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Standardization of Ethanol Extract 96% Cantigi Leaves (*Vaccinium varingiaefolium* Miq.)

(Standarisasi Eksrtak Etanol 96% Daun Cantigi (*Vaccinium varingiaefolium* Miq.))

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Abstract: Advantages of Cantigi (*Vaccinium varingiaefolium* Miq.) include anti-inflammatory, Spasmolytic, Antiviral, and Hypotensive Properties. The objective of this study was to standardize the Cantigi leaf extract. Non-specific parameters include total ash content, acid insoluble ash content, water content, dry loss, determination of heavy metals (Hg, As, Cd, Pb, hydroquinone), mold number, yeast, total plate count, and solvent residue. Specific parameters included extracts soluble in ethanol and water. Cantigi leaves are extracted by kinetic maceration using solvents such as n-hexane, ethyl acetate, and 96% ethanol. To obtain a thick extract, 96% ethanol extract was evaporated using a rotary evaporator. The experiment produced 44.5 g of the cantigi leaf extract in 96% ethanol. Phytochemical screening presents alkaloids, saponins, tannins, flavonoids, triterpenoids, steroids, essential oils, and quinones. Ethanol soluble extract was 9.49% and that of the water-soluble extract was 22.21%. A total of ash 3.66%, an acid insoluble ash 0.35%, water content 6.31%, and drying loss 6.49%. Results of the heavy metal contamination test revealed that no traces of Hg, As, Cd, Pb, or hydroquinone were found, along with 10 yeast molds, 10 plates, and 0.005% residual ethanol solvent. Based on the results, cantigi leaf extract met the standard of extract quality.

Keywords: Cantigi, ethanol extract, non-specific parameters, specific parameters

Abstrak: Cantigi (*Vaccinium varingiaefolium* Miq.) memiliki banyak manfaat, yaitu untuk antiradang, spasmolitik, antivirus, dan hipotensi. Penelitian ini bertujuan mendapatkan standarisasi ekstrak daun cantigi dengan menggunakan parameter spesifik dan non spesifik. Parameter spesifik meliputi ekstrak larut dalam etanol dan larut dalam air, sedangkan parameter non spesifik meliputi kadar abu total, kadar abu tidak larut asam, kadar air, susut kering, penetapan logam berat (Hg, As, Cd, Pb, hidrokuinon), angka kapang khamir, angka lempeng total, dan residu pelarut. Prosesnya ekstraksi daun cantigi dengan maserasi kinetik menggunakan pelarut n-heksana, etil asetat, dan etanol 96%. Ekstrak etanol 96% diuapkan menggunakan rotary evaporator hingga diperoleh ekstrak kental. Hasil diperoleh 44,5 g ekstrak etanol 96% daun cantigi. Selain itu, dilakukan penapisan fitokimia. Hasil pemeriksaan menunjukkan senyawa alkaloid, saponin, tanin, flavonoid, triterpenoid, steroid, minyak atsiri, dan kuinon. Hasil pengujian kadar sari larut etanol sebesar 9,49%, kadar sari larut air 22,21%. Hasil kadar abu total 3,66%, kadar abu tidak larut asam 0,35%, kadar air 6,31% , susut pengeringan 6,49%. Hasil uji cemaran logam berat Hg, As, Cd, Pb, hidrokuinon tidak terdeteksi, angka kapang khamir < 10, angka lempeng total < 10, sisa residu pelarut etanol 0,005%. Berdasarkan hasil penelitian, ekstrak daun cantigi (*Vaccinium varingiaefolium* Miq.) memenuhi standarisasi mutu ekstrak.

Kata kunci: Cantigi, ekstrak etanol, parameter nonspesifik, parameter spesifik

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INTRODUCTION

INDONESIA is the second country with the largest biodiversity in the world after Brazil[1]. This biodiversity is not only found on land but also in Indonesian waters. While on land, it covers the lowlands and highlands, including mountains and around volcanic craters. Abundant natural wealth both on land and at sea provides opportunities for the Indonesian people to take advantage of Indonesia's natural products, both for health maintenance and the treatment of diseases that are passed down from generation to generation. This plants has been popular from time to time, in case in Indonesia. This proves that nature, especially plants, can be used as a source of natural and easily obtained treatment[1].

The use of herbal products in the form of drugs, supplements, and cosmetics has increased both in developing and developed countries, so the potential and opportunities for the use of herbal products are still very broad[2]. The development of traditional medicines as the nation's cultural heritage continues to be improved and encouraged by the development and discovery of medicines, including the cultivation of traditional medicines with three conditions, namely safe, efficacious, and quality[3].

Standardization are needed to ensure quality, safe, and efficacious drugs. Standardization guarantees that the final product of traditional medicine (drugs, extracts, and extract products) produced through the scientific method has certain constant parameter values. Standardization of extract/simplicia quality consists of various general standard parameters and specific parameters[3].

Cantigi (*Vaccinium varingiaefolium* Miq.) is a plant that belongs to the *blueberry* and *cranberry* families, which is a *bearberry.* Cantigi grows in the highlands and native plant from Indonesia and usually grows around volcanic craters, for example in Tangkuban Parahu (North Bandung), Mount Papandayan, and Mount Patuha (South Bandung)[4]. Research that has been conducted on phytochemical screening shows that cantigi leaves contain phytochemical compounds such as anthocyanins, chlorophyll, phenols, saponins, steroids, tannins, triterpenoids, and flavonoids[5]. Previous research showed cantigi leaves extract has antioxidant activity and an IC_{50} of 16.84 ppm, which is categorized as having very strong antioxidant activity(6). Therefore, to obtain standardized cantigi leaf extract (*Vaccinium varingaefolium* Miq.*)*, specific and non-specific parameters were tested for ethanol 96% cantigi leaf extract. Standardized cantigi leaf extract can be used as a reference in the manufacture of powder preparations as a natural treatment in Indonesia.

MATERIALS AND METHODS

MATERIALS. The test material was cantigi leaves (*Vaccinium varingiaefolium* Miq.) obtained from Tangkuban Parahu (Bandung). The chemicals used were 96% ethanol (Brataco), acetone (Brataco), hydrochloric acid (HCl) (Brataco), aquadest (Brataco), ethyl acetate (Titan Biotech), 0.5% sodium acetate (CH_3COONa) (Brataco), aluminum chloride ($AlCl_3$) (Brataco), acetic acid (CH_3COOH) (Brataco), Dragendorf reagent (Titan Biotech), Bauchardat reagent (Titan Biotech), sodium hydroxide (NaOH) (Brataco), sulfuric acid (H_2SO_4) (Brataco), iron (III) chloride ($FeCl_3$) (Brataco), chloroform (CCl_3) (Titan Biotech), and *P*otato Dextrose Agar (Titan Biotech)

Tools. The tools used in the research include digital scales (Fujitsu), oven (Memmert), pH meter (Hanna Instrument), glassware (Pyrex), rotary evaporator (IKA Labortechnik), filter paper (Whatman), separating funnel (Iwaki), hotplate (Thermo), object glass (Slides), measuring pipette (Duran)

METHOD. Cantigi Leaf Extraction. An amount of 315 g of cantigi leaf simplicia powder (*Vaccinium varingiaefolium* Miq.) was kinetically macerated in stages with 3.15 L of n-hexane solution. Using a vacuum rotary evaporator, the filtrate from the maceration process was concentrated. The remaining n-hexane residue was then once more macerated with 96% ethanol and ethyl acetate as the solvents, respectively. To generate a thick extract of 96% ethanol, the extract was then evaporated using a vacuum rotary evaporator[4].

Organoleptic Testing. We noticed the cantigi leaf extract's color, scent, and shape[4]

Extract pH. A pH meter was utilized to measure the pH. A buffer for pH 4 and pH 7 was used to calibrate the pH meter initially. The pH meter electrode was dipped into the extract to take measurements[7].

Phytochemical Screening[8]. Alkaloids. The extract was heated for two minutes in a water bath before being added to an evaporating dish with 1 mL of 2 N hydrochloric acid and 9 mL of distilled water. A watch glass should contain 3 drops of the filtrate. If a brown to black precipitate forms after adding 2 drops of Bouchardat reagent, the extract includes alkaloids. After that, try Mayer reagent.

Saponins. A test tube containing 1 mL of extract was filled with 10 mL of hot water, cooled, and vigorously shaken for 10 seconds. If a steady foam forms that lasts for at least 10 minutes and is 1 cm to 10 cm high and does not vanish after adding 1 drop of 2 N hydrochloric acid, the extract contains saponins.

Tannins. 20 mL of 70% ethanol were used to extract a total of 1 gram of extract. Pipette 3 drops of

extract into a 5 drop solution of 1% iron (III) chloride. The creation of a green to blue-black tint signifies a successful reaction.

Flavonoids. 1 mL of the experimental solution should be evaporated and 1 to 2 mL of 95% P ethanol should be added. Add 2 mL of 2 N hydrochloric acid and 10 milligrams of zinc powder, give it a minute. Hydrochloric acid concentration, 10 drops, added. If it turns a bright red color within 2 to 5 minutes, flavonoids are present.

Terpenoids. 10 drops of sulfuric acid and 5 mL of anhydrous acetic acid P were combined with 100 mg of the extract. Terpenoids are present in the extract when it becomes blue or green.

Steroids. 10 drops of sulfuric acid were added to 100 mg of the extract together with 5 mL of anhydrous acetic acid. When the extract is crimson, steroids are present.

Essential oils. A total of ± 0.2 g of extract was put into an erlenmeyer (volume 20 mL), then 10 mL of petroleum ether solution was added, the mouth of the tube was fitted with a funnel which was given a layer of cotton that had been moistened with water, then heated for 10 minutes on a water bath and after cooling. filtered with filter paper. The filtrate obtained was evaporated in an evaporating dish, the residue was dissolved with 5 mL of alcohol solvent, then filtered through filter paper. The residue with an aromatic smell indicates the presence of volatile oil group compounds.

Quinone. A total of 5 mL of flavonoid experimental solution was put into beakerglass, added a few drops of 1N NaOH solution, an intensive red color was formed indicating the presence of the quinone group.

Specific Parameters. Ethanol-dissolved substances[8]. In a glass-stoppered flask, 5 grams of extract were macerated for 24 hours with 100 mL of 96% ethanol while being shaken for the first 6 hours and left to stand for the remaining 18. It was then swiftly filtered and let to dry in a small dish. At 105°C, the residue was cooked to a constant weight. From the first extract, the concentration of dissolved components was determined.

Dissolved substances in water[8]. Using a glass stopper flask, 5 grams of extract were macerated for 24 hours with 100 mL of water-chloroform solution while being shaken for the first 6 hours and left to stand for the remaining 18 hours. It was then swiftly filtered and let to dry in a small dish. At 105°C, the residue was cooked to a constant weight. From the first extract, the concentration of dissolved components was determined.

Non-specific Parameters[9]. Total Ash Content[9]. 2 grams of extract were added to a tare porcelain crucible. till the charcoal runs out, glowing. If the charcoal cannot be removed, boiling water should be added before filtering through ash-free filter paper. In the same crucible, the filter paper and residue are fired. In order to achieve a constant weight, the filtrate was placed into a crucible, evaporated, and burned at 450 °C. The substance is weighed in comparison to the ash content.

Analysis of Ash (Acid Insoluble)[9]. The ash used to calculate the total ash content was heated for 15 minutes in 25 cc of diluted sulfuric acid solution. The insoluble component was gathered, passed through a glass crucible or ash-free filter paper, rinsed in hot water, burned to a consistent weight, and weighed. Determine the air-dried material's acid-insoluble ash content.

Determination of Water Content (Karl-Fischer) [10]. By carefully weighing the extract up to 30–50 mg with glass weighing, and then adding the extract to the titration vessel, the water contained in the extract is allowed to react with the Karl–Fischer reagent to determine the water content using the Karl–Fischer titration method. Assess the water content in relation to the extract's weight.

Loss on Drying[11]. 1 gram of extract was equilibrated to a constant weight and heated for 60 minutes at 105 °C. After shaking the bottle until a coating between 5 and 10 mm thick has formed, place it in the oven set to 105 °C. Open the cover, and then dry at 105°C to a constant weight.

Heavy Metal Contamination[10,12]. The test sample is weighed 0.5 grams, put into the Erlenmeyer. Then they added HNO_3 solution and H_2O_2 30%, and destroyed it. Then it was allowed to stand at room temperature, transferred to a 50.0 mL flask, rinsed using aquademineralization and added aquademineralization to the mark, filtered if the solution was cloudy, put into a test tube, and measured the test solution using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). Then content was calculated.

Mercury (Hg) was determined by making standard solution in 50 mL volumetric flask from the standard 1000 ppm. Create a standard series of 0, 1, 5, 10, 20, and 40 ppb of standard 1 ppm mercury solution. Prepared standard solutions for As, Cd and Pb of 10 ppm in a 50 mL volumetric flask. Then standard series of As, Cd and Pb were made, namely 0, 5, 25, 50, 100, 200 ppb.

Hydroquinone analysis was determined by adding 10 mL of solvent to the 0.2–0.3 g of the sample solution into a screw tube, and the mixture was vortexed for one minute. After that, place for 15 minutes in a 60°C water bath. Once the solvent had been added to the desired level, the solution was

transferred to a 25.0 mL volumetric flask, filtered with Whatman filter paper, and then put into the vial. It is then injected into the HPLC (High Performance Liquid Chromatography. A 5 mL volumetric flask was filled with 5 mg of hydroquinone standard. The mark was then homogenized after being added methanol. A series of curves were made: 10, 20, 30, 40, 50 ppm into a 5 mL volumetric flask using a solvent. Furthermore, the solution is transferred into the vial and injected into the HPLC.

Microbial contamination. A total of 1 gram of extract was dissolved in phosphate buffer to a volume of 10 mL. If the mixture obtained is clear, continue the experiment with the plate method. If the solution mixture is not a clear solution, continue the experiment with the tube method. Then prepare 5 test tubes, each containing 9 mL of phosphate buffer diluent. From the results of homogenization in the preparation of the sample, 1 mL of the dilution was pipetted into the first tube. Repeat the same procedure until the dilution. From each dilution, 1 mL was pipetted into a triple petri dish. In the petri dish, add 15-20 mL of *Nutrient Agar* for the total plate number and 15-20 mL of *Potato Dextrose Agar* liquid (45°C) for yeast mold numbers, then homogenize. Incubated at 35-37°C for Nutrient Agar for 24-48 hours and 25-27°C for Potato Dextrose Agar for 5-7 days after solidifying in air.

Residual Solvent Analysis. Gas-liquid chromatography was used to identify the solvent. A 30 m x 0.32 mm glass column containing the stationary phase flowing by TR-WAX with a particle size range of 100 mesh to 200 mesh was added to the gas chromatograph along with a flame ionization detector. The carrier gas used is nitrogen.

RESULTS AND DISCUSSION

Pharmaceutical standardization consists of a number of parameters, processes, and measurement techniques that produce elements related to the pharmaceutical paradigm and quality in terms of satisfying standard requirements (chemical, biological, and pharmaceutical), including ensuring stability limits for generic pharmaceutical products. In other words, the process of guaranteeing that the finished drug product (drug, extract, or extract product) has specific constant and specified parameter values is included in the definition of standardization. The biological component of the ingredients of medicinal plant origin and the chemical content of the ingredients are the two aspects that have an impact on the quality of the extract.

To reach the same level of quality, standardization is the process of choosing properties based on particular criteria. Two parameters, called particular parameters and non-specific parameters, were used to standardize the extract. Identity, organoleptic, water-soluble, and ethanol-soluble chemical compounds are some of the specific criteria. Drying shrinkage, moisture content, ash content, and metal contamination are examples of non-specific characteristics.

At the time of the extraction process from cantigi leaf simplicia, it was carried out using the maceration method. The extraction process aims to extract secondary metabolites from cantigi leaves. A total of 315 grams of cantigi leaf simplicia powder was kinetic macerated. This method is a simple filtration method in which the solvent used is n-hexane, ethyl acetate, and 96% ethanol, which is then filtered using Whatman filter paper. This kinetic maceration process begins with the simplicia immersion process using a solvent. Using constant stirring, fluid will penetrate the cell wall and the cell cavity containing the active substance, which then compounds in the simplicia can be attracted or diffuse out of the cell during the maceration process. The results of maceration of cantigi leaf extract was shown in Table 1.

Table 1. Preparation of Cantigi Leaf Extract.

No	Examination results	Results
1	Weight of cantigi dry leaves (g)	315
2	Amount of thick extract (g)	44.5
3	Der-*native*	7.08
4	Yield (%)	14.13

The cantigi leaf extract underwent organoleptic examination (Table 2), which was the first step in the identification of a particular extract, to ascertain its color, scent, and shape using the five senses. Organoleptic testing revealed that cantigi leaf extract is a dark red color. The rich color of the cantigi leaves, which have the same color as the extract, is what gives it its dark red hue. The aroma of the cantigi leaves is what gives the extract its characteristic scent. The solvent from the liquid extract of cantigi leaves was evaporated using a rotary evaporator at 40°C, 60 rpm, 175 mbar of pressure, and then cooled at 40°C to produce the thick extract form.

Table 2. Organoleptic Examination of Cantigi.

No	Oranoleptic	
1	Color	Red
2	Smell	Typical
3	Shape	Thick

A pH examination was carried out using the *Hanna Instrument* HI 2211 pH meter, which had previously been calibrated using pH 7 and pH 4 buffers. Based on the examination (Table 3), the average pH was 3.23, an acidic pH. This acidic nature is due to cantigi leaves containing organic acid compounds such as oleic acid and palmitic acid (*hexadacanoic acid*).

Tabel 3. pH Examination.

No	pH	
1	3.24	
2	3.22	3.23
3	3.23	

In the screening results for alkaloid by Bouchardat reagent, a brownish orange precipitate was formed, while using Dragendorff's reagent an orange precipitate was formed. There was indeed contain alkaloids. It is known that the samples of cantigi leaf extract contain saponins. Saponins are surface-active substances that can be identified by their capacity to produce stable foam after shaking for ten minutes. The findings of this screening revealed that the samples of cantigi leaf extract contained saponins. When screening for tannin compounds, a green-black solution forms as a sign that tannins are present. According to the findings of the phytochemical screening, flavonoids were also detected in the cantigi leaf extract samples. The presence of a red hue in the amyl alcohol layer points to this outcome. Red and green precipitates appeared on the steam dish during the screening of triterpenoid and steroid compounds, indicating that they contained triterpenoids and steroids affirmatively. The appearance of an intense red tint on the screening findings indicated that quinone screening had produced favorable results.

Tabel 4. Phytochemical Screening.

No		Parameter	Serbuk simplisia	Ekstrak etanol
1	Phytochemical Screening	Alkaloids	+	+
2		Saponins	+	+
3		Tannins	+	+
4		Flavonoids	+	+
5		Triterpenoids	+	+
6		Steroids	+	+
7		Essential oil	+	-
8		Quinones	+	+

In the determination of the ethanol soluble extract content and the water soluble extract content, the results were 9.49 and 22.21. The results of this test met the requirements for determining the ethanol soluble extract content, namely 4.4 for water soluble extracts and 15.4. The test on the ethanol soluble extract content was used to determine whether the material could be extracted in alcohol, while the water soluble extract content was used to determine the ability of the material to be extracted in water solvent.

The total ash concentration was 3.66 according to the test. The outcomes are in compliance with the 7.2 maximum total ash content guideline. The total ash content test is performed to provide a comprehensive analysis of the material. Additionally, the amount of acid-insoluble ash was calculated, and the outcome was 0.35. The test findings were in compliance with the required limit of 0.9 for acid insoluble ash concentration. To give a general idea of the mineral content that isn't dissolved in acid, the determination of acid insoluble ash content is made[13].

According to the analysis of the cantigi leaf extract's water content, it has a water content of 6.31%. The findings obtained are in accordance with the standards for cantigi leaves, which must have a moisture content of less than 10%. The water content of the extract can vary depending on the humidity of the storage space as well as the amount of water still present in the extract. Water content has an impact on microbial development in extracts since it is a favorable substrate for microbe growth. Therefore, if an extract has a high water content, it is more likely that the extract will get overgrown with germs[8,14].

A maximum limit regarding the number of compounds lost during the drying process is intended to be provided by the measurement of extract drying shrinkage. In order to calculate drying shrinkage, we just weigh the substance after it has dried at 105°C

Table 5. Determination of Specific and Non-Specific Quality Parameters for Extracts.

No	Examination	Unit	Terms	Results
1	Ethanol soluble extract content	%	4.4 (MMI)	9.49
2	Water soluble essence	%	15.4 (MMI)	22.21
3	Concentration total ash	%	7.2 (MMI)	3.66
4	Acid insoluble ash content	%	0.9 (MMI)	0.35
5	Moisture content	%	10 (MMI)	6.31
6	Drying shrinkage	%	10 (FHI)	6, 49
7	Mercury (Hg)	mg/kg	0.5 BPOM No 12 (2014)	Not Detected
8	Arsenic (As)	mg/kg	5 BPOM No 12 (2014)	Not Detected
9	Cadmium (Cd)	mg/kg	0.3 BPOM No 12 (2014)	Not Detected
10	Lead (Pb)	mg/kg	10 BPOM No 12 (2014)	Not detected
11	%	0.33	(PerBPOM No 12 (2019)	Not Detected
12	Yeast	Colony Mold Number/g	10^3 BPOM No 12 (2014)	< 10
13	Total plate count	Colony Plate Number/g	10^3 BPOM No 12 (2014)	< 10
14	Solvent ethanol	%	1% BPOM No 17 (2019)	0.005

to a constant weight and then calculate the residual amount, which is then expressed as a percentage. The drying shrinkage result was 6.49% when calculating the cantigi leaf extract's drying shrinkage parameter. This heating has the potential to cause the mass of water molecules, essential oils, and ethanol solvents to be lost[11].

Mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb) were not found at the time of testing, according to the results of the study of cantigi leaf extract for these elements. (Pb). Heavy metals are examined since there should be a limit on their presence in the body due to the neurotoxicity and poisoning they may cause. Cantigi leaf extract may contain heavy metals that come from the environment where the original plant grows or that are created during the production process. If heavy metals are found to build in the body, they can impair the kidneys, nerves, and lungs. The levels of mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb) are 0.5 mg/kg, 5 mg/kg for arsenic (As), 0.3 mg/kg for cadmium (Cd), and 10 mg/kg for lead (Pb) according to BPOM No. 12 (2014)[11,15].

In testing the hydroquinone content of cantigi leaf extract, hydroquinone not detected in the extract. Some aglycones of natural glycosides contain a characteristic content of phenolic compounds. In other Ericaceae plants, hydroquinone is produced as the aglycone[16].

Based on the results of the microbial contamination test with most probable number and yeast parameters <10, it was found that the microbes in the cantigi leaf extract were both bacteria and fungi or fungi. Testing for microbial contamination is done to ensure that the extract doesn't have more microorganisms than allowed. The stability of the extract can be impacted by high levels of microbial contamination in the extract[17,18].

By employing liquid gas chromatography to measure the residual percentage of ethanol solvent, it was discovered that the thick extract of cantigi leaves contained 0.005% of ethanol solvent. The inspection's findings are in compliance with the 1.0% maximum residual solvent limit[15]. Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products [19,20].

CONCLUSION

Vaccinium varingiaefolium Miq., the source of cantigi leaf extract, satisfied the standardization of extract quality, according to the study's findings.

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