_{50} values of the ethanol extract of surian leaves

R1	R2	R3	mean±SD	Vitamin C
12.455	12.280	12.218	12.351±0.1229	7.805

CONCLUSION

The ethanol extract of Surian leaves contains flavonoid compounds with remarkably potent antioxidant activity. Therefore, it holds promise for the development of standardized anti-aging cosmetic formulations.

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AUTHORS CONTRIBUTIONS

Conceptualization: M. M, A. Y. C and W. S.; Methodology: U. L., M. M., A. Y. C and W. S; Data curation: U. L; M. M, W. S and A. Y. C; Writing-Original Draft Preparation: U. L.; Writing-Review and Editing: M. M, A. Y. C and W. S; Supervision: M. M., A. Y. C., and W. S.; Funding Acquisition: M. M, A. Y. C and W. S; All authors have read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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