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The Potential of Cantigi Leaves Extract (*Vaccinium varingiaefolium* Miq.) as a Skin-Lightening Agent Through Tyrosinase Enzyme Inhibition

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ABSTRACT

Introdiction*: Cantigi (Vaccinium varingiaefolium Miq.) was an Indonesian endemic plant that grows in mountainous regions and contains flavonoids, tannins, and anthocyanins as secondary metabolites. These compounds are potential antioxidants and natural skin-lightening agents that inhibit tyrosinase enzymes.* ***Aims****: This study aimed to evaluate the tyrosinase inhibition activity of cantiga leaf extract and compare it with that of kojic acid as a positive control.* ***Methods****: Cantigi leaves were extracted sequentially using n-hexane, ethyl acetate, and 96% ethanol, followed by concentration using a rotary evaporator. Evaluations included in vitro evaluation of organoleptic properties, pH, solubility, total flavonoid content, and tyrosinase inhibition activity in vitro.* ***Result****: The results showed that the 96% ethanol extract was dark red, had a characteristic odor, and had a viscous form with an average pH of 3.12. The total flavonoid content was determined to be 4.03% using the colorimetric method with quercetin as the standard. Tyrosinase inhibition testing showed that kojic acid had an IC_{50} value of 28.219 ppm, whereas cantigi leaf extract had an IC_{50} value of 1987.775 ppm.* ***Conclusion****: Although the tyrosinase inhibition activity of cantigi leaf extract is lower than that of the positive control, the findings demonstrate its potential as a natural active ingredient for skin-brightening cosmetic products. Further research is recommended to isolate the main active compounds and enhance effectiveness through innovative formulations for optimized use in topical cosmetic preparations.*

KEYWORDS*: Cantigi, tyrosinase, flavonoid, IC_{50}, ethanol extract*

INTRODUCTION

Cantigi (*Vaccinium varingiaefolium* Miq.) is an endemic plant native to Indonesia, particularly in volcanic mountain regions such as Tangkuban Parahu in West Java. This plant belongs to the Ericaceae family and shares similarities with other species, such as bilberry (*Vaccinium myrtillus*), which is well-known for its therapeutic properties. Cantigi contains various bioactive compounds, including flavonoids, anthocyanins, and other phenolic compounds, which have significant potential in both medicinal and cosmetic applications, particularly in skin care (Kosasih, Redja, & Farida, 2022; Roziaty & Agustina, 2023).

Flavonoids found in cantigi leaves exhibit potent antioxidant activity, which plays a crucial role in counteracting free radicals that cause cellular damage and premature aging. Several studies have demonstrated that cantiga extract can help rejuvenate the skin by reducing the effects of free radical damage, while also enhancing skin elasticity and hydration (Roziaty & Agustina, 2023; Kosasih, Sumaryono, & Supriono, 2019). Furthermore, anthocyanins in cantigi leaves provide additional protection against UV-induced skin damage, one of the primary causes of premature aging and skin cancer (Kosasih et al., 2021).

Cantigi leaf extract also shows significant potential as a skin-brightening agent in cosmetic applications. Hyperpigmentation caused by excessive melanin production often leads to dark spots and uneven skin tone. Cantigi leaf extract inhibits tyrosinase activity, the enzyme responsible for melanin production. By inhibiting tyrosinase, cantigi extract can reduce excessive melanin formation, providing a skin-brightening effect and a more even skin tone (Yulyana et al., 2023; Christian, Rahmat, Farida, 2022).

A key aspect of this research is the use of n-hexane and ethyl acetate as solvents during the extraction process. These solvents were selected for their ability to bioactive compounds from cantigi leaves. N-hexane is used to extract non-polar compounds, whereas ethyl acetate is used to extract semi-polar compounds, allowing for the extraction of several bioactive constituents present in cantigi. After the extraction process, the 96% ethanol extract is used for further testing because ethanol is highly effective in extracting polar flavonoids and phenolic compounds, which were of particular interest for this study (Mokhamad M & Shelly T, 2022; Efri Roziaty, 2022).

The novelty of this study lies in the use of n-hexane and ethyl acetate as extraction solvents, a less commonly explored approach in previous studies of cantigi leaf extracts. This research focuses on evaluating how these solvents affect the extraction efficiency of bioactive compounds, particularly about tyrosinase inhibition and skin-brightening potential. Furthermore, this study provides new insights into the mechanisms of action of cantigi extract in inhibiting melanin formation, laying the groundwork for the development of more effective and natural skin care products.

METHODS

Equipment

The equipment used in this study included an analytical balance (AND HR-120), a rotary evaporator (Büchi R-300), a magnetic stirrer (IKA), a vacuum filtration setup (Whatman), a UV-Vis spectrophoto-

meter (Shimadzu UV-1800), a micropipette (Eppendorf), an evaporation dish (IWAKI PYREX), a dropper pipette (IWAKI PYREX), a 96-well microtiter plate (IWAKI, Japan), an ELISA reader (ELx 800TM, US), an incubator (Memmert), an oven (Memmert), precision and non-precision glassware (IWAKI PYREX), an analytical balance (Mettler Toledo), a digital thermometer (Hanna Instruments), a water bath (Memmert), a UV lamp (UVP), and a spatula (Sigma-Aldrich).

Chemicals and Reagents Used

The materials used in this study included cantigi leaves (Bandung), n-hexane (Merck), ethyl acetate (Merck), 96% ethanol (Merck), DPPH reagent (Sigma-Aldrich), quercetin (Sigma-Aldrich), kojic acid (Sigma-Aldrich), distilled water (Merck), ethanol (Fisher Scientific), methanol (Fisher Scientific), aluminum foil (Sigma-Aldrich), yellow tips (Eppendorf), and blue tips (Eppendorf). These materials are used for the extraction process and testing, including antioxidant activity and tyrosinase inhibition assays.

Sample Collection and Identification

Approximately 3 kg of fresh cantigi leaves were collected from the Center for Natural Resources Conservation (Balai Besar Konservasi Sumber Daya Alam) and Mount Tangkuban Parahu, North Bandung, West Java. Subsequently, the sample was authenticated through botanical identification by qualified personnel at the designated herbarium institution, following standard taxonomic procedures. Documentation was completed to ensure the correct classification of the plant species used in this study.

Extraction of the Cantigi Leaves

A total of 250 grams of powdered cantigi leaf simplicia was extracted sequentially using solvents of varying polarity. The process began with maceration in 2.5 L of n-hexane in a closed container. The sample was left to stand at room temperature for 72 h, with occasional stirring to enhance solvent penetration into the plant matrix. After maceration, the mixture was vacuum filtered to separate the liquid extract from the plant residue. The n-hexane filtrate was then concentrated using a rotary vacuum evaporator at a temperature of 40–45°C under reduced pressure to obtain a thick n-hexane extract (Christian & Rahmat, 2022). The residue from the n-hexane extraction was then re-extracted using 2.5 liters of ethyl acetate, following the same steps: 72-hour soaking, occasional stirring, filtration, and solvent evaporation using a rotary vacuum evaporator to produce a thick ethyl acetate extract.

Next, the residue from the ethyl acetate extraction was macerated in 2.5 liters of 96% ethanol. This final maceration was also carried out for 72 hours at room

temperature, followed by filtration, and concentration of the filtrate using a rotary vacuum evaporator to obtain the thick 96% ethanol extract. The ethanol extract was stored in a tightly sealed container at refrigeration temperature (approximately 4°C) until further analysis.

This multi-stage extraction method was employed to obtain a broad spectrum of phytochemical compounds based on their polarity, with n-hexane targeting non-polar compounds, ethyl acetate for semi-polar compounds, and 96% ethanol for polar compounds. This concentrated extract was weighed, and its yield was calculated to determine the efficiency of the extraction process using the equation

$$\%\text{Extract yield} = \frac{\text{Crude extract weight (g)}}{\text{Sample dry weight (g)}}\ x\ 100$$

Characterization of Cantigi Leaf Extract

Organoleptic evaluation was conducted to observe the physical characteristics of *Vaccinium varingiaefolium* Miq. (cantigi) leaf extract based on its color, odor, and consistency. The color was visually observed under normal lighting and described, such as dark red or brownish. The odor was assessed by directly smelling the extract to determine its intensity and characteristics, such as a leafy or herbal scent. The consistency was examined to determine whether the extract appeared thick, semi-solid, or solid, and its texture and homogeneity were also noted. These observations served as preliminary information before conducting further chemical analysis and bioactivity testing (Christian & Rahmat, 2022).

pH Measurement of Cantigi Leaf Extract

The pH measurement was conducted to determine the acidity or alkalinity of the cantigi leaf extract (*Vaccinium varingiaefolium* Miq.). This procedure was carried out using a digital pH meter, which was first calibrated with buffer solutions having pH values of 4 and 7 to ensure the accuracy of the instrument. After calibration, the pH meter's electrode was immersed in the cantigi leaf extract, which had been dissolved in a neutral solvent or distilled water, depending on the form of the extract (thick liquid or semi-solid). The electrode was left in the solution until the reading on the pH meter stabilized, and the pH value of the extract was recorded. This measurement is crucial for understanding the chemical stability of the extract and its application in topical or oral formulations, as pH can influence the effectiveness of active compounds and user comfort (Christian et al., 2022).

Solubility of Cantigi Leaf Extract

The solubility of cantigi leaf extract (*Vaccinium varingiaefolium* Miq.) was tested to determine the extract's ability to dissolve in various solvents. The ethanol

extract of cantigi leaves was evaluated by dissolving the extract in several different solvents to identify the solvent that provides the best solubility. The solvents used in this test include distilled water, 96% ethanol, isopropyl myristate, olive oil, virgin coconut oil, and oleic acid. Each solvent was tested by adding the extract into the solvent and mixing it until a uniform solution was achieved. Observations were made to determine whether the extract dissolved completely or formed a suspension. The results of this solubility test are crucial for selecting the most appropriate solvent for formulating the extract into the desired preparation, whether for topical or oral formulations (Yulyana et al., 2023).

Preparation of Tyrosinase Inhibition Activity Test

A phosphate buffer solution was prepared by dissolving 1.3609 g of potassium dihydrogen phosphate in 100 mL of distilled water, diluting it to 200 mL, and then adding 22.4 mL of 0.2 N NaOH. For the L-DOPA substrate, 39.438 mg of L-DOPA (MW 197.19) was dissolved in 10.0 mL of phosphate buffer (pH 6.8), then diluted to obtain concentrations of 10, 5, 2.5, 1.25, and 0.625 mM while protected from light using aluminum foil. The tyrosinase enzyme solution was prepared by dissolving 0.0859 mg tyrosinase in phosphate buffer to obtain a concentration of 496 U/mL, then further diluted to 310, 155, and 75 U/mL, also protected from light using foil during preparation to maintain stability (Prasetyo, 2021; Hidayatri, Rahmat, & Wibowo, 2021).

Tyrosinase Inhibition Activity Kojic Acid

To prepare the sample solution, 5 mg of kojic acid was dissolved in 10.0 mL of phosphate buffer (0.1 M, pH 6.8) to obtain a 500 µg/mL stock, which was then serially diluted to 125, 62.5, 31.25, and 15.625 µg/mL. For the tyrosinase inhibition assay, 80 µL of phosphate buffer, 40 µL of kojic acid solution, 40 µL of L-DOPA solution, and 40 µL of tyrosinase enzyme were added to each well of a 96-well microplate. Control wells received the same components excluding tyrosinase. Blank wells contained 120 µL of buffer, 40 µL of L-DOPA, and 40 µL of tyrosinase enzyme. Positive controls included all reagents except the enzyme. Absorbance was measured using an ELx 800 microplate reader at the appropriate wavelength after incubation at 37°C. This in vitro assay was used to determine the IC_{50} value of the cantigi leaf extract against tyrosinase (Puspitasari & Dari, 2022).

Tyrosinase Inhibition Activity Cantigi Leaf

As much as 80 mL of phosphate buffer solution (0.1 M, pH 6.8), 40 mL of extract solution, 40 mL of L-DOPA solution, and 40 mL of tyrosinase enzyme solution were added into a 96-well microtiter plate in 10.0

mL volumetric flasks to achieve concentrations of 4000, 2000, 1000, 500, 200, 100, and 25 μg/mL. The blank control and control solutions were also tested. The control solution consisted of 40 mL of 1% DMSO, 80 mL of phosphate buffer solution, 40 mL of L-DOPA solution, and 40 mL of tyrosinase enzyme solution. The blank control only contained the same materials as the control solution, but without the enzyme. Absorbance was measured using an ELx 800 microplate reader at the appropriate maximum wavelength during incubation at the ideal temperature of 37°C.

Tyrosine Inhibition Activity (IC_{50}) of Cantigi Leaf extreact

The inhibition activity was calculated using the formula in the equation, where the inhibition percentage (% inhibition) can be determined (Prasetyo, 2021**;** Fatmawati, Jafar, & Riantini, 2020).

$$\% \text{ Inhibition} = \frac{(A - B) - (C - D)}{(A - B)} \; x \; 100$$

Where:

A : Absorbance of the blank solution
B : Absorbance of the blankcontrol solution
C : Absorbance of the sample solution
D : Absorbance of the sample control solution

RESULTS AND DISCUSSION

The cantigi leaf simplicia extraction (*Vaccinium varingiaefolium* Miq.) was conducted using a kinetic maceration process to extract secondary metabolites from the leaves. The extraction was performed sequentially using solvents that included 96% ethanol, n-hexane, and ethyl acetate to obtain an extract rich in bioactive compounds such as flavonoids, phenolics, and other antioxidant components. After the extraction, the filtrate was processed further using a rotary evaporator at 40°C, with a pressure of 175 mbar, a rotation speed of 60 rpm, and cooling set to 10°C to prevent any degradation of active compounds due to excessive heat. This process demonstrated that cantigi leaves contain bioactive compounds that can be effectively extracted using polar solvents like ethanol, with careful control of temperature and pressure to preserve the integrity of the compounds (Christian et al., 2022).

The extraction yielded 30.5 g of thick extract from 250 g of cantigi leaves, resulting in a yield of 12.2% and a DER-native (Dry Extract Ratio) of 8.19, indicating a high concentration of active ingredients in the extract (Table 1). A higher DER-native ratio suggests the extract is rich in active compounds, which enhances its potential for therapeutic applications. The filtrate obtained had a greenish color, indicating the presence of compounds soluble in 96% ethanol, and showed that this solvent was most effective for obtaining a good yield of bioactive compounds, such as flavonoids and phenolics, from the leaves. Additionally,

Table 1. Results of thick cantigi leaf extract

Examination Result	Description
Weight (g)	250 g
Thick extract (g)	30,5
DER-*native*	8,19
Yield (%)	12,2

the use of n-hexane and ethyl acetate in the early extraction stages allowed for the extraction of non-polar compounds, while ethanol was used to extract more polar compounds (Christian et al., 2022).

The conditions under which the extraction was carried out are crucial for ensuring that the bioactive compounds in the cantigi leaf extract remain intact. The use of a rotary evaporator allowed the solvent to evaporate under reduced pressure, which helped prevent the loss of volatile compounds. The extraction temperature of 40°C was maintained to ensure the stability of the active compounds without causing thermal degradation. This careful control of temperature and pressure conditions during the extraction process ensured that the active compounds were extracted efficiently without being damaged.

In conclusion, the maceration kinetic extraction process using n-hexane, ethyl acetate, and 96% ethanol yielded a high-quality extract with a 12.2% yield and a DER-native of 8.19. This process successfully produced an extract rich in bioactive compounds, making it a promising candidate for use in pharmaceutical and cosmetic applications due to its high flavonoid and antioxidant content. The findings underscore the importance of controlling the extraction conditions to maximize the concentration of bioactive compounds in the cantigi leaf extract, enhancing its therapeutic potential (Christian & Rahmat, 2022)(Christian et al., 2022).

In this study, organoleptic evaluation was performed to provide an initial identification of the cantigi leaf extract. The results showed that the extract had a dark red color, which is characteristic of cantigi leaves (*Vaccinium varingiaefolium* Miq.). This dark red color is attributed to the presence of flavonoids and anthocyanins, compounds commonly found in the leaves. The color arises due to the interaction of these phenolic compounds with ethanol solvent, producing a distinctive hue in the extract. These phenolic compounds have the ability to absorb light at specific wavelengths, which is why they give the extract its characteristic color.

The odor of the extract was noted as being specific to cantigi leaves, which may indicate the presence of essential oils and other volatile compounds that are dissolved in the extract. These compounds contribute to the characteristic aroma and may also play a role in various biological activities, such as antimicrobial and antioxidant

Table 2. Examination results of thick cantigi leaf extract

Examination	Examination result
Organoleptic:	
Color	Dark red
Odor	Characteristic cantigi leaf
Texture	Thick
pH	3,12
Solubility in solvents:	
Distilled water	(1:100) Slightly soluble
Ethanol (96%)	(1:30) Soluble
Isopropyl myristate	(1:100) Slightly soluble
Olive oil	(1:100) Poorly soluble
Virgin coconut oil	(1:100) Poorly soluble
Asam oleat	(1:100) Poorly soluble

properties.

The consistency of the extract was described as thick, which suggests that the active compounds in cantigi leaves were effectively extracted using 96% ethanol. This thick consistency is indicative of the presence of bioactive compounds like flavonoids, terpenoids, and other bioactive molecules that are soluble in polar solvents like ethanol. The use of the rotary evaporator at 40°C and 175 mbar pressure ensures that the extract's bioactive compounds remain stable and do not degrade due to excessive heat.

pH Measurement of Cantigi Leaf Extract

The pH measurement was carried out using a Hanna instrument HI 2211 pH meter, which was calibrated with buffer solutions at pH 7 and pH 4. The results showed that the pH of the extract ranged from 3.10 to 3.13, indicating that the extract is slightly acidic. This acidity is likely due to the presence of phenolic acids and other organic acids such as gallic acid, caffeic acid, and oleic acid that are commonly found in plant extracts. The slightly acidic pH of the extract is favorable for topical applications, as the skin's natural pH is also slightly acidic, which makes the extract safe for skin use (Christian et al., 2022).

Solubility of Cantigi Leaf Extract in Different Solvents

The solubility of the cantigi leaf extract was tested in several solvents, including distilled water, 96% ethanol, isopropyl myristate, olive oil, virgin coconut oil, and oleic acid. The extract was found to be highly soluble in 96% ethanol and isopropyl myristate, indicating that it contains polar compounds soluble in these solvents (Table 2). Ethanol, being a polar solvent, is highly effective in extracting hydrophilic compounds such as flavonoids, which explains the solubility observed in the extract. Additionally, the use of isopropyl myristate, which is also a non-polar solvent, suggests that the extract contains lipophilic components that can be used in cosmetic formulations.

On the other hand, the extract was not soluble in distilled water, indicating that the bioactive compounds in the extract are

more soluble in organic solvents than in water. The extract also showed minimal solubility in olive oil, virgin coconut oil, and oleic acid, which further suggests that the extract is more compatible with formulations based on organic solvents or mixtures that contain bioactive compounds (Christian et al., 2022).

Based on the results from the organoleptic evaluation, pH measurement, and solubility testing, the cantigi leaf extract demonstrates promising characteristics as an active ingredient for various therapeutic and cosmetic applications. The dark red color and distinct odor suggest the presence of active bioactive compounds, while the slightly acidic pH aligns with the natural pH of the skin, making the extract suitable for topical use. The excellent solubility in 96% ethanol confirms that the extract is rich in polar compounds, which are responsible for its antioxidant and anti-inflammatory properties. The extract's solubility also highlights its potential use in formulations involving organic solvents or lipophilic components.

Tyrosinase Enzyme Inhibition Activity

The tyrosinase inhibition activity of cantigi leaf extract was tested to evaluate its potential in inhibiting the activity of the tyrosinase enzyme, which plays a crucial role in melanin formation. Melanin, produced by tyrosinase, is responsible for skin pigmentation, and inhibiting this enzyme can be used to address pigmentation disorders like hyperpigmentation. In this study, kojic acid was used as a positive control, as this compound is widely known as an effective tyrosinase inhibitor (Noor & Magdalena, 2018).

Tyrosinase inhibition was first tested using kojic acid at concentrations of 125, 62.5, 31.25, 15.625, and 7.8125 μg/mL (Table 3). The results showed an IC_{50} value of 28.219 ppm, indicating that kojic acid is quite effective in inhibiting tyrosinase activity. The low IC_{50} value (Table 3) demonstrates kojic acid's strong inhibition effect, providing a benchmark for comparing the effectiveness of cantigi leaf extract as a tyrosinase inhibitor (Fatmawati et al., 2020).

The inhibition effect of cantigi leaf extract was tested at higher concentrations (Table 3), about 312.5, 625, 1250, 2500, and 5000 ppm, resulting in an IC_{50} value of 1987.775 ppm, which is higher compared to the kojic acid control. This indicates that while the extract of cantigi leaves shows inhibitory effects on tyrosinase, its effectiveness is still lower than that of the positive control. However, the result still shows the potential of cantigi leaf extract to inhibit tyrosinase, which could be utilized in cosmetic applications, especially for skin whitening products. The inhibition effect is likely due to the presence of flavonoids and

Table 3. Tyrosinase enzyme inhibition test results of cantigi leaf extract compared to kojic acid as positive control.

Substance	Concentration (µg/L)	(%) Inhibition	Function	IC_{50} (µg/L).
Kojic acid	125	87.092	y = 27.997 ln(x) -43.509	28.219
	62.5	75.981	R^2 = 0.9818	
	31.25	57.866		
	15.625	30.583		
	7.8125	12.761		
Cantigi leaf extract	10000	82.353	y = 18.36 ln(x) -89.437	1987.775
	5000	62.273	R^2 = 0.9877	
	2500	54.430		
	1250	43.936		
	625	28.468		

phenolic compounds in the extract, which are known to bind to metal ions such as copper, crucial for tyrosinase activation (Bayu, Amrozi, and Nur, 2024).

Comparison with positive control (kojic acid), cantigi leaf extract showed a relatively higher IC_{50} value of 1987.775 ppm compared to 28.219 ppm for kojic acid. This indicates that although cantigi leaf extract exhibits inhibitory activity, it is less effective than kojic acid. However, the active compounds in cantigi, particularly flavonoids like quercetin, are likely responsible for this inhibitory effect. The structure of flavonoids, which includes an aromatic conjugated system, allows them to interact with the tyrosinase enzyme, inhibiting its activity (Fatmawati et al., 2020).

Based on the results, cantigi leaf extract has potential as a tyrosinase inhibitor, though its effectiveness is lower than that of kojic acid. The IC_{50} of 1987.775 ppm for the extract indicates that while the active compounds in the extract can inhibit tyrosinase, a higher concentration is required to achieve effective inhibition. Nevertheless, these results show that cantigi leaf extract holds promise for the development of cosmetic products targeting skin pigmentation disorders. Moreover, the nanoemulgel formulation containing cantigi leaf extract significantly enhanced tyrosinase inhibition, further demonstrating the potential of nanoparticle-based formulations to improve the efficacy of plant-derived bioactive compounds. Further studies are needed to isolate and identify the key active compounds in cantigi leaves responsible for this activity, as well as to optimize the extract for better application in cosmetic and dermatological products (Amin, 2024).

CONCLUSION

Cantigi leaf extract shows moderate tyrosinase inhibition with an IC_{50} of 1987.775 ppm, lower than the positive

control kojic acid (IC_{50} = 28.219 ppm), suggesting better bioavailability. Further research should isolate active compounds and optimize formulations for enhanced efficacy in cosmetic applications

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