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Standardization And Phytochemical Test Of Red Betel Leaf Simplicia (*Pipper Ornatum)*

By
Riza Vetisari[1], Heldi Candra[2]
[1,2]Pharmacognosy Laboratory, Faculty of Health Sciences, University of Batam
E-mail: [1]rizaveti2003@gmail.com

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ABSTRACT

Abstract: *Red betel (Piper ornatum) is a medicinal plant widely utilized in traditional medicine because of its potential pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities. This study aimed to standardize red betel leaf simplicia and evaluate its phytochemical characteristics to determine its quality as a raw material for herbal medicinal products. An experimental laboratory study was conducted using red betel leaves processed into simplicia through harvesting, wet sorting, washing, drying, and grinding. Ethanolic extracts were prepared using maceration followed by Soxhlet extraction. Standardization was performed by evaluating non-specific parameters, including moisture content, drying loss, ash content, water-soluble extractive value, and ethanol-soluble extractive value. Thin-layer chromatography (TLC) and qualitative phytochemical screening were employed to identify the presence of major secondary metabolites. The results showed that several quality parameters complied with the Indonesian Herbal Pharmacopoeia standards, while the moisture content and drying loss exceeded the recommended limits, indicating that the drying process requires further optimization. TLC analysis successfully produced characteristic chromatographic profiles, confirming the presence of bioactive compounds in the extract. These findings suggest that red betel leaf possesses considerable potential as a source of herbal medicine; however, improvements in post-harvest handling and drying techniques are essential to produce standardized simplicia with consistent quality and safety*

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Corresponding Author:
Riza Vetisari
Pharmacognosy Laboratory, Faculty of Health Sciences, University of Batam
E-mail: rizaveti2003@gmail.com

1. INTRODUCTION

The history of traditional medicine in the world includes the use of a variety of natural ingredients, such as plants, minerals, and animals, for medicine and health care. This practice has been around since prehistoric times and has continued to evolve over time. In prehistoric times, humans have relied on natural ingredients as medicines, including plants, fruits, roots, and leaves. The use of natural remedies used by prehistoric humans can be found in archaeological records, such as plant remains at prehistoric sites, such as the archaeological discovery that in Iraq people used mallow (Malva lalai) and yarrow (Achillea millefolium) about 60,000 years ago to treat wounds, respiratory infections, digestive problems, skin diseases, and liver disease. (Nanda Setyani. 2022.)

In the Mesopotamian civilization (3500-300 BC) the Sumerians in Mesopotamia used plants and minerals for medical purposes. Prehistoric records on clay prisms provide information on the use of traditional medicine at the time, an example of which is one well-documented nail-shaped medical source is the Uruanna Lexicon: maštakal). It contains about 1300 terms for drugs of plant origin that, taking into account variants and synonyms, describe about 340 different plants. Plants (plants, trees and shrubs, spices, grasses, algae, aromatic plants and fungi). (Yuli Widiastuti. 2017.)

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Traditional medicine continues to develop in modern civilization as it is today. Over time, traditional medicine has developed and adapted to social, cultural, and scientific changes, and traditional medicine is still used today.

Traditional Chinese Medicine (TCM) which uses a variety of herbal plants, such as ginseng, ginkgo biloba, and echinacea, to treat a wide variety of diseases, including heart disease, stroke, and cancer, in India Ayurveda is a traditional Indian medicine system that has been used for more than 5,000 years. Ayurveda uses a wide variety of herbal plants, such as turmeric, tamarind, and ginger, to treat a wide variety of ailments, including digestive diseases, skin diseases, and heart disease, in Japan Kampo is a traditional Japanese medicine system that has been used for over 1,000 years. Kampo uses a wide variety of herbal plants, such as ginseng, gingko biloba, and tamarind, to treat a wide variety of ailments, including digestive diseases, skin diseases, and heart disease. At this time traditional medicine in the world is often used as an alternative or complementary medicine to modern medicine. Traditional medicine can be a good option for people who have allergies to modern medicines or who want to avoid the side effects of modern medicines. (Ministry of Health of the Republic of Indonesia. 2000.)

Traditional medicine has been used by the Indonesian people since ancient times. Evidence of the use of traditional medicine has been found at various archaeological sites in Indonesia, including in the Leang-leang Cave in South Sulawesi, which is estimated to be more than 40,000 years old. In ancient times, traditional medicine was often used as the only treatment available. Modern medicine only began to develop in the 19th century. However, traditional medicine remains popular in Indonesia, even today. Types of Traditional Medicines in Indonesia.One of the traditional medicines in Indonesia is:

1. Herbs

Jamu is a traditional Indonesian medicine derived from various kinds of herbal plants. Herbal medicine has been used by Indonesian people since ancient times to treat various diseases, including stomach diseases, skin diseases, and rheumatic diseases. (Ministry of Health of the Republic of Indonesia. 2000.)

The objectives of this research are to thoroughly investigate the health potential of red betel leaves (Pipper ornatum) as a herbal plant, by focusing on aspects of chemical composition, pharmacological properties, and the potential for the development of alternative plant-based therapies.

b. Special Purpose

Analyzing the kiia composition of red betel leaf kiia (Pipper ornatum), determining and identifying the active compounds in red betel leaves to understand their chemical composition in depth

II. LITERATURE REVIEW

Farmakognosi

Pharmacognosy was originally defined as the study of raw medicines obtained from plants, animals, and minerals and their contents. But the new historical research states that pharmacognosy describes the study of medicinal plants and their properties. The term Pharmacognosy comes from two other words, namely pharmakon "medicine" and gignoso "to gain knowledge of" Then it means "knowledge or science about medicine". (Nugroho & Hartini, 2021).

Simplisia

Simplicia or herbs are natural ingredients that have been dried and used for treatment and have not undergone processing, unless otherwise stated that the drying temperature of simplicia is not more than 60C. The term simplicia is used to refer to natural medicinal ingredients that are still in their original form or have not changed their shape (Gunawan, 2010).

Simplicia is a natural ingredient that is used as a medicine that has not undergone any processing and unless otherwise stated, in the form of a material that has been dried.simplia si is divided into three groups, namely vegetable simplia, animal simplia, and mineral simplia (Melinda, 2014).

Safe and efficacious simplicia is that simplicia does not contain chemical, microbiological and physical hazards and contains efficacious active substances. A good characteristic of simplicia is in dry conditions (%water<10%). For leaf simplicia when squeezed and rustle, fruit simplicia and rhizomes (slices) when squeezed are easily broken. Another characteristic of a good simplisia is that it is not moldy and has a distinctive berbubu, which resembles its fresh ingredients (Herawat *et al.* In Paramita *et al.*, 2022).

Standardization of Simplification

Standardization of simplicia is the process of establishing the required quality and quantity parameters of a plant raw material or simplicia. The standardization of red betel leaf simplicia is the process of determining the quality and quantity parameters that must be met by the raw materials of the plant. In the context of red betel leaves, standardization is carried out to ensure that the quality and safety of the raw materials are in accordance with the standards that have been set. (Winangsih & Parman, 2013).

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Ethnopharmacy

Ethnopharmacy is the study of the knowledge and practices of traditional societies in utilizing plants and animals for medicine. Ethnopharmacy is a field of science that combines pharmaceuticals, ethnoborean, and ethnozoology (Islami *et al.,* 2017).

Ethnopharmacy has several goals, including:

- Documenting traditional medicine knowledge and practice
- Identifying plants and animals that have the potential to be medicinal
- Studying the chemical content and mechanism of action of traditional medicine
- Developing modern traditional medicine

Ethnopharmacy has an important role in the development of traditional medicine. Ethnopharmaceutical research can help identify plants and animals that have the potential to be medicinal, as well as study the chemical content and mechanism of action of traditional medicine. This can be the basis for the development of modern traditional medicine that is safer and more effective (Ningsih, 2016).

Medicinal plants

Medicinal plants refer to plants that are used for medicinal or health purposes. This plant has chemical compounds that can provide therapeutic or health effects when used correctly. The use of medicinal plants has existed since ancient times and is part of the medicinal traditions in many cultures around the world (Savitri, 2016).

The medicinal plant of red betel leaf (*Piper ornatum*) is a vine native to Southeast Asia (Moeljanto, 2003). It is used in various medicinal traditions, especially in Asia, both in chewable form and as an ingredient in traditional herbs. It is known to have antioxidant, anti-inflammatory, and antiseptic properties. Used for oral problems, respiratory disorders, and certain skin problems. It is often used in traditional medicine practices, such as in wedding ceremonies or religious ceremonies (Sangi *et al.,* 2019).

Extract

In botanical or pharmaceutical contexts, "extract" often refers to compounds or substances extracted from plants. The extraction process involves the separation or extraction of certain substances from natural materials, such as plants, for use in the pharmaceutical, cosmetic, or food industries (Hasan *et al.,* 2023).

In chemistry or chemical engineering, "extract" can refer to a liquid or compound obtained from the extraction process. For example, liquid extracts from an ingredient can be used to obtain certain substances from complex mixtures. (Moulia, 2018).

Screening Phytochemistry

Phytochemical screening is a process of qualitative testing or analysis carried out to identify and detect the presence of certain chemical compounds in plants or other natural products. The main goal of phytochemical screening is to determine the groups of chemical compounds present in plant materials, especially those that have bioactive potential or therapeutic effects. Phytochemical screening is the first step in research on medicinal or herbal plants. (Padmasari) *et al.,* 2013).

III. RESEARCH METHODS

Types and plans of material collection

a. Types of Material Collection

This research is a type of experimental research carried out in the laboratory of the University of Batam using a one-group pre-test and post-test design.

b. Material Collection Plan

- Material preparation
- Manufacture of red betel leaf extract
- Standardization and phytochemical tests on red betel leaf extract

Number of Plants and Number of Samples

a. Total Plants

The total number of plants taken from 3 pots is taken from the leaves

b. Number of Samples

1. Inclusion Criteria and Exclusion Criteria
   - Criteria Inclusion

     The criteria for sample inclusion needed in this study are the part that is still good, does not wither and is not eaten by caterpillars.
   - Exclusion Criteria

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The criterion for excluding samples that is not needed in this study is that the growth of basil has a bad part such as leaves that are eaten by caterpillars and the stems have wilted.

2. Large Sample
   The sample size is ± 17 cm in each plant
3. Sampling Techniques
   The technique used in sampling was treated for 3 months and 12 weeks

Sample Collection Location and Time

The sample collection location was carried out at the Batam University Laboratory on November 17, 2023, at 13:00 WIB. Where the duration of this sample collection lasts approximately 3 months.

Materials and Tools

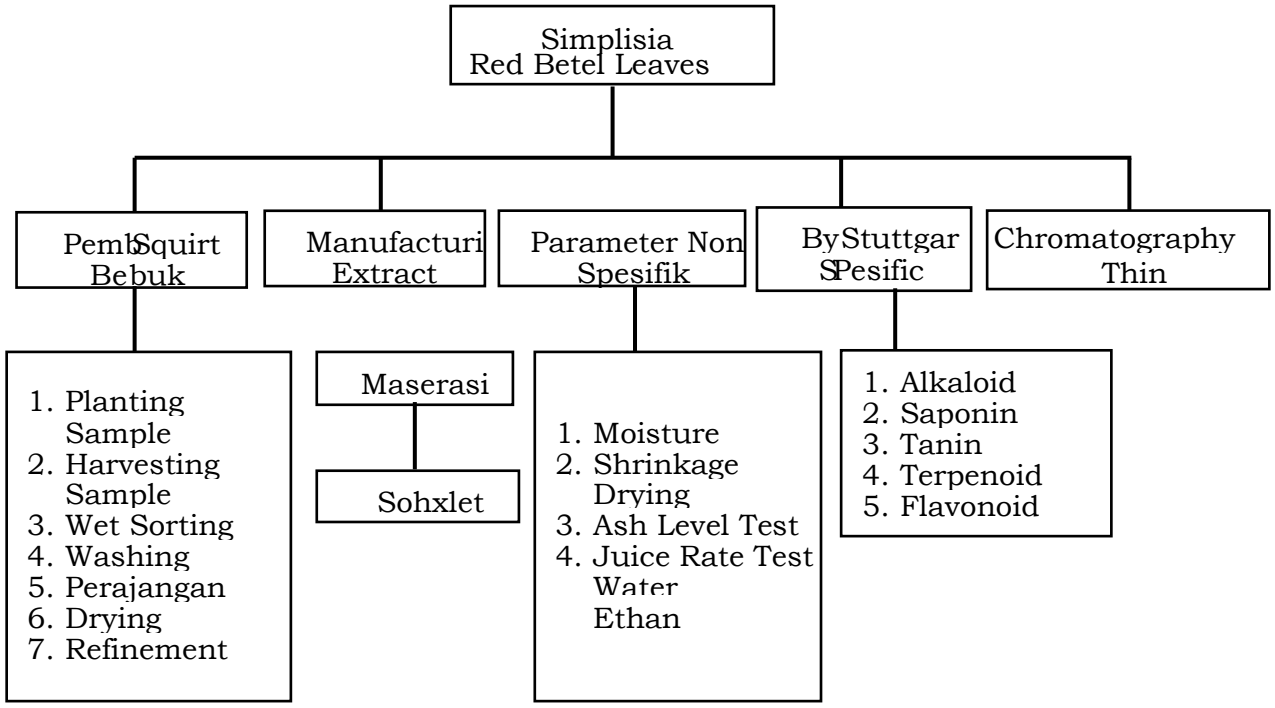
The ingredients used during the praticum are red betel leaves, 96% ethanol. The tools used during the practising are plastic bags, mobile phone cameras, scissors, woven bamboo, beaker glass, measuring cups, whiteman paper, black bottles, glass funnels, prosline cups, crusher pliers, 3 legs, gauze wire, spirit lamps and matches.

How It Works

In this study, the procedures used are as follows:

1. Powder making consists of simplicia planting, simplicia harvesting, wet sorting, washing, drying, and powder making.
2. The manufacture of extracts consists of maceration and socleration.
3. Non-specific parameters consist of moisture content test, drying shrinkage test, ash content test, total, water soluble juice content test and ethanol soluble juice content test.

The working procedure is as shown in the following chart:



TechniqueSample collection

The technique for collecting this sample is by planting red betel leaf plants with a target of 250 grams. The principle of determination is the same as accidental sampling. But the researchers first determined the number of samples needed. The advantage of using this technique in sampling is that it is practical because the research sample is known beforehand.

Sample collection in this study was carried out with various research methods such as observation and documentation, requiring tools as instruments. The instruments in question are cameras, mobile phones for videos, pens and so on.

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IV. RESULTS AND DISCUSSION

Harvesting Results of Simplicia

On August 21, 2023 at 08:00 WIB, red betel leaves have been planted, and red betel leaves have been planted from good seeds in potted media. The planting location was carried out in the Hawaii garden at a height of 14.8 m.

The way simplicia planting works is to prepare the necessary tools and materials such as seeds, black soil, and pots. Prepare the pot to be used, then sprinkle the seeds into the pot and then water.

The number of red betel leaf plants planted was 2 main plants and 1 reserve plant, the 3 plants managed to survive until harvest in the 3rd month. Planting results in appendix **1**.

Table 1. Identity Check Results

Yes	Plant Identity	Examination Results
1	Plant names	Red betel leaves
2	Latin Names	*Pipper Ornatum*
3	Plant parts used	Daun
4	Name Indonesia	Red betel plant

Results Material collection

The leaves are sorted and washed, the weighing result is 21 grams, after 5 days of drying the weighing results of red betel leaves are as much as 17 grams so that the total dry plants that are ready to be used as simplicia only amount to 17 grams of whole plants planted for 4 months, which is very less if you look at the applicable provisions, namely the total plants that should be needed is as much as 250 grams, So to cover this shortcoming, 350 grams of red betel leaf powder was purchased on one of the online applications that provide herbal products.

Maceration Test Results

In the practicum on maceration carried out at the pharmaceutical laboratory at the University of Batam on Thursday, December 14, 2023, the simplicia powder that had been provided was put into a chocolate bottle, ethanol was added 3 fingers on top of the sillia.on the first day of maceration using 150 ml of ethanol, after soaking for 1x24 hours, filtered using filter paper and producing 125 ml.

On the 2nd day of maceration, using 150 ml of ethanol, after soaking 1x24 hours it is filtered using filter paper and produces 200 ml. On the 3rd day of maceration using 160 ml of ethanol, after soaking for 1x24 hours, it is filtered using filter paper and the result is 130 ml. After 1x24 hours it is filtered and the results are obtained, the filtrate is collected.

Results of the Scoliation Test

The way it works is to prepare tools and materials. Prepare the boiling stones and put them in the base pumpkin. Prepare the filtered solution then put the boiling stone and solution in a 150 ml base flask then turn on the heater. Allow the solvent to evaporate to the top. If there is a solution in the sokhlet tube, turn off the heater if it has almost passed the predetermined limit. Do this repeatedly when the maceration solution has been exhausted and the solution in the sokhlet tube has almost crossed the predetermined limit. Put the solution in the sokhlet tube into the container. The existing solution is boiled and condensed to 20 ml and after 20 ml is put into a vial bottle. The socleration procedure reaches 5 cycles, as shown in the table below. The socle procedure is found in appendix **8.**

Table 2. Results of Chocolate Extraction

Siklus		Start time	Temperature	Clock Completion	Ethanol yield
1		11.22	75°C	11.40	45 ml
2		11.42	60°C	12.03	37 ml
3		12.04	60°C	12.21	40 ml
4		14.14	50°C	14.32	38 ml
5		14.34	50°C	14.58	36 ml
6		14.59	50°C	15.16	36 ml
7		15.18	60°C	15.36	39 ml
8		15.38	60°C	15.58	37 ml
9		15.58	60°C	16.16	34 ml
					342 ml

Results of Water Soluble Juice and Ethanol Test

In this practicum, 10 grams of simplicia are needed, each test requires 5 grams. In the water-soluble cider content test, 100 ml of saturated solution derived from a mixture of aquadest and chloroform was given, while in the ethanol

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soluble content test, 100 ml of 96% ethanol solution was given. The 2 liquids that have been mixed with powder as much as 5 grams, then shake for 6 hours, by stirring every 15 minutes every hour.

After being shaken, the liquid is then filtered and the filtrate is taken as much as 20 ml, to be evaporated with a vaporizer cup that has been tempered at a temperature of 250°C. The evaporation will produce a scale on the cup which is then weighed, and redissolved with 10 drops of ethanol. The result of the vaporizer cup scale and the crust that has been produced at the evaporation of the water-soluble juice content is 39,081 grams, while the ethanol soluble content is 59,480 grams. The results of the water and ethanol soluble juice test are found in appendix **4**.

Table 3. Results of Determination of Water Soluble Juice Rate

No	Specific Parameter Results	Determination Results
1.	0.227 gr	< 22.6%

Table 4. Results of Ethanol Soluble Determination

No	Specific Parameter Results	Determination Results
1.	10 gr	< 13%

Ash Level Test

On January 12, 2024, an ash content test practicum was carried out at the University of Batam Laboratory. In the ash content test, a vaporizer cup and purple leaf powder were used as much as 2 grams. Before the evaporator cup, it is ovened for 10 minutes, and placed in the desilator after being ovened for 5 minutes. Then 2 grams of purple leaf powder are put in a cup, and baked for 30 minutes at 250°C, then the cup and the powder that has become ash are weighed.

Measurement of ash content can be done with the following formula:

➢ Final Weight = 79.004 g – 78.570 g = 0.434 g

$$\% \text{ up to Abu} = \frac{W1}{W} x100\%$$

$$\% \text{ up to Abu} = \frac{0{,}434}{2} x100\%$$

$$= 21{,}7\%$$

So the percent of ash content obtained is 21.7%. In the 2017 edition of the herbal pharmacopoeia, the ash content was 15.6%

Table 5 Results of Determination of Ash Levels

Yes	Specific Parameter Results	Determination Results
1.	21,7 %	< 15.6%

Drying Shrinkage Test

In the drying shrinkage test, a cross cup was used with a lid, and 3 grams of purple leaf powder was used. Before conducting the test, the cross cup is heated 3 times, namely for 10 minutes, 5 minutes, 5 minutes, and placed in the desilator every time it has been ovened for 5 minutes. After heating the cup, the simplicia powder is put in the cup for 30 minutes and put in the desilator for 5 minutes after the oven. Repeat the step 3 times at 105°C.

Drying shrinkage measurement can be done using the following formula:

- Total Weight = 73.91 gr – 71.49 gr = 2.42 gr
  = 73.91 gr – 71.49 gr = 2.42 gr
  = 73.16 gr – 71.49 gr = 1.67 gr
  = 6.51 gr
- Final weight = 6.51 g ÷ 3 g
  = 2.17 gr
- % Drying shrinkage = $\frac{W-W1}{W} x100\%$

$$= \frac{3-2{,}17}{3} x100\%$$

$$= 27{,}6\%$$

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Table 6 Drying Shrinkage Rate Results

No		Specific Parameter Results	Determination Results
1.		27,6 %	> 10%

Moisture Content Test

In the moisture content test, a cross cup with a lid and 2 grams of simplicia powder was used. Before conducting the test Before conducting the test, the cross cup is heated 3 times, namely for 10 minutes, 5 minutes, 5 minutes, and placed in the desilator every time it has been ovened for 5 minutes. After heating the cup, the simplicia powder is put in the cup for 30 minutes and put in the desilator for 5 minutes after the oven. Repeat the step 3 times at 105°C. The results of the moisture content test are found in appendix 7.

The measurement of the moisture content test can be carried out using the following formula:

- Berat total = 73.27 gr – 71.51 gr = 1.76 gr
  = 73.00 gr – 71.51 gr = 1.49 gr
  = 73.00 gr – 71.51 gr = 1.49 gr
  = 4.74 gr
- Final weight = 4.74 g ÷ 3 g
  = 1.58 gr
- % Up to Air = $= \frac{W-W1}{W} x100\%$

$= \frac{3-1,58}{3} x100\%$

$= 47,3\%$

So the moisture content test in purple leaf simplicia was 47.3%. In the 2017 edition of the hebal pharmacopoeia, the moisture content was >10%.

Table 7 Results of Determination of Moisture Content

Yes	Specific Parameter Results	Determination Results
1.	47,3 %	>10%

Thin-Layer Chromatography Test

On January 19, 2024, a thin-layer chromatography test practicum was carried out. In this practicum using a KLT tool with a size of 1x10 cm, and a developer with a ratio made of methanol: ethyl acetate: n-hexane which is 6:2:2 which has been left for 30 minutes with a closed state.

This is done so that the vapor pressure of the solvent during the separation process will be the same, so that the solvent will rise at the same time and get more accurate results. Meanwhile, the chamber containing the eluent is saturated and closed with the aim that the solvent used does not evaporate so that it does not affect the separation process. Then insert the sample into the KLT plate that has been inserted into the developer using a capillary pipette. This pipette is used because the polishing results are more accurate. Then wait for the sample to move to the specified limit. After that, it detects under 254 nm UV light. Then observe where the color comes out, measure, and calculate the result by dividing the color distance by the KLT distance. In the KLT test, RF 1 results were obtained which was 0.625 cm, RF 2 was 0.75 cm, RF 3 was 0.8125 cm. The results of the KLT test are in appendix **8**.

V. CONCLUSION

From this study, it can be gathered that:

1. The manufacture of basil powder starts from the stages of planting, harvesting, wet sorting, washing, framing, drying, dry sorting, and packing. The yield of basil powder is obtained 125 grams.
2. The non-specific parameter value of the basil powder moisture content test was 13.3%, drying shrinkage was 18.5%, ash content was 10%, water-soluble juice content was 70%, and ethanol soluble juice content was 61%. The result of the extract by the soklet method was 25 ml. The KLT value of RF1 was obtained 0.25 and RF2 was obtained 0.25 under 254 nm UV light. All tests carried out are

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eligible according to the 2nd edition of the herbal pharmacopoeia of 2017, except for the moisture content test, and the drying shrinkage test.

VI. SUGGESTIONS

1. It is necessary to re-confirm the state of simplia whether the dry hearing is perfect or not. In order to obtain results that are in accordance with the existing references.
2. Provide more care and pay attention to the internal and external factors of the cultivation of medicinal plants so that the results obtained are not less than specified.
3. It is hoped that further research can be carried out carefully from the previous one

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