Characterization of the Manufacturing Process Simplicia Syzygium aromaticum as Raw Material for Wound Healing Drug Preparations

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ABSTRACT

Characterization is the first step before standardization. Standardization of a drug substance is a requirement to achieve parameters that will ensure constant quality in a preparation. The main compounds contained in clove leaves have analgesic and antibacterial properties to accelerate wound healing. This study aims to characterize the process of making clove leaf simplicia and Simplicia (Syzygium aromaticum) originating from Lemukutan Island, Bengkayang Regency, West Kalimantan as raw material for wound healing drug preparations. Clove leaf Simplicia was characterized biologically including habitat and morphology, physically including organoleptic, microscopic, moisture content, ash content, and acid insoluble ash content, and chemically, namely phytochemical screening. Making clove leaf Simplicia consists of harvesting, wet sorting, washing, drying, dry sorting, packaging, and storage. The yield of clove leaf Simplicia was obtained as much as 70.90%. The results of the characterization of clove leaves showed that the clove tree grows in the highlands, with a tropical climate, with a temperature range of 25 C-32 C. Clove leaves are oval in shape, tapered tip, pinnate leaf bone, leaf length is approximately 9-12 cm and width 4 cm-4.5 cm. The moisture content, ash content, and insoluble ash content of the clove leaf Simplicia powder were 9.21%, 3.05%, and 0.69%, respectively. The outcomes of the phytochemical screening of clove leaf Simplicia powder have been superb for holding flavonoids, saponins, triterpenoids, and tannins.

Keyword: Characterization, Simplicia Clove leaf (Syzygium aromaticum), Phytochemical screening, Medicinal raw materials

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1. Introduction

Characterization is an initial step before the standardization process. Standardization of drug preparation in the form of Simplicia, extract, or product is a requirement to achieve parameters that will ensure constant quality in the preparation[1]. The number of plants that empirically have beneficial pharmacological effects but are not standardized so that the varying safety profile, efficacy, and quality of herbal sources of each product and difficult to control the quality is a drawback of using herbs as medicine[2]. Differences in screening test results Clove leaf phytochemicals (Syzygium aromaticum) in several studies can be influenced by geographical conditions and where clove leaves are planted. Characterization became
achieved to decide the fine of the simplicia in order that it became the identical and secure for use time and again as a medicinal element in each drug guidance and might be used as a reference aimed toward growing in addition research[3,4,5].

Figure 1. Clove leaves (*Syzygium aromaticum*)

Wounds are one of the damages to skin tissue. The occurrence of injuries can be caused by contact with heat sources (chemicals, hot water, fire, radiation, and electricity), the result of medical procedures, and even changes in physiological conditions. Wounds based on time and the healing process are divided into acute wounds and chronic wounds. Factors that affect wound healing include local factors and systemic factors. The prevalence of acute and chronic wounds is increasing every year. The utilization of efficacious plants as wound care therapy is more effective and easy to obtain. The diversity of herbal plants in Indonesia can be used as wound healing medicine[6].

Herbal medicine is a form of alternative medicine that includes the use of a different plant or plant extract[3]. Traditional medicine comes from plant materials, animal ingredients, minerals, or mixtures of these materials that have been used for generations for disease prevention, health maintenance, or treatment[7]. According to research conducted by Andrie and Taurina (2019), the preparation of snakehead fish (*Channa striata*) with a combination of kelulut honey (*Heterotrigona Itama*), golden sea cucumber extract (*Stichopus Hermanii*), betel leaf extract (*Piper betel*), and clove oil (*Syzygium aromaticum*) proven to help wound healing. The ethanol extract of betel leaf (*Piper betle*) and clove oil (*Syzygium aromaticum*) had the finest antibacterial pastime with a big inhibitory area price on each styles of bacteria (*Staphylococcus aureus*, and *Pseudomonas aeruginosa*), which indicated that clove oil had broad-spectrum antibacterial pastime[8]. The analgesic and antibacterial properties of clove oil can be used to accelerate wound healing[9][10]. The main compound contained in clove leaves is eugenol which acts as an antiseptic, anti-inflammatory and immunomodulatory[11,12].

Clove (*Syzygium aromaticum*) is a plant native to Indonesia[11]. Cloves belong to the Myrtaceae of aromatic spice plants[13]. The clove tree is conical in shape, medium in size with straight stems, the clove tree height is 10 to 12 meters. Clove leaves are oval to elliptical, yellowish-green to dark green, about 2.35-2.41 cm long, clove leaf width about 0.57-0.64 cm, and weight about 0.58-0.82 g[13][14]. Analysis of clove leaf compounds using the GC-MS method, namely the eugenol compound 74.28%[12]. Clove leaf (*Syzygium aromaticum*) is one of the raw materials that can be used in the preparation of wound-healing drugs. The standardization of clove leaf Simplicia (*Syzygium aromaticum*) has never been done before. In this study, a characterization of the process of making clove leaf simplicia and Simplicia (*Syzygium aromaticum*) was carried out which was obtained from clove plantations in Batu Barat Hamlet, Pulau Lemukutan Village, Sungai Raya Islands District, Bengkayang Regency, West Kalimantan. characterize the process
of making clove leaf simplicia and Simplicia (*Syzygium aromaticum*) to obtain products with consistent, sustainable results and guaranteed efficacy and quality. In this study, the simplicia characterization of clove leaves (*Syzygium aromaticum*) was carried out biologically which included morphology, habitat, and microscopy, physically, namely organoleptic, water content, ash content, and acid insoluble ash content, and chemically, namely phytochemical screening. This study aims to characterize the processing of clove leaf Simplicia (*Syzygium aromaticum*) and clove leaf Simplicia (*Syzygium aromaticum*) which will be used as raw materials for wound healing drug preparations.

2. Research Methods

**Materials**

Tools used on this research are a microscope a heater and a desiccator. The materials used in this study included clove leaves, chloral hydrate solution, 2N hydrochloric acid, distilled water, Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, magnesium powder, concentrated hydrochloric acid, amyl alcohol, hot water, clove leaf Simplicia powder. macerated with 20 ml of n-hexane, Liebermann-Burchard reagent, 1% iron(III) chloride reagent, toluene, and dilute hydrochloric acid.

**Methods**

The characterization of the process of making Simplicia begins with observing the clove plant growing habitat, observing the clove plant growing habitat, which is dry/tropical climate, the air temperature is around 25°C-28°C[15]. Furthermore, morphological observations of cloves were carried out. The clove tree has a height of 20-30 meters The clove leaves are oval with an elongated oval shape with an angled tip and base, an average of 2 to 3 cm wide, and a leaf length without stems of 7.5 to 12.5 cm[16]. Clove leaves do not have upih or midrib but have petioles, leaf blades (lamina). Clove leaf determination was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Tanjungpura University to make sure the correctness of the samples used. Sample collection was carried out by harvesting clove leaves that had fallen in the morning. After that, wet sorting was carried out and then the good clove leaves were weighed to be used as samples, and each impurity was weighed. Wash the cloves with clean and running water. The next stage is the drying process of clove leaves that have been washed by air drying. Then dry sorting is done to sort the leaves that can be used for further processing. Simplicia that has gone through the dry sorting process is then packaged. Clove leaf packaging uses a glass jar with a tight lid, then covered with aluminum foil to avoid the clove leaf Simplicia from light. Furthermore, the clove leaf Simplicia is stored in a dry room and not exposed to direct sunlight. Clove leaves that are stored are whole clove leaves that have been dried but have not been mashed. Stored at a temperature of 26°C to 28°C.

Characterization of clove leaf Simplicia, namely microscopic, organoleptic, phytochemical screening, water content, ash content, and acid insoluble ash content. Microscopic observations were made with many powders placed on a slide, then added a few drops of chloral hydrate solution, fixed over a spirit lamp, and then left and covered with a coverslip. The preparations were then observed under a microscope. The
organoleptic examination of clove leaves (Syzygium aromaticum) was carried out using the senses to describe the taste, color, and aroma of clove leaves (Syzygium aromaticum).

Phytochemical screening consists of checking out the material of alkaloids, flavonoids, saponins, steroids/terpenoids, and tannins. Testing for the content of alkaloids was carried out with 0.5 g of the test sample weighed then brought 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated for 2 minutes on a bath, cooled, after which filtered. The filtrate obtained for the alkaloid test was tested, and 0.5 ml of the filtrate was added to each of the 3 test tubes as follows: [16] Test tube 1 was added with 2 drops of Mayer reagent; Test tube 2 was added with 2 drops of Bouchard at reagent; Test tube 3 was added with 2 drops of Dragendorff's reagent. Alkaloids are positive if there is a precipitate or cloudiness in at least two of the three experiments[16].

The flavonoid test was carried out with 10 g of the test sample added with 10 ml of hot water, boiled for 5 minutes, and filtered in hot conditions, 0.1 grams of magnesium powder and 1 ml of concentrated hydrochloric acid, and 2 ml of amyl alcohol were put into 5 ml of the filtrate and then shaken and left to separate. It is stated to be superb if there may be a red or yellow or orange color at the amyl alcohol layer[17].

Testing for saponins is performed with 0 five grams and placed right into a take a look at tube, then introduced 10 ml of warm water and cooled then shaken vigorously for 10 seconds. If the froth is fashioned as excessive as 1-10 cm that is strong for much less than 10 mins and does now no longer disappear with the addition of one drop of two N hydrochloric acid, it shows the presence of saponins. with 10 mL of N-hexane for 1 hour and then filtered. The obtained filtrate was evaporated, the remaining filtrate was added with 10 drops of anhydrous acetic acid reagent and 1 drop of concentrated sulfuric acid. The changes that occur are observed, if the powder is positive for terpenoid compounds, it will be marked by the formation of a purple or red color that changes to blue-green[18].

The tannin content test was carried out by weighing 11 grams of the sample and then boiling it for 3 minutes in 100 ml of distilled water, then cooled and filtered. Take 2 ml of the solution and add 1-2 drops of 1% iron (III) chloride reagent. The presence of tannins is indicated with the aid of using the prevalence of blue-black or green-black color [17].

Measurement of the moisture content of clove leaf Simplicia powder was carried out using the toluene distillation method which was saturated with water, as much as 5 g of simplicia was then put into a round bottom flask and added toluene. which has been saturated. Heat the flask for 15 minutes, wait until the toluene begins to boil, the distillation is set to 2 drops per second, then 4 drops/second. When all the water has been distilled, continue heating for up to 5 minutes. Allow the tube to cool to room temperature. The volume of water was calculated after the toluene and water were completely separated[19].

Measurement of the ash content of clove leaf Simplicia powder using the gravimetric method by weighing an empty crucible and then putting the sample into an empty crucible then weighed, heated in an oven at 105°C for 3 hours to constant weight, put into a desiccator then weighed, covered with porcelain crucible, put in the furnace and then heated at 600 C for 8 hours until it becomes ash and reaches a constant weight. Put in a desiccator, weighed, and calculated % of clove leaf Simplicia ash content. Examination of the insoluble ash content material of clove leaf simplicia acid, specifically with the ash acquired within the dedication of the ash content material after which
boiled the use of 25 mL of dilute hydrochloric acid LP for five minutes. The acid-insoluble element became accumulated and filtered the use of ash-unfastened clear out paper. The acid-insoluble ash content material is calculated in opposition to the burden of the check fabric and is expressed in %w/w[17].

3. Result and Discussion

Habitat observation

Clove plant habitat is based on the results of observations and interviews conducted in clove plantations in West Batu Hamlet, Pulau Lemukutan Village, Sungai Raya Islands District, Bengkayang Regency, West Kalimantan, namely this clove plant grows in highland areas with lowlands with beaches, with a climate tropical climates, and temperatures ranging from 25°C-29°C.

Morphological observation the morphology

Clove plants were observed, namely, the tree has a height ranging from 15 to 25 meters with a distance between trees of 6 meters, the age of the clove plant is more than 50 years. Clove leaves are oval with a pointed tip, pinnate leaf bones, clove leaves with a leaf length of approximately 9 cm to 12 cm, and a width of 4 cm to 4.5 cm.

Determination

Determination Clove sample determination was carried out to ensure that the plant was used as *Syzygium aromaticum*. Based on research conducted at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak the samples used were confirmed to be true clove leaves (*Syzygium aromaticum* (L.) Merr. Perry)

The sample collection

Clove leaves (*Syzygium aromaticum*) used in this study were taken from a clove plantation in Batu Barat Hamlet RT 003/RW 001, Pulau Lemukutan Village, Sungai Raya Islands District, Bengkayang Regency, West Kalimantan. Harvesting of clove leaves (*Syzygium aromaticum*) is carried out in the morning, the clove leaves (*Syzygium aromaticum*) used in this study are clove leaves that are deciduous or yellowish. Weigh the clove leaves that have been collected. Obtained clove leaves as much as 2,145 grams.

Making simplicia

Wet sorting is done to sort out clove leaves with impurities such as leaves of other plants, parts of clove stems, roots, and soil or gravel. Separate the part of the impurity and the part of the clove leaf that will be used, weighed each part. It is known that 486 grams of clove leaves are too dry, 17 grams of twigs from clove trees and other plants, 13 grams of damaged clove leaves, and 10 grams of other leaves. So that obtained as much as 1,619 grams of clove leaves that can be used for the next process. The next step is washing. Clove leaves that have gone through a wet sorting process are then washed to reduce dirt that sticks to the harvesting process and wet sorting. Washing clove leaves using clean running water. Clove leaf washing is not carried out for too long, because it is feared that certain substances contained in the ingredients will dissolve with water.
which can lead to a decrease in the quality of the material. Clove leaf drying is done by air drying. Clove leaves are dried by air-drying because the clove leaves used are fallen clove leaves that are already dry. Clove leaf drying in this study was carried out for 1 day. Dry sorting is done after the drying process. The dried clove leaves were sorted dry to separate the clove leaves from the impurities involved in the drying process. Obtained as much as 97 grams of clove leaves that are not damaged so they can not be used for the next stage. Clove leaf simplicia that has gone through several stages is then packaged. A total of 1522 grams of dry clove leaves are packaged. Packaging aims to maintain material quality, improve material protection, maintain material safety from impurities, and maintain material quality. Clove leaf packaging uses a glass jar with a tight lid, then covered with aluminum foil to avoid the clove leaf simplicia from light. Storage is done in a dry room and not exposed to direct sunlight. Clove leaves that are stored are whole clove leaves that have been dried but have not been mashed. Stored at a temperature of 26°C to 28°C.

**Microscopic**

Microscopic observations had been finished on the Pharmacy Biology Laboratory, Faculty of Medicine, Tanjungpura University. In clove leaf simplicia, there are calcium oxalate crystals, glandular oil cells, sclerenchyma, transport bundles with dotted parenchyma, upper epidermis with stomata, transport bundles with spiral thickening. According to the research of Wirnawarti, in 2020, microscopic Clove leaf simplicia includes palisade tissue, with calcium oxalate crystals, lower epidermis fragments with stomata.

**Organoleptic**

Observations of clove leaf simplicia using the five senses, in this study clove leaf simplicia has a bitter and slightly spicy taste, has a dark brown color, with a distinctive aroma of clove leaves, and is in the form of a slightly coarse powder.

**Phytochemical screening**

Dried clove leaves were mashed using a blender to pass 60 mesh. The results of the above test showed that clove leaf simplicia obtained from clove plantations in Dusun Batu Barat, Pulau Lemukutan Village, Sungai Raya Islands District, Bengkayang Regency, West Kalimantan was positive for flavonoids, saponins, triterpenoids, and tannins and was declared free of alkaloids (Table 1). Phytochemical screening of clove leaf simplicia was carried out using the color reagent method.

**Table 1. Results of phytochemical screening of clove leaf simplicia powder**

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<th>No</th>
<th>Test Parameters Test</th>
<th>Results</th>
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<tr>
<td>1</td>
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<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>+</td>
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</tbody>
</table>
Water Content

Moisture content that does not exceed the maximum limit, which is not more than 10% will be useful for extending the durability of the material during storage. Results of the examination of the water content in this study, namely 9.21%. Clove leaf simplicia has a good water content that does not exceed 10%. Water content that exceeds 10% is feared to accelerate the growth of bacteria[20].

Ash content

In this study, the average ash content of clove leaf simplicia was 3.05%. Based on research conducted by Wirnawarti, et al ash content of clove leaf simplicia, is 11.6%. Based on the Indonesian Herbal Pharmacopoeia ash content of simplicia leaves of the same genus, namely Syzygium but different genus, namely bay leaf simplicia (Syzygium polyanthum), the ash content of bay leaf simplicia is not more than 2.5%[20].

Acid insoluble ash content

Ash content material objectives to decide infection originating from outside elements which include dust, sand, or soil, throughout the drying process. In this study, the acid insoluble ash content material in clove leaf simplicia changed into 0.69%. Acid insoluble ash content material displays the presence of steel or mineral infection that isn't soluble in acid in a material.

4. Conclusion

Characterization of the process of making clove leaf simplicia (Syzygium aromaticum) obtained from Pulau Lemukutan Village, Bengkayang Regency with a tropical climate and in the highlands with a temperature range of 25 C-32 C. Clove leaves are oval with a pointed tip, pinnate leaf bones, have a length of 9-12 cm and a width ranging from 4-4.5 cm. The manufacture of clove leaf simplicia (Syzygium aromaticum) includes harvesting, moist sorting, washing, drying, dry sorting, packaging, and storage. The effects of the calculation of the share yield of clove leaf simplicia received as a great deal as 70.95%. The simplicia characterization of clove leaves (Syzygium aromaticum) received from Pulau Lemukutan Village, Bengkayang Regency consisted of organoleptic examination, microscopic examination, phytochemical screening, water content material, ash content material, and acid insoluble ash content material. The effects of the organoleptic examination, particularly darkish brown clove leaf simplicia, barely highly spiced sour taste, and a unique aroma of clove leaves. The water content material, ash content material, and insoluble ash content material in clove leaf simplicia acid (Syzygium aromaticum) have been 9.21%, 3.05%, and 0.69%, respectively. The effects of phytochemical screening of clove leaves (Syzygium aromaticum) have been declared superb for holding flavonoids, saponins, triterpenoids, and tannins.

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