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Original Article

Standardization Extracts and Simplicia of Limau Sundai Peel (*Citrus x aurantiifolia* 'sundai'), Determine Content of Nobiletin and Antibacterial Activity Test

Elidahanum Husni*, Friardi Ismed, Awaliana

ABSTRACT

Introduction: One type of oranges typical of West Sumatra, which is widely used as traditional medicine is sundai lime (*Citrus x aurantiifolia* 'sundai'); **Aims:** therefore, it is necessary to standardize extracts and Simplicia, determine the content of nobiletin, and antibacterial activity test. **Methods:** The standardization method was used refer to Farmakope Herbal Indonesia. TLC Densitometry was used to determine the content of the nobiletin, and the diffusion method to antibacterial activity test. To get a good standardization, the sundai lime was taken from three regions: Bukittinggi, Pariaman, and Solok. **Results**: From these three regions, conclusions drawn from the macroscopic fruit peel slices were uneven and had distinctive odors. The outer surface is brown, and the inner surface is yellowish-white. From the microscopic was identified fragments in sundai lime peels consisting of hair covering, ladder-shaped transport, parenchyma with secretion cells, oxalate crystals, and parenchyma tissue and stomata. Water-soluble extract content of Simplicia ≤ 24.90 %, and ethanol-soluble extract content ≤ 17.66 %. Non-specific parameters are loss on drying ≤ 5.65 %, total ash content ≤ 5.14 %, and acid insoluble ash content ≤ 0.80 %. The specific parameters were crude extract, black, characteristic odor, Rf of nobiletin was 0.75. Rendement extract ≥ 18.80 %. Non-specific parameters of extract were water content ≤ 18.37 %, total ash content ≤ 3.93 %, and non-acidic ash content ≤ 0.27 %. The nobiletin content in the sundai lime extract Pariaman was 0.33 %, Solok 0.59 %, and Bukittinggi 0.47 %. The antibacterial test with diffusion method in three regions has moderate activities as concentrations of 20% and 15%. **Conclusion:** Sundai lime had Antibacterial activity.

Key words: Standardization, Sundai lime peel fruit, *Citrus x aurantiifolia*('sundai'), Nobiletin, TLC Densitometry, Antibacterial activity.

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INTRODUCTION

Orange is a medicinal plant with high production. Oranges have an essential role in the world market and the country, both in fresh and processed form. Based on data from the Food and Agriculture Organization (FAO), the prospects for the development of Indonesian oranges in the ASEAN arena are quite good, considering Indonesia is a country with the largest harvest area and production for oranges in ASEAN[1]. The pulp of citrus fruit is the most widely used part, whether it is consumed directly (sweet orange), made for juice (muskrat), preservatives (lime), and the leaves as a cooking spice (kaffir lime)[2]. The use of citrus fruit peels is still very little, even though the orange peels' chemical content has higher biological effectiveness than the edible parts. Polyphenol compounds are secondary metabolites of oranges that contain many biologically active compounds, such as anti-inflammatory, anti-microbial, cardioprotective, neuroprotective, anti-adipogenesis, anti-diabetes, hepatoprotective, etc[3].

Sundai lime is a cross between *Citrus aurantiifolia* and *Citrus hystrix*. The identification results obtained from the Herbarium of Andalas University said that the sundai lime has mixed characteristics of the two parents, with the lime's features being more dominant. Data related to sundai lime is very minimal, but in a review of the Citrus clan in the Madura area by Arifin Surya, the juice of sundai lime is traditionally used by the surrounding community as a cough, medicine, and cooking spices[4]. Harrumi Novita said in the Solok sundai lime leaf used in traditional medicine[5]. Due to the large availability of sundai lime in West Sumatra and the lack of data related to sundai lime with wide use, researchers are interested in conducting this research. In general, the orange group contains quite flavonoids and has bioactivity. One of these flavonoids is nobiletin[6].

Polyphenol compounds that are usually found in citrus fruits are flavonoids such as flavanone aglycone (hesperitin, naringenin), flavone aglycone (acacetin, quercetin, diosmetin), polymethoxyflavones (quercetogetin, nobiletintangeretin), flavanone-O-glycosides (neohesperidin), , and flavone-C- and flavone-Cglucoside[3]. Each plant has different levels and compositions so that in its use as a medicinal raw material, it is necessary to characterize and standardize both extracts and its simulations, including citrus. One of the typical oranges and is widely used in West Sumatra but there is no standardization in the Indonesian Herbal Pharmacopoeia is sundai lime, so it is necessary to standardize it so that its use as a medicinal raw material can be developed again. For standardization, samples were taken from three regions, namely Pariaman, Bukittinggi, and Solok.

Nobiletin is a methoxy flavone class of flavonoids that has activity as anti-carcinogenic, anti-inflammatory, antidiabetic[7], anti-cancer, antiviral[6], antibacterial[8].

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One of the essential parts of standardization is the determination of levels. One of the compounds that are likely to be present in sundai lime is nobiletin, so it is necessary to determine how much nobietin is in the sundai lime peel extract. The determination of nobiletin levels used the TLC - Densitometry method following the Indonesian Herbal Pharmacopoeia provisions.

The flavonoids in oranges generally have anti-microbial activity; therefore, it is necessary to test the sundai lime peel extract to determine how significant its antibacterial effect is. The method used is the diffusion method with the test bacteria *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas auruginosa* ATCC 27853.

MATERIAL AND METHOD

Place and time

The research conducted in five months in February-September 2020. The preparation and extraction of sundai lime peel to determinating the standardization from sundai lime peel's extract were conducted at Sumatra Biota Laboratory Andalas University.

Tools and materials

The tools used in the research were Rotary evaporator (Buchi®), vortex (Etech®), sonicator (Elma®), filter paper (Whatman ™), analytical scales (Kern®), hot plates, cover and slide glass curs, vaporizer cup, desiccator, chromatographic vessel, aluminum foil, microscope (Bel Engineering®), Laminar Air Flow (LAF) (Biobase®),, measuring flask (Pyrex®), measuring cup (Pyrex®), micropipettes (Biokit proline®), UV lamps 254 and 366 nm, UV-Vis spectrophotometer (Shimadzu®), capillary tube, TLC Scanner (Camag®), corn yarn, grinder, cotton, oven (Memmert®), furnaces (1500 furnaces Barnstead thermolyne®), tweezers, paper discs, autoclaves (All American®), magnetic stirrers, ose needles, long bars, Petri dishes, spatel, gauze, plastic wrap, spiritus lamps, standard glassware.

The materials used in the research were sundai lime peel, aqua dest, nobiletin, chloroform (Merck®), toluene (Merck®), ethyl acetate (Merck®), chloral hydrate, formic acid (Merck®)), citroborate, hydrochloric acid (Merck®), Nutrient Agar (NA) (Merck®), Ethanol (Merck®), dimethyl sulfoxide (DMSO Silica gel 60 F254 plate (Merck®), physiological NaCl, Mc Farland Solution,), Test microbes: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas auruginosa* ATCC 27853

Extracting

The simplicia powder of sundai lime peel was extracted by maceration method using ethanol 70%. Ethanol is added in a ratio of 1: 10^9. Then filtered using filter paper and the filtrate was concentrated using a rotary evaporator. The rendemen obtained is the weight percentage (w/w) between the extract obtained and the weight of the simplicia used.

Simplicia and extract standardization

1. Macroscopic properties:

Identifying simplicia include color, shape and smell

2. Microscopic characterizations:

Identifying the simplicia powderidentification fragment using chloral hydrate reagents with magnification 400x.

3. Loss on drying:

1-2 g of simplicia powder is put into a weighed bottle that has been tared. Flatten the simplicia powder in a bottle with a thickness of 5-10 mm then weigh it. Then dried at 105 ° C in an open container using an oven until the weight remains[10].

4. Total Ash:

2-3 g of simplicia or extract is put into the tapered rate, then glow the sample until the charcoal runs out. If the charcoal can't be removed, wash it with hot water and filter it with ash-free filter paper. The filtrate and filter paper are input in the same exchange, then incandescent with a temperature of 800 ± 25^{0}C until the weight remains. Cool in a desiccator with a closed container and weigh the ash that has been obtained from annealing. Ash content is expressed in% w / w[10].

5. Acid-insoluble ash:

The ash obtained from the total ash content determination is boiled with 25 mL dilute hydrochloric acid for 5 minutes. Collect the insoluble part of the acid, filter it using an ash-free filter paper, wash it with hot water, glow the ash with a temperature of 800 ± 25^{0}C until fixed weight. Acid insoluble ash content is expressed in% w / w[10].

6. Ethanol-soluble Extract:

Simplicia powder of approximately 5 g which has been dried in air. Place in a clogged flask, add 100 mL of ethanol, repeatedly shake for the first 6 hours and leave for 18 hours. Then filter and evaporate 20 mL of the filtrate to dry in a steaming cup that has been tared at 105 ° C until the weight remains[10].

7. Water-soluble Extract:

Simplicia powder of approximately 5 g which has been dried in the air. Put in a clogged flask, add 100 mL of chloroform saturated water, repeatedly shake for the first 6 hours, and leave for 18 hours. Then filter and evaporate 20 mL of the filtrate to dry in a steaming cup that has been tared at 105 ° C until the weight remains[10].

8. Moisture content:

The extract, which is estimated to contain 1-4 mL of water, is weighed and then put into a dry flask. For substances that can cause sudden fluctuation when boiling, add boiling stone. Put about 200 mL of saturated toluene water into the flask, attach the set of tools. Heat the squash gently for 15 minutes. After the toluene has boiled, adjust the distillation to approximately 2 drops per second until most of the water is distilled and increase the distilling speed to 4 drops per second. After all the water has been distilled, the inside of the condenser is washed with water-saturated toluene while cleaning it with a tube brush attached to a copper wire and moistened with water-saturated toluene. Continue to distill for 5 minutes. Cool the receiving tube to room temperature. If water drops adhere to them, scrub the cooling tube and receiver tube with a rubber band tied to a copper wire and moistened with water-saturated toluene until the water drops drop. Read the volume of water after the water and toluene have completely separated. Water content is calculated in% v / w[10].

Quantification of hesperidin by TLC-densitometry

1. Validation of Nobiletin Content

a. Accuracy

The accuracy is marked on the TLC plate, eluted with the optimal mobile phase, measured three times repetition. The calculated value is recoverability.

b. Precision test

The precision test uses three concentrations using three concentrations, namely 20, 50 and 80 µg / ml. Then the% RSD is calculated

c. Linearity

A good linearity value is r> 0.99

d. Limit of Detection (LoD) and Limit of Quantification (LoQ)

LoD and LoQ equations. LoD = 3,3 SD / slope LoQ = 10SD / slope [11].

2. Calibration Curves

Perform TLC work on comparators made in a concentration of 20,30,50,60,70,80 (µg / ml) bottles with 1 µl each volume. The TLC plate was then scanned using a densitometer with a maximum nobiletin absorption wavelength of 334 nm. The curve between the concentration and AUC formed must be linear (r> 0.99) and determine the regression equation.

3. Determination of Nobiletin Contents

The TLC plate had a sample eluted with a concentration of 1% and the comparison was scanned using a densitometer at the maximum nobiletin absorption wavelength. Then the histogram area data obtained from the nobiletin compound in the test solution (y). The histogram area data of the test compounds were entered into the regression equation y = a + bx. The compound (x) content can be determined.

Antibacterial activity assay for diffusion method[12]

1. Preparation of Nutrient agar (NA) media:

Nutrient agar powderas much as 20 grams dissolved with 1 L aqua dest in Erlenmeyer,then heated on a hot plate using a magnetic stirrer to form a clearsolution. Next, it was sterilized in an autoclave at 121 ° C with apressure of 15 lbs for 15 minutes.

2. Preparation of microbial suspensions:

As much as one microbialtest loop was taken from pure culture and suspended into sterilephysiological NaCl, then homogeneous using a vortex. The suspension's turbidity is compared using the standard 0.5% Mc-Farlandon a black background and bright light. A 0.5% Mc-Farland strengthstandard was created by adding 99.5% mL of 1% H_2SO_4 to 0.5mL of 1.175% $BaCl_2$ solution. The test extract was dissolved withDMSO solution with concentrations of 15 and 20%. A total of 100µL of the microbial test suspension was inserted into the petri dish and then added with enough NA media to cover the lower surface of the petri dish and then homogenized. After the solid media isplaced, a sterile blank disc has dropped 10 µL of the testsolution. For positive control, chloramphenicol is used. While thenegative control was used, a sterile blank disc dropped by 10 µLDMSO. The Petri dishes were incubated at 35-37°C for 18-24 hours.

Antibacterial activity is positive if there is a barrier zone in a clear area around the blank disc. Barriers are measured usingcalipers. The smallest concentration that still shows the clear area isused to determine the Minimum Inhibitory Concentration (MIC).All tests were done in triplicate.

RESULT AND DISCUSSION

The first step is carried out in simplicia, samples from the three wet sorted areas to separate the sundai limes from dirt or foreign materials. Then the sundai lime is peeled to separate the skin from the flesh. Furthermore, the skin of the sundai lime is washed in a short time and chopped. Chopping that is too thin can cause the loss of nutritious substances that are volatile, while chopping that is too thick can slow down the drying process and provide opportunities for the growth of fungi in the simplicia. Therefore the simplicia is chopped not too thick and thin[9]. The macroscopic simplicia of fruit peel slices was uneven and had distinctive odors. The outer surface is brown, and the inner surface is yellowish-white—microscopic observation to identifying fragments from the simplicia of finely ground sundai lime. The simplicia powder was observed using chloralhydrous reagent with a magnification of 400x.

From the microscopic was identified fragments in sundai lime peels consisting of hair covering, ladder-shaped transport, parenchyma with secretion cells, oxalate crystals, and parenchyma tissue and stomata. We can see at figure 1. How many of these identifying fragments are also present in other citrus species, like *Citrus aurantiifolia*[10], *Citrus jambhirii* Lush[13], *Citrus micocarpa* Bunge[14].

The next specific parameter of standardization is water-soluble content. Determination of water-soluble content describes the amount of compound content in simplicia which is polar or has a polarity similar to water. The filtrate is evaporated in an evaporating cup to form an extract, then heated in an oven for 105^{0}C until the change in weight is not more than 0.25%. The solvent used is water-saturated chloroform; chloroform functions to attract non-polar impurities. The compounds found in the water are polar compounds that are impurity-free. The data contained in table 1 shows that the water-soluble extract content in Pariaman is 24.90 ± 0.45%, Bukittinggi 23.13 ± 0.68%, and Solok is 23.18 ± 0.58%. From the three samples' data, it can be concluded that the water-soluble extract content in the simplicia of Sundai lime peel is not less than 23.12± 0.58 %.

The determination of ethanol-soluble extract illustrates the number of compounds that are soluble in ethanol. In terms of quality, the determination of ethanol-soluble extract content is almost the same as water content determination. Table 1 shows that the content of ethanol-soluble extract is smaller than that of water, this shows that the compounds in the skin of sundai lime contain more polar compounds than semipolar non-polar. Ethanol soluble extract content in Pariaman was 17.66 ± 0.21%, Bukittinggi 15.70 ± 0.96%, and Solok 16.77 ± 0.70%. From the data obtained from the three regions, the ethanol-soluble extract content in the simplicia of sundai lime skin is not less than 15.70 ± 0.96%.

Loss of drying is one of the non-specific parameters that limit the amount of compound lost in the drying process. The loss on drying parameter is the measurement of the remaining substance after drying at a temperature of 105^{0}C to constant weight. Loss on drying in some conditions is often equated with moisture content. The sample used does not contain volatile substances such as essential oils. This method is called the gravimetric method. However, because citrus peels in general and sundai lime contain volatile substances, the drying loss in simplicia of sundai lime peels only describes the amount of compounds lost in the heating process. It does not represent the moisture content of the sympathizers. Table 1 shows that the largest drying loss value is found in simplicia from Solok amounting to 5.65 ± 0.80%, then Pariaman at 5.45 ± 0.69%, and Bukittinggi simplicia at 4.83 ± 0.28%.

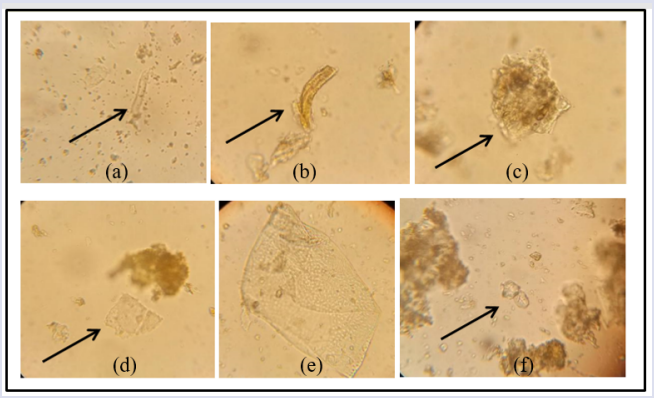

Figure 1: (a) Hair covering, (b) ladder-shaped transport, (c) parenchyma with secretion cells, (d) oxalate crystals, (e)parenchyma tissue and (f) stomata.

Table 1: Standardization simplicia.

Sample	Standardization Parameter	Result %		
		Bukittinggi	Pariaman	Solok
Simplicia	Specific Parameters			
	Water soluble extract	23.13 ± 0.68	24.90 ± 0.45	23.12 ± 0.58
	Ethanol soluble extract	15.70 ± 0.96	17.66 ± 0.21	16.77 ± 0.70
	Non Specific Parameters			
	Loss on drying	4.83 ± 0.28	5.45 ± 0.69	5.65 ± 0.79
	Total ash content	4.96 ± 0.43	4.23 ± 0.08	5.14 ± 0.04
	Acid insoluble ash content	0.76 ± 0.06	0.72 ± 0.03	0.80 ± 0.12

Table 2: Standardization extract.

Sample	Standardization Parameter	Result %		
		Bukittinggi	Pariaman	Solok
Extract	Specific Parameters			
	Nobiletin Content	0.47 ± 0.45	0.33 ± 0.24	0.59 ± 0.16
	Non Specific Parameters			
	Rendemen	20.83	21.08	18.80
	Water content	16.73 ± 0.99	15.63 ± 0.95	18.38 ± 0.86
	Total ash content	3.60 ± 0.66	3.87 ± 0.76	3.93 ± 0.92
	Acid insoluble ash content	0.26 ± 0.04	0.26 ± 0.03	0.27 ± 0.08

Loss on drying value will affect the storage time of the simplicia. The smaller the value, the less likely substances such as water and volatile substances are so that the growth of microorganisms during the storage process can be avoided. From the data of the three regions, it can be concluded that the drying loss of sundai lime peel simplicia is not more than 5.65± 0.80%.

The determination of the ash content aims to provide an overview of the internal mineral content. The extract is heated at a high temperature until the organic compounds and their derivatives are digested and evaporated until the mineral and inorganic elements remain. Complete combustion of organic compounds will produce CO_2 and H_2O, while inorganic compounds will produce ash. This combustion process is called combustion, which uses a furnace with a very high temperature of 800^{0}C. Ash is a metal oxide which is a residue or combustion residue. The ash content's determination aims to determine the total inorganic compound content in the form of metal oxides [15]. The filter paper used in the processing of the ash content is Whatman filter paper no.40. Whatman paper has a relatively small ash content. If it is annealed again with simplicia ash, it will not have a significant effect, while ordinary filter paper, if it is annealed, will leave ash; this will affect the data's accuracy. From the data for the three regions in table 1, the total ash content in the sundai lime skin is not more than 4.23 ± 0.08%. As with simplicia, the ash content in the extract also needs to be determined because the minerals and inorganic metals found in high extracts can accumulate in the body if consumed continuously. Metals that accumulate in the body will make the kidneys work harder and disrupt the body's physiological functions. From table 2, it can be concluded that the total ash content in the sundai lime extract is not more than 3.93 ± 0.92%.

Acid insoluble ash content reflects mineral or metal contamination that is insoluble of acid in simplicia. Acid insoluble ash content is one of the criteria in determining the level of cleanliness in the processing of a product. Acid insoluble ash is reflected by the presence of mineral or metal contamination that is not acid soluble in a product. Insoluble levels in acids usually contain silicates derived from soil, environment, or sand. The amount of dirt, soil, clay and metal elements Ag, Pb and Hg[15]. Determining the acid insoluble ash content is a continuation of the determination of the total ash content. The rest of the entire ash content is boiled with dilute HCl for 5 minutes, then filtered with Whatman filter paper, then the part that does not dissolve the acid along with the filter paper is annealed until constant weight. Like the total ash content in the acid-insoluble ash content, the acid-insoluble simplicia ash content from Solok has a high value compared to the other two regions (table 1). The poor sorting process can cause this. From the data of the three regions in table 1, the acid insoluble ash content in the sundai lime skin is not more than 0.72 ± 0.04%. From the data in table 2 it can be concluded that the acid insoluble ash content in the sundai lime peel extract is quite high, but from these data it can also be concluded that the acid insoluble ash content of the ethanol extract sundai lime is not more than 0.268 ± 0.08%.

The extraction process from simplicia uses the maceration method with 70% ethanolThe rendemen obtained with a weight of 400 grams of dry simplicia from the three regions, namely Pariaman: 21.08%, Bukittinggi: 20.832%, Solok: 18.80%.

Determination of moisture content is very important in ensuring the drug's quality because high water content will increase humidity and increase the risk of unwanted microorganism growth. A good extract is an extract that has a small moisture content. According to the 2014 BPOM Regulation, a good moisture content for extracts used in herbal medicinal preparations is ≤ 10%[16]. Therefore, to be used as raw material for traditional medicine, the ethanol extract of sundai lime peel must undergo treatment first because the levels exceed the provisions (table 2). Sundai lime extract is a thick extract with a moisture content of 5 - 30%[17]. Determination of the moisture content of this sundai lime extract using a distillation method with toluene. Toluene was chosen as a solvent because of the immiscible nature of toluene and had a lighter specific gravity than water, making it easier to observe the volume of water read in this process. The boiling point of toluene is also higher than water, which is 110 ^{0}C, this causes the water to completely evaporate and be cooled by the condenser so that the water content determination of the extract is accurate. This method was chosen because it is easiest to perform than the Karl Fischer titration and more accurate than the gravimetric method. The data obtained from the three regions, it can be concluded that the water content in the sundai lime peel extract is not more than 18.38 ± 0.86%.

The specific parameters of extract were crude extract, black, characteristic odor. TLC profile is one of the specific parameters that is

important for extracts. TLC profile can describe the components of the compounds contained in the extract. Figure 2 shows the TLC profile of the ethanol extract of sundai lime peel with a nobiletin comparison using chloroform: ethyl acetate (7: 3) eluent and a few drops of formic acid. The eluent is used because the compounds in the extract are semipolar and non-polar, such as nobiletin. While the ratio of 7: 3 was chosen because, in that comparison, the separation between the stains on TLC was visible. The addition of formic acid to the eluent serves to minimize tailings when eluting the compound. The addition of acid will reduce the interaction between the phenol groups present in the flavonoids and the silanol groups in the silica gel stationary phase. [18] The stain used was citroborate, which is a specific appearance for the flavonoid class. After elution and drying, the TLC plate was sprayed with the appearance of citroboric stains (5 grams of citric acid and 5 grams of boric acid in 100 ml of ethanol), then heated in an oven at 105^{0}C and observed under uv 365 nm[10].

Nobiletin has effectiveness as antiviral, anti-inflammatory[6], anti-cholesterol, antibacterial[8], antidiabetic, neuroprotective, as well as anti-teratogenic and anti-cancer[19]. To determine the purity and levels of nobiletin, HPLC and TLC Densitometry can be used. In this research, nobiletin level determination used TLC Densitometry. For sample preparation, 50 mg of the extract is dissolved with 5 ml of methanol in a volumetric flask, so that the final concentration is 10,000 µg / ml. The comparison is 5 mg nobiletin dissolved with 5 ml methanol, so that the concentration is 1000 µg / ml, then dilution is done so that the concentration is 20 µg / ml, 30 µg / ml, 50 µg / ml, 60 µg / ml, 70 µg / ml and 80 µg / ml. The elluent used was chloroform: ethyl (7: 3) and then added a few formic acid drops. The volume of the sample that was bottled was 1 µl, with three repetitions for each concentration (triplo). The sampling is carried out from small concentrations to large concentrations to be more accurate. After the pinching process, the plates are dried first and then eluted in a vessel containing the eluent and are already saturated. The plates were then scanned using a TLC Scanner with a maximum wavelength of 334 nm nobiletin[20].

The AUC of nobiletin is then plotted so that a linear regression equation is obtained. According to AOAC, the regression equation is said to be linear if the coefficient of correlation is> 0.99[21]. The nobiletin correlation coefficient obtained is 0.9971 (Figure 3) and meets the specified criteria.

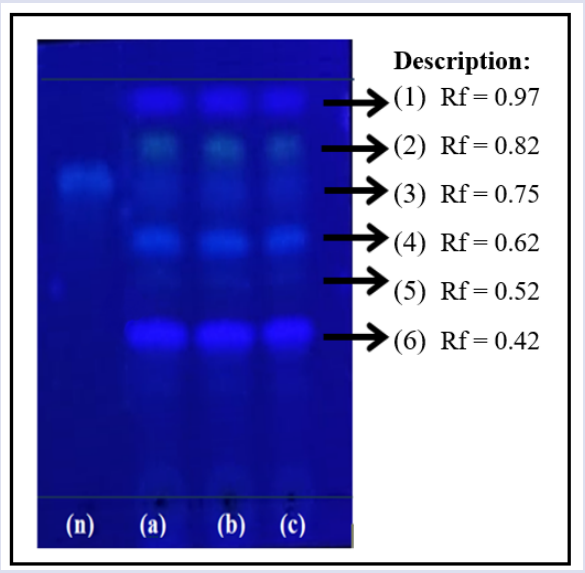

Figure 2: Profile TLC (a) Extract from Bukittinggi, (b) Extract from Pariaman, (c) Extract from Solok, and (n) Comparrison : Nobiletin.

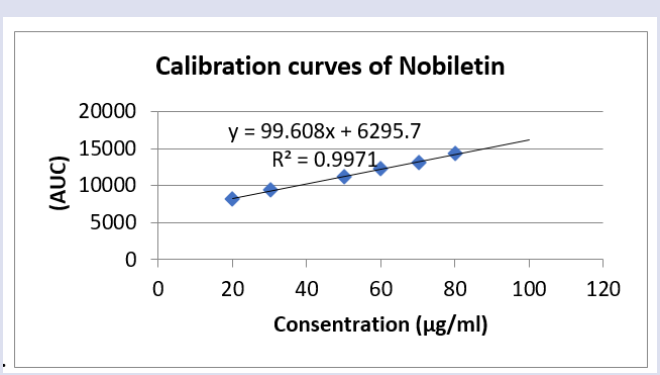

Figure 3: Calibration curves of nobiletin.

The regression equation obtained from the calibration curve (Y = 99.609X + 6295.7) can be used to determine the nobiletin content in the extract. From the results of these calculations, it can be seen (table 2) that the nobiletin content in the sundai lime peel extract from Bukittinggi is 0.47%, Pariaman 0.33% and Solok is 0.59%. The difference in nobiletin levels is influenced by the soil nutrients where the plants are grown, the climate and weather for each area. From all the data (table 3), the method of determining the nobiletin content in the sundai lime peel extract is valid.

One of the biological activities that the genus Citrus usually has is its antibacterial activity. Therefore, screening for the antibacterial activity of the sundai lime peel extract is carried out. The method used is the diffusion method using discs because it is relatively easy and fast in processing. The assessment results are carried out based on the clear inhibition zone measured using a caliper. The clear inhibition zone is a transparent/clear area because anti-microbials (bacteria, fungi or viruses) cause ring-like formation, which is an obstacle in the dense bacterial growth area. No bacteria grow in the ring. According to David and Stout (1971) method, anti-microbial activity is grouped where the inhibition zone diameter ≥ 20 mm indicates extreme activity, 10-20 strong, 5-10 moderate, and ≤ five weak[24].

The four bacteria were chosen because of the large number of resistant cases of this type of bacteria. Chloramphenicol 0.3% was used as a positive control because chloramphenicol antibiotics have a broad spectrum. DMSO is used for positive control because DMSO is a solvent used to dilute extracts and does not have antibacterial activity. It can be seen in Table 4 that the largest inhibition zone of the extract is 7.6 mm, and the smallest is 6.2 mm. This shows that the antibacterial activity is moderate with a concentration of 20% and 15%.

In this study, only screening was carried out on the sundai lime peel extract, to determine whether there was antibacterial activity in the extract. After doing the antibacterial test, it was found that the extract had antibacterial activity. This is thought to be due to the flavonoid content in sundai lime. One of the flavonoids reported to have antibacterial activity is nobiletin. In his research, Johann (2007) proved that the flavonoids nobiletin and tangeretin had activity against *S.aureus* and *E. coli* bacteria with an MIC of 500 µg / ml. [8]. Therefore, it is better to determine the compounds contained in sundai lime extract and determine their antibacterial activity.

CONCLUSION

To get a good standardization, the sundai lime was taken from three regions: Bukittinggi, Pariaman, and Solok. From these three regions, conclusions drawn from the macroscopic of fruit peel slices were uneven and had distinctive odors. The outer surface is brown, and the inner surface is yellowish-white. From the microscopic was identified fragments in sundai lime peels consisting of hair covering, ladder-

Table 3: Validation of Determine nobiletin content.

Parameter	Requirements	Result
Liniearitas	coefficient of correlation> 0,99 [21]	Regression equation Y = 99.609X + 6295.7 r = 0.9971
LOD (Limit Of Detection)	3,3 x SD / slope	5.2686 µg
LOQ (Limit Of Quantification)	10 x SD / slope	15.9654 µg
Intra day precision	3 standard concentrations made by triplo, for small compound levels of RSD <15%[22]	Concentration 20 µg / ml: 19.48 µg, RSD 3.45% Concentration 50 µg / ml: 46.85 µg, RSD 3.13% Concentration 80 µg / ml: 7.,28 µg, RSD 0.74%
Accuration	Data taken at least three concentrations with three repetitions, and calculated as the percentage of recovery [23] Recovery for a 0.01% concentration is 85 - 110%[21]	Concentration 20 µg / ml Recovery: 97.42% Concentration 50 µg / ml Recovery: 93.70% Concentration 80 µg / ml Recovery: 99.11%

Table 4: Inhibition zone of extract.

Bacteria	Inhibition zone (mm ± SD)						
	Bukittinggi		Pariaman		Solok		K + (Chloramphenicol)
	20%	15%	20%	15%	20%	15%	
E. coli ATCC 25922	6,7± 0,17	6,3 ± 0,1	6,63± 0,21	6,23 ± 0,23	7,33 ± 0,31	6,33 ± 0,12	24 ± 0.0984
E. faecalis ATCC 12228	6,73 ±0,12	6,4 ± 0,1	6,83± 0,06	6,5 ± 0,1	7,53 ± 0,06	6,63 ± 0,15	
P. aeruginosa ATCC 27853	6,8 ± 0,1	6,43 ± 0,06	6,5 ± 0,1	6,23 ± 0,12	7,6 ± 0,1	6,67 ± 0,15	
S. aureus ATCC 25923	6,7 ± 0,1	6,27 ± 0,06	6,47± 0,06	6,17 ± 0,06	7,7 ± 0,1	7,53 ± 0,15	

shaped transport, parenchyma with secretion cells, oxalate crystals, and parenchyma tissue and stomata—water-soluble extract content of simplicia ≤ 24.90 %, and ethanol-soluble extract content ≤ 17.66 %. Non-specific parameters are loss on drying ≤ 5.65 %, total ash content ≤ 5.14 %, and acid insoluble ash content ≤ 0.80 %. The specific parameters were crude extract, black, characteristic odor, Rf of nobiletin was 0.75. Rendement extract ≥ 18.80 %. Non-specific parameters of extract were water content ≤ 18.37 %, total ash content ≤ 3.93 %, and non-acidic ash content ≤ 0.27 %. The nobiletin content in the sundai lime extract Pariaman was 0.33 %, Solok 0.59 %, and Bukittinggi 0.47 %. The antibacterial test with diffusion method in three regions has moderate activities as concentrations of 20% and 15%.

SUMMARY

Research on the Standardization Study of Simplicia and Extract of Sundai lime (*Citrus x aurantiifolia* 'sundai')) Peel, Quantification of Nobiletin and Antibacterial Assay was carried out. Sundai lime had Antibacterial activity.

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CONFLICTS OF INTEREST

The author(s) declare(s) that there is no conflicts of interest regarding the publication of this article.

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GRAPHICAL ABSTRACT

SIMPLICIA

EXTRACT

Sample	Standardization Parameter	Result %		
		Bukittinggi	Pariaman	Solok
Simplicia	Specific Parameters			
	Water soluble extract	23.13 ± 0.68	24.90 ± 0.45	23.12 ± 0.58
	Ethanol soluble extract	15.70 ± 0.96	17.66 ± 0.21	16.77 ± 0.70
	Non Specific Parameters			
	Loss on drying	4.83 ± 0.28	5.45 ± 0.69	5.65 ± 0.79
	Total ash content	4.96 ± 0.43	4.23 ± 0.08	5.14 ± 0.04
	Acid insoluble ash content	0.76 ± 0.06	0.72 ± 0.03	0.80 ± 0.12

Sample	Standardization Parameter	Result %		
		Bukittinggi	Pariaman	Solok
Extract	Specific Parameters			
	Nobiletin Content	0.47 ± 0.45	0.33 ± 0.24	0.59 ± 0.16
	Non Specific Parameters			
	Rendemen	20.83	21.08	18.80
	Water content	16.73 ± 0.99	15.63 ± 0.95	18.38 ± 0.86
	Total ash content	3.60 ± 0.66	3.87 ± 0.76	3.93 ± 0.92
	Acid insoluble ash content	0.26 ± 0.04	0.26 ± 0.03	0.27 ± 0.08

Bactery	Inhibition zone (mm ± SD)						
	Bukittinggi		Pariaman		Solok		K + (Kloramfenikol)
	20%	15%	20%	15%	20%	15%	
E. coli ATCC 25922	6,7± 0,17	6,3 ± 0,1	6,63± 0,21	6,23 ± 0,23	7,33 ± 0,31	6,33 ± 0,12	24 ± 0.0984
E. faecalis ATCC 12228	6,73 ±0,12	6,4 ± 0,1	6,83± 0,06	6,5 ± 0,1	7,53 ± 0,06	6,63 ± 0,15	
P. aeruginosa ATCC 27853	6,8 ± 0,1	6,43 ± 0,06	6,5 ± 0,1	6,23 ± 0,12	7,6 ± 0,1	6,67 ± 0,15	
S. aureus ATCC 25923	6,7 ± 0,1	6,27 ± 0,06	6,47± 0,06	6,17 ± 0,06	7,7 ± 0,1	7,53 ± 0,15	

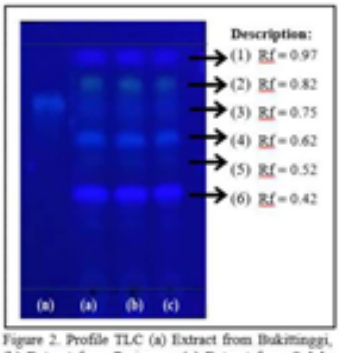

Figure 2. Profile TLC (a) Extract from Bukittinggi, (b) Extract from Pariaman, (c) Extract from Solok, and (n) Comparison : Nobiletin

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