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STANDARDIZATION OF SIMPLICIA AND LIME PEEL EXTRACT

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ABSTRACT

Lime (*Citrus aurantifolia Swingle*) is one of the plants that can be used as traditional medicine. Parts of the lime plant that can be used as traditional medicine include the fruit, leaves, and peel. Lime peel waste is known to contain chemical compounds and as a raw material for medicine, standardization is needed to ensure its quality. The 70% ethanol extract of lime peel was made using the maceration extraction method. This study aimed to standardize both simplicia and 70% ethanol extract of lime (*Citrus aurantifolia Swingle*) peel according to the Indonesian Herbal Pharmacopoeia (2017) guidelines to ensure quality for pharmaceutical raw material applications.Lime peel was processed into simplicia and extracted using maceration with 70% ethanol. Standardization was performed on both simplicia and extract based on specific (organoleptic, phytochemical screening) and non-specific parameters (drying loss, water content, total ash, acid-insoluble ash, ethanol-soluble extract, water-soluble extract, and specific gravity). All analyses were performed in triplicate ($n = 3$) and statistical significance was tested using SPSS version 26 with a confidence level of 95% ($p < 0.05$).Both simplicia and extract met the pharmacopoeial requirements and can be recommended as standardized raw materials for herbal pharmaceutical formulations. These findings provide a reference for the quality control of herbal raw materials and promote sustainable utilization of lime peel waste.

Keywords: extract, lime peel, raw materials for medicine, simple medicine, standardization.

INTRODUCTION

Herbal medicines are widely used as alternatives or complements to conventional therapies. Standardization of herbal raw materials is essential to ensure quality, safety, and efficacy (WHO, 2023). Lime (*Citrus aurantifolia* Swingle) peel, a by-product of the juice industry, has demonstrated significant pharmacological properties including antioxidant and antimicrobial activities (Smith et al., 2024). However, variability in the chemical composition and quality parameters of herbal materials can affect their therapeutic performance. International guidelines, such as those from USP (2023) and WHO (2023), emphasize stringent quality control for herbal products to maintain consistency and efficacy.

Lime (*Citrus aurantifolia* Swingle) is a plant widely used by the community as a traditional medicine. Lime is reported to contain beneficial chemical compounds, such as citric acid, resin, glycosides, citric acid, sulfur, flavonoids, amino acids (tryptophan, lysine), vitamin A, vitamin C, vitamin B1, calcium, potassium, phosphorus, iron, copper, essential oils (citral, limonene, fellandren, terpineol, camphene), saponins, hesperidin glycosides, tangeretin, naringin, eriositrin, eriositrocid, alkaloids. Saponins, flavonoids and polyphenols in lime have antiseptic, antibacterial, antioxidant activity and can inhibit bleeding in the skin (Abdullah et al., 2023; Kurniawati et al., 2020; Sari & Asri, 2022; Sari et al., 2021) .

Parts of the lime plant with medicinal properties include the fruit, leaves, and even the peel. Lime peel is a part of the plant that is underutilized. Lime peel is known to contain various secondary metabolites, such as flavonoids, alkaloids, tannins, steroids, and saponins, which are thought to provide antibacterial and antioxidant activity. The concentration of flavonoids in lime peel is higher than in other parts (Ashfia et al., 2019; Wardani et al., 2018; Zhang et al., 2017).

Lime peel can be utilized and processed into raw materials for traditional medicines in the form of simple preparations or extracts. Simple preparations and lime peel extracts are natural ingredients used as raw materials for traditional medicines that require standardization to ensure quality. The quality of simple preparations and extracts must meet the standardization requirements of the Indonesian Herbal Pharmacopoeia. Standardization of simple preparations and extracts can be carried out based on specific and non-specific parameters (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017). The aim of this study was to standardize simplicia and lime peel extracts, so that they can serve as a reference for the quality standards of medicinal raw materials.

METHOD

A. Sample Preparation

Lime peel was collected, dried, and powdered to produce simplicia. Extraction was performed using 70% ethanol by maceration (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017; Sari et al., 2021).

B. Standardization of Simplicia

1. Simplicia Identity

   Identification of simplicia by describing the nomenclature which includes the name of the simplicia, the Latin name, the required plant part, and the Indonesian name (Ance et al., 2019; Issulingtyas et al., 2024; Sari et al., 2024).

2. Macroscopic Test

   Macroscopic tests through direct observation are related to the characteristics of simplicia (Ance et al., 2019; Issulingtyas et al., 2024; Sari et al., 2024).

3. Drying Loss of Simplicia

   The weighing bottle is heated at 105°C for 30 minutes. The weighing bottle is reweighed until a constant weight is obtained or the difference between the two weighings does not exceed 0.005 g. 1 gram of simplicia is placed in the weighting bottle as a test material. The test material is dried at 105°C for 5 hours and then reweighed. The drying process is continued and reweighed for 1 hour until the difference between successive weighings is no more than 0.25%. Good simplicia has a drying loss of ≤ 10% (Ministry of Health of the Republic of Indonesia, 2000). The drying loss can be calculated using the equation below:

   $$\text{Drying Loss (\%)} = \frac{a - b}{b} \times 100\%$$

   Information :
   a = initial weight of the simple substance (g)
   b = final weight of the simple substance (g)

4. Total Ash Content

   Two grams of powdered simplicia was placed into a silica crucible that had been incandescent and tared. The powdered simplicia was incandescent at 600 ° C, then cooled and weighed until a constant weight was obtained. The total ash content was calculated against the initial powder weight in %w/w (Cahyani et al., 2019; Ministry

of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).

5. Acid Insoluble Ash Content
   The ash obtained from the total ash content determination was boiled in 25 mL of dilute hydrochloric acid for 5 minutes. The insoluble part of the acid was collected and filtered using filter paper. The filtrate was heated until a constant weight was obtained, then cooled and reweighed. The acid-insoluble ash content was calculated for the air-dried material (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).
6. Water Soluble Content
   5 grams of simplicia powder was macerated for 24 hours with 100 mL of water-chloroform (2.5 mL of chloroform in distilled water to 100 mL) in a stoppered flask while occasionally shaking for the first 6 hours, then left for 18 hours and filtered. 20 mL of filtrate was evaporated to dryness in a heated and tared flat-bottomed evaporator dish. The remaining filtrate was heated at 105°C until the weight remained constant. The percentage of water-soluble extract was calculated against the air-dried material (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).
7. Ethanol soluble Content
   5 grams of simplicia powder was macerated for 24 hours with 100 mL of 95% ethanol in a stoppered flask while shaking occasionally for the first 6 hours, then left for 18 hours and filtered. 20 mL of the filtrate was evaporated to dryness in a heated and tared evaporator dish. The remaining filtrate was heated at 105°C until a constant weight was obtained. The percentage of soluble extract in 95% ethanol was calculated for the air-dried material (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).

C. Extraction
   Lime peel extraction using the maceration method with 70% ethanol solvent with a ratio of 1:10 (w/v) of simplicia powder: solvent. 100 grams of simplicia powder was put into the macerator, added with 70% ethanol solvent until the simplicia was completely submerged. The simplicia soak was left for 3 x 24 hours and every 24 hours the solvent was replaced with fresh solvent until a clear filtrate was obtained. The solvent was evaporated using a vacuum rotary evaporator until a thick extract was obtained and the yield was calculated. The extract yield was calculated using the following formula (Agustia & Mardiana, 2021; Hindun et al., 2017; Ministry of Health of the Republic of Indonesia, 2017; Sari et al., 2021a) :

$$\%\text{Rendemen} = \frac{\text{Thick extract weight (gram)}}{\text{Simplicia weight (gram)}} \times 100\%$$

D. Extract Standardization
   1. Specific Parameters
      a. Identity
         Extract identity parameters include a description of the extract name, the Latin name of the plant, and the part of the plant used (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).
      b. Macroscopic Test
         Macroscopic test parameters of extracts using the five senses aim to describe shape, color, smell, and taste (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).

c. Qualitative Identification of Chemical Compounds

1) Flavonoid Test
0.5 grams of extract was dissolved in 5 mL of 96% ethanol, then several drops of $FeCl_3$ were addeduntil the color changed to green, red, purple, or dark black (Habibi et al., 2018; Sari et al., 2024) .

2) Alkaloid Test
0.5 grams of extract added with Dragendroff's reagent produces an orange to reddish brown precipitate (Haryati et al., 2015; Sari et al., 2024) .

3) Phenol Test
0.5 grams of extract was dissolved in 5 mL of distilled water and added with 1% $FeCl_3$ reagent until the color changed to blackish green (Haryati et al., 2015; Sari et al., 2024).

4) Saponin Test
0.5grams extract was added with 5 mL of distilled water, then shaken vigorously for 10 seconds to form a stable foam for no less than 10 minutes with a height of 1-10 cm. The extract showed positive results for saponins with the addition of 1 drop of 2N HCl the foam did not disappear (Haryati et al., 2015; Sari et al., 2024).

5) Steroid Test
0.5 grams extract was macerated with 10 mL of n-hexane for 1 hour and filtered. The filtrate was evaporated in an evaporator dish and 1-2 drops of concentrated sulfuric acid were added to the remainder until a green or blue color was formed (Sari et al., 2024; Ulya et al., 2023).

6) Tannin Test
0.5 grams of extract was boiled with 20 mL of water, filtered and a few drops of 1% $FeCl_3$ were added until a greenish brown or blackish blue color was formed (Ikalinus et al., 2015; Sari et al., 2024).

7) Glycoside Test
1 gram of extract dissolved in 5 mL of anhydrous acetic acid and added with 10 drops of sulfuric acid will form a blue or green precipitate (Kumalasari et al., 2018; Sari et al., 2024).

8) Triterpenoid Test
2 mL of the extract was evaporated, then the residue was dissolved with 0.5 mL of chloroform and 0.5 mL of anhydrous acetic acid. 2 mL of concentrated sulfuric acid was added through the wall of the test tube until a brownish or violet ring formed at the border of the solution (Minarno, 2015; Sari et al., 2024).

9) Essential Oil Test
0.5 grams of extract was put into a test tube, adding a few drops of Sudan III reagent until a red solution was formed (Kurnianingsih et al., 2020; Minarno, 2015; Sari et al., 2024).

2. Non-Specific Parameters

a. Extract Drying Loss
A weighing bottle was prepared, heated at 105°C for 30 minutes, and reweighed. One gram of extract was weighed and placed into the weighing bottle. The extract was spread evenly in the weighing bottle by shaking the bottle until a layer 5 mm to 10 mm thick was obtained. The extract was dried at 105°C until a constant weight was achieved. The drying loss can be calculated using the equation below (Marpaung & Septiyani, 2020; Wahyuni & Marpaung, 2020) :

$$\text{Drying Loss (\%)} = \frac{a - b}{b} \times 100\%$$

Information :
a = weight of dry extract (g)
b = weight of wet extract (g)

b. Specific Gravity
A clean, dry, empty pycnometer and a pycnometer filled with water at 25oC are weighed and calibrated (W1). The extract is placed in the empty pycnometer and the filled pycnometer is set to 25°C, then weighed (W2) (Ministry of Health of the Republic of Indonesia, 2000) . The specific gravity calculation is as follows:

$$\text{Specific gravity (d)} = \frac{W2 - W0}{W1 - W0} \times 100\%$$

Information :
W0 = weight of empty pycnometer

W1 = weight of pycnometer + water

W2 = weight of pycnometer + extract

c. Water content
Water content test using toluene distillation method (Azeotropy). The distillation apparatus consists of a 500 mL round bottom flask, a container, a cooler, a connecting tube and a 10 mL receiving tube. Toluene is saturated by adding water and shaking the mixture. The mixture is left to stand until a toluene layer and a water layer are formed. 200 mL of saturated toluene is put into the round bottom flask. 5 grams of extract is put into the round bottom flask that has been given 200 mL of saturated toluene, then heated for 15 minutes. The drip rate is set at 2 drops per second until most of the water is distilled. The distillation rate is increased to 4 drops per second until all the water is distilled. The receiving tube is allowed to cool to room temperature. The water volume is read with an accuracy of 0.05 mL. The difference between the two water volumes read corresponds to the water content in the material being examined. Water content is calculated in percentage using the following formula (Anggraeni, 2020; Ministry of Health of the Republic of Indonesia, 2000):

$$\text{Water content (\%)} = \frac{\text{water volume (mL)}}{\text{sample weight (g)}} \times 100\%$$

d. Total Ash Content
The extract was weighed accurately, 2 to 3 grams, and placed in a porcelain crucible that had been heated and tared. The porcelain crucible was heated at 600°C, then cooled and weighed until a constant weight was obtained. The ash content was calculated for the air-dried material (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).

e. Acid Insoluble Ash Content
The ash obtained from the total ash content determination was boiled in 25 mL of dilute hydrochloric acid for 5 minutes. The acid-insoluble portion was collected and then filtered through filter paper. The filtrate was heated until a constant weight was obtained, then cooled and reweighed. The acid-insoluble ash content was calculated for the air-dried material (Ministry of Health of the Republic of Indonesia, 2000; Ministry of Health of the Republic of Indonesia, 2017).

RESULTS AND DISCUSSION

A. Simplicia

The lime peel used in this study was peel that had been separated from the fruit flesh. The 6-month-old limes came from Glempang Village, Maos. Lime peel crude material was obtained through a process of wet sorting, slicing, drying, dry sorting, and pollination. Wet sorting aims to clean and remove dirt adhering to the lime peel. Dry sorting aims to separate unwanted lime peel or other remaining impurities. The slicing process aims to increase the surface area, thereby accelerating the sample drying process (Ministry of Health of the Republic of Indonesia, 2017; Luketsi & Rohmah, 2019; Oriana et al., 2021; Pujiastuti & El'Zeba, 2021).

Lime peels are arranged in a non-stacked manner and dried in the sun covered with a black cloth until dry, so that the heat from the ~~sun spread~~s evenly and maintains the compound content in the lime peel. Lime peels are dried in the sun when the weather is sunny at an air temperature of 30-40^{o} C for 3 days until the lime peels are dry and can be broken. Lime peel simplicia is ground and sieved with a No. 50 sieve to obtain a fine and homogeneous powder. The purpose of the simplicia powder is to expand the surface, thereby speeding up the extraction process (Issulingtyas et al., 2024; Sa'adah & Nurhasnawati, 2017; Sari et al., 2024; Syarifuddin et al., 2020).

The resulting lime peel powder yielded 554 grams, with a yield of 33.57%. This yield was higher than that of previous research, which reported a yield of 24%. The high yield indicated a consistent drying temperature (Dewatisari et al., 2018; Sari et al., 2021b).

B. Lime Peel Extract

Lime peel extract was obtained using the maceration extraction method. Maceration extraction is used to extract active ingredients that are not damaged by heating. The soaking process causes the cell walls and membranes to break down due to the pressure difference between the outside and inside of the cell, causing secondary metabolites in the cytoplasm to break down and dissolve in the organic solvent used (Chairunnisa et al., 2019; Novitasari et al., 2021).

Lime peel extraction used 70% *food-grade ethanol solvent* with a ratio of 1:10, so that the solvent has a greater diffusion rate into the material to extract active substances more effectively. Maceration is carried out for 3 x 24 hours, this is the optimum time for the ethanol solvent to extract active compounds maximally. 70% ethanol solvent is a solvent that can extract polar and non-polar compounds, has a low boiling point of 78oC so it is easily evaporated from the extract. *Food-grade solvents* are used for food and/or beverage production. 70% ethanol can be used to extract phenolic compounds with medium polarity such as flavonoids. The yield of 70% ethanol extract of lime peel was 20.7% and met the requirements (> 15%) (Ardyanti et al., 2020; Chairunnisa et al., 2019; Ministry of Health of the Republic of Indonesia, 2017; Marpaung & Septiyani, 2020; Noviyanti, 2016; Sari et al., 2021b).

C. Standardization of Simplicia and Lime Peel Extract

Standardization of the simpicia and 70% ethanol extract of lime peel was carried out to determine the quality of the simplicia and extract to be used as raw materials for medicines to meet the requirements according to the monograph in the Indonesian Herbal Pharmacopoeia. Standardization of simplicia was carried out based on specific and non-specific parameters. Specific parameters tested included the identity of the simplicia and extract, macroscopic tests, microscopic tests, and qualitative identification of compounds. Non-specific parameters tested included drying loss, total ash content, acid-insoluble ash content, water-soluble extract content, and ethanol-soluble extract content, water content, and specific gravity (Ministry of Health of the Republic of Indonesia, 2000; Jannah et al., 2021; Kariem & Maesaroh, 2022; Marpaung & Septiyani, 2020) .

1. Identity of Simplicia and Extract

The identity of the simplicia and extract was obtained from the results of plant determination conducted at the Faculty of Pharmaceutical Biology, Muhammadiyah University of Purwokerto. The determination of *Citrus aurantifolia* (Christm.) Swingle lime aims to prove the accuracy of the materials used to avoid errors in research materials (Fidyasari et al., 2017; Kariem & Maesaroh, 2022; Marpaung & Septiyani, 2020; Oriana et al., 2021). The identity of the simplicia and 70% ethanol extract of lime peel can be seen in Table 1.

Table 1. Identity of Simplicia and Lime Peel Extract

Identity	Lime Peel Simplicia	Lime Peel Extract
Name	Citri Aurantifoliae Pericarpium	Citri Aurantifoliae Pericarpii Extractum Spissum
Latin Name of Plant	*Citrus aurantifolia*	*Citrus aurantifolia* Swingle
Parts Used	Rind	Rind
Indonesian Names of Plants	Lime	Lime

The identity of the simplicia and 70% ethanol extract of lime peel is in accordance with the identity of the implicia and lime peel extract listed in (Ministry of Health of the Republic of Indonesia, 2017) .

2. Macroscopic Test

The macroscopic test aims to determine the color, taste, odor, and shape of the simlicia and 70% ethanol extract of lime peel. The results of the macroscopic test of the simplicia and 70% ethanol extract of lime peel can be seen in Table 2.

Table 2. Macroscopic Test of Simplicia and Lime Peel Extract

Macroscopic Test	Lime Peel Simplicia	Lime Peel Extract
Form	Thin Slices with Uneven Edges	Thick
Color	Brownish Green	Chocolate
Smell	Typical Lime	Typical Lime
Flavor	Bitter and slightly sour	Bitter and slightly sour

The results of macroscopic tests showed that the simplicia and 70% ethanol extract of lime peel used were in accordance with the description of the simplicia and 70% ethanol extract of lime peel in (Ministry of Health of the Republic of Indonesia, 2017).

3. Qualitative Identification of Chemical Compounds in Simplicia and Lime Peel Extract

Qualitative identification of compounds or phytochemical tests aims to determine the secondary metabolite content in simplicia and extracts (Ariani et al., 2020) . The results of phytochemical tests on simplicia and 70% ethanol extract of lime peel can be seen in Table 3.

Table 3. Qualitative Identification of Chemical Compounds in Simplicia and Lime Peel Extract

Qualitative Test	Lime Peel Simplicia (+/-)	Lime Peel Extract (+/-)
Flavonoid	A thick black color is formed (+)	A thick black color is formed (+)
Alkaloid	Orange to reddish brown sediment forms (+)	Orange precipitate (+) is formed
Phenol	A blackish green color (+) is formed	A blackish green color (+) is formed
Saponin	A brownish ring (+) is formed	Foam forms (+)
Steroid	Green color (+) is formed	A reddish brown color (+) is formed.
Tannin	A greenish brown color (+) is formed.	A greenish brown color (+) is

		formed.
Glycosides	Green color (+) is formed	Green sediment formed
Triterpenoid	A brownish ring (+) is formed	A violet ring (+) is formed
Essential oil	A red color (+) is formed	Red color (+)

Information :
+ : contains chemical compounds
- : does not contain chemical compounds

a. Flavonoid

The 70% ethanol extract of lime peel positively contains flavonoid chemical compounds, seen in the dark black color change after the addition of $FeCl_3$. The flavonoid chemical compounds in the 70% ethanol extract of lime peel have a ^{-}OH group thatreacts with the Fe^{3+} ionfrom $FeCl_3$. The resulting color complex is dark black (Djoko et al., 2020; Fatmawati et al., 2021; Habibi et al., 2018; Sari et al., 2021b).

b. Alkaloid

The 70% ethanol extract of lime peel contains alkaloid chemical compounds with the presence of orange to brownish-red potassium alkaloid deposits after the addition of Dragendorf reagent. Potassium alkaloid deposits occur due to the replacement of ligands between nitrogen atoms in alkaloids. Nitrogen atoms in alkaloids have lone electron pairs that will replace iodine ions in Dragendorf reagents. Nitrogen atoms are used to form coordinate covalent bonds with K^+ metal ionsin Dragendorf reagents (Djoko et al., 2020; Fatmawati et al., 2021; Habibi et al., 2018; Nuralifah et al., 2020; Sari et al., 2021b).

c. Phenol

The 70% ethanol extract of lime peel contains chemical compounds of the phenol group as evidenced by a color change to blackish green after the addition of $FeCl_3$ reagent. The hydroxyl group bound to benzene will react with $FeCl_3$ to form a blackish green color complex (Anjani et al., 2018; Desinta, 2015; Djoko et al., 2020; Fatmawati et al., 2021; Habibi et al., 2018; Nuralifah et al., 2020; Sari et al., 2021b).

d. Saponin

The 70% ethanol extract of lime peel contains the chemical compound saponin, as evidenced by the formation of a stable foam. Saponin is a glycoside consisting of an aglycone called sapogenin. The glycosides in saponins can form foam in water because they have hydrophilic and hydrophobic groups. The hydrophilic group binds to water, while the hydrophobic group binds to air, forming foam. 2N HCl aims to increase polarity so that the hydrophilic groups will bind and the foam becomes more stable (Arnida et al., 2021).

The saponin test results between the crude drug and the extract differed. The crude drug produced less stable foam because the active saponin compound had not been extracted. A 70% ethanol extract of lime peel produced stable foam because the saponin compound had been extracted during the maceration process (Bintoro et al., 2017; Sari et al., 2021b).

The next saponin test used the Liebermann-Burchard reagent. The crude drug and 70% ethanol extract of lime peel contain saponin, a chemical compound indicated by the presence of a brownish ring after the Liebermann-Burchard reagent was added. The brownish ring indicates the presence of triterpene saponins (Minarno, 2015; Sari et al., 2021b).

e. Steroid
The 70% ethanol extract of lime peel contains steroid chemical compounds, which produce a green or reddish-brown color. Steroid chemical compounds are non-polar, so they are extracted after maceration with n-hexane. When n-hexane extract containing steroids is added to anhydrous acetic acid, the $^{-}$OH group in the steroids acetylates,Sulfuric acid hydrolyzes water, which reacts with the acetyl derivative to form a green or reddish-brown color (Sari et al., 2021b; Sulistyarini et al., 2019).

f. Tannin
The 70% ethanol extract of lime peel contains tannin chemical compounds, as evidenced by a color change to greenish brown after the addition of $FeCl_3$ reagent. Tannin chemical compounds are polyphenol compounds that have a phenol group. The phenol group will react with Fe^{3+} ionsto form a strong green, red, purple, and black color complex (Halimu et al., 2017; Sari et al., 2021b).

g. Glycosides
The 70% ethanol extract of lime peel contains glycoside chemical compounds. Glycoside compounds are compounds that bind to sugar compounds through glycosidic bonds. Flavonoids act as aglycones in glycosidic bonds. The glycoside compounds in the 70% ethanol extract of lime peel are included in flavonoid glycosides. The 70% ethanol extract of lime peel contains flavonoid compounds, indicating the presence of glycosidic compounds (Julianto, 2019).

h. Triterpenoid
The 70% ethanol extract of lime peel contains triterpenoid compounds with the formation of violet or brown rings after the administration of Liebermann-Burchard reagent. The principle of the triterpenoid test reaction with Liebermann-Burchard reagent is characterized by the presence of oxidation in the triterpenoid compound group which will form conjugated double bonds. Oxidation by SO_2 occurs after the administration of Liebermann-Burchard reagent, where Liebermann-Burchard reagent consists of H_2SO_4 in anhydrous acetic acid (Habibi et al., 2018).

i. Essential oil
The 70% ethanol extract of lime peel contains essential oils due to the formation of a red color. Essential oils were identified using Sudan III reagent. Sudan III has the ability to dissolve oils, fats, and waxes (Nugraheni et al., 2016).

4. Drying Loss of Simplicia and Lime Peel Extract

Drying loss is a non-specific parameter used to determine the maximum amount of compound lost during the drying process. Drying loss is carried out at a temperature of 105 °C to evaporate water and other compounds such as essential oils and ethanol (Ministry of Health of the Republic of Indonesia, 1989; Ministry of Health of the Republic of Indonesia, 2017; Marpaung & Septiyani, 2020). The results of the extract drying loss can be seen in Table 4.

Table 4. Drying Loss of Simplicia and Lime Peel Extract

Sample	Drying Loss Content (%) ± SD
Lime Peel Simplicia	8.6000 ± 0.0000
Lime Peel Extract	28.3930 ± 0.4280

The drying loss of lime peel simplicia was 8.6000% and meets the requirements, namely, no more than 10% (Ministry of Health of the Republic of Indonesia, 2017).

Drying loss shows the content of active compounds that can be lost during the heating process, including essential oils, water, and ethanol (Ministry of Health of the Republic of Indonesia, 2000, 2017; Ministry of Health of the Republic of Indonesia, 2017; Rahel et al., 2022).

The 70% ethanol extract of lime peel has a drying loss of 28.3930% ± 0.4280. The higher drying loss value of the extract than the drying loss of the simplicia can be caused by the presence of a higher ethanol content, which causes a lot of ethanol to evaporate or be lost (Ministry of Health of the Republic of Indonesia, 2017; Marpaung & Septiyani, 2020; Nugroho et al., 2020).

5. Total Ash Content of Simplicia and Lime Peel Extract

Total ash content is a parameter that aims to determine the total impurity content originating from the initial process until the formation of the extract. The ash content test uses a dry ashing method at high temperatures in an ashing furnace until a grayish-white dry ash and constant weight are obtained. The ash content indicates the amount of minerals such as calcium, phosphorus, potassium, sodium, lead, mercury, cadmium, and aluminum (Cahya & Prabowo, 2019; Ministry of Health of the Republic of Indonesia, 2000, 2017; Ernawaningtyas & Yulinar, 2019) . The results of the total ash content of 70% ethanol extract of lime peel can be seen in Table 5.

Table 5. Total Ash Content of Simplicia and Lime Peel Extract

Sample	Total Ash Content (%) ± SD
Lime Peel Simplicia	6.7550 ± 0.0769
Lime Peel Extract	5.8451 ± 0.1211

The total ash content of lime peel simplicia of 6.7550% ± 0.0769 has met the requirements (<7%). The ash content can be affected by the drying temperature. The drying method of simplicia uses solar heat and is carried out outdoors. The drying method carried out outdoors allows for contamination of sand, soil, gravel and silica. Contamination of sand, soil, gravel, and silica affects the total ash content of simplicia because it is included in non-physiological ash (Cahya & Prabowo, 2019; Ministry of Health of the Republic of Indonesia, 2017; Manfaati et al., 2019).

The total ash content of 70% ethanol extract of lime peel was 5.8451 ± 0.1211 and met the requirements (<6.6%). The ash of the extract is different from the ash of the crude drug which can be affected by the solvent and extraction. 70% ethanol solvent can extract lime peel optimally and the impurities extracted are in small amounts. The temperature used during the extraction process can affect the evaporation process of the active substances including the minerals contained. The extraction method is carried out by maceration and remaceration which involves a filtration process to separate the impurities from the macerate (Ministry of Health of the Republic of Indonesia, 2017; Erni et al., 2018; Ivana et al., 2018).

6. Water Content of Lime Peel Extract

Water content is a parameter that aims to determine the water content in the extract. Water content is carried out using the toluene distillation method (Azeotrope) because it is effective with repeated distillation and there is a reverse cooler to prevent excessive evaporation. Toluene is first saturated to prevent toluene from binding water in the distillation process. Toluene has a specific gravity of 0.87 g/mL, a boiling point of 110^{o}C, is non-polar, and is insoluble in water. The specific gravity of toluene is smaller than the specific gravity of water, making it easier to separate the water layer from toluene. The water layer is below and the toluene layer is above (Ministry of

Health of the Republic of Indonesia, 2017; Handayani et al., 2017; Ridwanto et al., 2016).

The 70% ethanol extract of lime peel has a water content of 1.4% ± 0.1428 and meets the requirements of ≤10%. A low water content indicates a low water content, making it less susceptible to contamination (Ministry of Health of the Republic of Indonesia, 2017; Kariem & Maesaroh, 2022).

7. Acid-Insoluble Ash Content of Simplicia and Lime Peel Extract

The acid-insoluble ash content aims to determine the amount of ash content obtained from external factors such as sand and soil. The ash obtained from the total ash content test is reacted with HCl. HCl can affect the decrease in the acid-insoluble ash content value because it can extract minerals such as calcium and phosphorus. The acid-insoluble part is filtered and washed in filter paper using water. The water is used for the demineralization process. Demineralization is carried out to remove water-soluble minerals so that acid-insoluble ash remains (Ministry of Health of the Republic of Indonesia, 2017; Kariem & Maesaroh, 2022). The results of the acid-insoluble ash content of lime peel simples can be seen in Table 6.

Table 6. Acid-Insoluble Ash Content of Simplicia and Lime Peel Extract

Sample	Acid insoluble Ash Content (%) ± SD
Lime Peel Simplicia	0.3000 ± 0.0050
Lime Peel Extract	0.0493 ± 0.0005

Lime peel simplicia has an acid-insoluble ash content of 0.3000% ± 0.0050 and meets the requirements (<0.4%). The results of the acid-insoluble ash content of 70% ethanol extract of lime peel are 0.0493 ± 0.0005 and meet the requirements (<0.1%). The low acid-insoluble ash content can indicate minimal contamination due to sand or soil (Ministry of Health of the Republic of Indonesia, 2017).

8. Water-soluble and Ethanol-soluble Content of Lime Peel Simplicia

The water-soluble extract content is a test to determine the amount of polar compounds (soluble in water). The medicinal plant is macerated using a water-chloroform solvent. Chloroform is used to inhibit the growth of microorganisms that could interfere with the research process (Ministry of Health of the Republic of Indonesia, 2017; Djoko et al., 2020; Kariem & Maesaroh, 2022).

The ethanol-soluble extract content is a test to determine the amount of semi-polar and non-polar compounds (soluble in ethanol). The sample was macerated using 96% ethanol, a solvent with a low polarity that can bind non-polar compounds (Riwanti et al., 2020). The results of the water-soluble extract content and the ethanol-soluble extract content of lime peel are shown in Table 7.

Table 7. Water-Soluble and Ethanol-Soluble Content of Lime Peel Simplicia

Lime peel Simplicia	Content (%) ± SD
Water-soluble	26.5333 ± 0.3055
Ethanol-soluble	23.7333 ± 0.3055

The water-soluble extract content of lime peel simplicia met the requirements, as it was not less than 25.6%. A high water-soluble extract content indicates a high content of polar active compounds such as flavonoids, alkaloids, and tannins (Ministry of Health of the Republic of Indonesia, 2017; Suputri et al., 2021).

Factors that can affect the water-soluble extract content include evaporation temperature. High evaporation temperatures can damage active compounds, such as tannins, flavonoids, and phenolic compounds. Other factors that can affect the water-soluble extract content include particle size and the maceration method. Smaller particles have a wider contact with the solvent, resulting in more active compounds being extracted. The maceration method used causes the sample to be in contact with the solvent for a longer period, resulting in more active compounds being extracted (Chairunnisa et al., 2019; Sari & Triyasmono, 2017; Dewi et al., 2016; Warnis et al., 2021) .

The ethanol-soluble extract content in lime peel simplicia of 23.7% has met the requirement of not less than 18%. The ethanol-soluble extract content is lower than the water-soluble extract content indicating that the active compounds in lime peel are more polar. Non-polar active compounds include terpenoids, steroids, and essential oils. The ethanol-soluble extract content can be affected by the maceration process. Maceration for 3 days can cause the sample to be in contact with 96% ethanol for longer and contain more active compounds extracted (Chairunnisa et al., 2019; Ministry of Health of the Republic of Indonesia, 2017; Handayani et al., 2017; Utami et al., 2017).

9. Specific Gravity of Lime Peel Extract

Specific gravity is the ratio of the mass of a sample volume to the mass of water at the same temperature and volume. A 70% ethanol extract of lime peel has a specific gravity of 0.8830 g/mL. The specific gravity results can indicate the number of compound components contained in the extract. The specific gravity of a 70% ethanol extract of lime peel indicates that many of the compounds contained are polar (Sari & Triyasmono, 2017; Widyasanti et al., 2018).

CONCLUSION

Both simplicia and 70% ethanol extract of lime peel met the pharmacopoeial quality requirements. The study establishes a standardized protocol for lime peel, providing a foundation for future herbal pharmaceutical formulations and sustainable resource utilization.

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