Standardization of Dengen Bark Extract (Dillenia serrata) for Emulgel Product Development

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Standardization of Dengen Bark Extract (*Dillenia serrata*) for Emulgel Product Development

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ABSTRACT

Medicinal raw materials derived from plants must be standardized in order to guarantee the quality, stability and safety of medicinal preparations containing these raw materials. Extract standardization includes two parameters, namely specific parameters and non-specific parameters. Dengen plant is one of the endemic plants of the island of Sulawesi which has been used by the community for several treatments. For the development of medicinal raw materials from this part of the dengen plant, it first needs to be standardized. This research is an experimental research. The ethanol extract of the dengen bark was obtained by extraction method and then tested for specific and non-specific parameters. The results obtained on organoleptic examination are brownish red color, characteristic odor, thick texture, and astringent taste. The content of soluble compounds in water is 6.16 ± 0.125%, the content of soluble compounds in ethanol is 44.61 ± 0.15%, contains flavonoid compounds, saponins and tannins, ash content is 4.17 ± 0.0285%, ash content is not soluble in acids. 0.024 ± 0.03%, moisture content 21.08 ± 0.209%, specific gravity 0.8256 ± 0.002 (5%), 0.8248 ± 0.005 (10%), total bacterial and mold/yeast contamination < 1.0 x 10^1 with negative mold/yeast culture, heavy metal content (mercury-Hg) 0.3335 g/g, Copper-Cu < 0.01 g/g, Lead-Pb 0.094 g/g. Based on the results of the study, the ethanolic extract of the stem bark of dengen (*Dillenia serrata*) has met the applicable standardization requirements for extracts which include specific and non-specific parameters as medicinal raw materials.

Key words : Ethanol extract of stem bark, Dengen Plant (*Dillenia serrata*), Extract Standardization

INTRODUCTION

The development of medicine is currently growing rapidly along with the increasing types of diseases suffered by humans. In addition to using modern medicine, treatment made from plant raw materials is also no less competitive for use by the community[1]. Several medicinal plants have been clinically proven to provide pharmacological effects for the treatment of several diseases. With the understanding that the side effects caused by the use of traditional medicines in small levels lead *to Back to Nature*[2].

Treatment with traditional medicine is a legacy of the cultural ancestors of the Indonesian nation. Therefore it needs to be developed and preserved. This is supported because the raw material resources of plants in Indonesia thrive so that it supports the quantity and quality of the active compounds contained in these medicinal plants.

The current use of medicinal plants still has drawbacks, one of which is that there are not so many medicinal raw materials that have been clinically or preclinically tested, where their use is only limited to hereditary use from the family[3]. To obtain medicinal raw materials from plants with high quality, standardization of medicinal raw materials is required.

An extract before being processed into a drug preparation, must first be standardized. Extract standardization is a series of parameters, procedures and measurement methods aimed at ensuring the quality, stability and safety of the drug preparation. Standardized extracts will be safer to be processed into preparations than if they are not standardized. Extract standardization includes two categories, namely specific parameters and non-specific parameters. The quality of an extract, especially regarding the specification of the content of the active ingredient and certain levels, such as water content, is indicated by the specific and non-specific parameters of the standardized

extract. These two parameters can be used by the traditional medicine industry as a national reference for plant raw materials to be further processed in the manufacture of natural medicines [4].

Dengen (*Dillenia serrata*) is one of the plants that has the potential to be used as medicine. Its use in society is still limited to the fruit, which is used as an acid in cooking. In addition to the fruit, the bark of dengen is claimed by local people as a medicine for vomiting blood[5]. Dengen is a plant endemic to the island of Sulawesi. This plant contains many active substances including polyphenolic compounds and flavonoids [5]. Some basic research has been done in exploring the ability of this plant, especially the bark.

Dengen plant is one of the local resources of the island of Sulawesi which has been used by local people both for food and as medicine. Before a plant is developed as a raw material for phytopharmaca drugs, the extract must be standardized first, then if it meets the requirements set by the POM RI, then it can then be developed into a phytopharmaca by going through several standardized tests both *in vitro* and *in vivo*.

The results of the standardization of this dengen plant extract will be the initial data and basic data for further tests. The purpose of this study was to determine the data on specific parameters and non-specific parameters of the extract of the bark of Dengen (*Dillenia serrata*) that met the requirements or did not comply with the provisions of the applicable extract standardization.

METHODS AND MATERIALS

Research design

This research is a laboratory experimental research. This research has been carried out at the Biopharmaceutical Laboratory and Chemical Laboratory of the Health Polytechnic of the Ministry of Health Makassar and there are tests at the Makassar BBLK

Population and Sample

The sample used was the bark of the dengen plant (*Dillenia serrata*) originating from Luwu Regency

Materials

The tools that have been used in this research are in the form of glassware, porcelain utensils (porcelain cup, porcelain exchanger), oven, kiln, LAF and AAS spectrophotometer. While the materials used in this study were Dengen bark extract, 96% ethanol and qualitative reagents

Procedur

1. **Making Dengen Bark Simplicia**
   The bark of the dengen plant is cleaned with running water and drained until it is not too wet. Then the simplicia is cut into small pieces of a certain size. After that, it is dried by aerating it to dry.
2. **Making Dengen Extract**
   The ethanol extract of the dengen bark was obtained by soaking the dried stem bark simplicia with 96% ethanol solvent in a maceration container until the solvent did not attract color from the simplicia residue. In this study, the remaceration process was carried out for 5 days with the help of stirring. Separation of solvent and residue is carried out by a filtering process and continued by concentration using a rotary evaporator until a thick extract is obtained. Then calculated the extract yield

$$\text{Extract yield} = \frac{\text{Total Extract}}{\text{Simplicia Weigth}}\ x\ 100\%$$

3. **Extract Standard[6,7,8]**
   a. **Specific Parameter**
      1. **Organoleptic Test**
         Organoleptic testing by involving the five senses of the extract that has been obtained. This data includes the smell, color, texture, shape and taste of the extract.
      2. **Content of Water Soluble Compounds**
         The ethanol extract of the Dengen bark was weighed as much as 2 grams. Then macerated with 50 mL of chloroform-water LP solvent for 24 hours. In the first 6 hours, repeated shaking was carried out using a *shaker* and the next 18 hours the solution was left. After 24 hours, the solution was filtered. The filtering results were pipetted as much as 20 ml. The filtrate was then heated at a temperature of 105°C until a constant weight was obtained. The concentration of water-soluble compounds was obtained against the weight of the initial extract.

$$Water\ Soluble\ Compound = \frac{(\text{container weight} + \text{residue}) - \text{container weight}}{\text{Extract Weight}} x\ 100\%$$

3. **Content of Compounds Soluble in Ethanol**
   ± 1 gram of the ethanol extract of the Dengen stem bark was weighed and then macerated in 50 mL of 95% ethanol solvent. In the first 6 hours the solution was shaken repeatedly and then left for 18 hours later. The solution is filtered as quickly as possible to avoid evaporation of the ethanol. 20 ml of the filtrate was taken and then evaporated to leave a residue. The resulting residue was heated at a temperature of 105°C until a constant weight was obtained.
   The concentration of ethanol-soluble compounds (95%) was calculated by weight of the initial extract in percent.

$$Ethanol\ Soluble\ Compound = \frac{(\text{container weight} + \text{residue}) - \text{container weight}}{\text{Extract Weight}} x\ 100\%$$

4. **Identification of alkaloids[9]**
   A number of extracts were dissolved in 2 N HCl in a test tube. If the solution is added Mayer reagent and forms a yellow or white precipitate, it is positive for alkaloids. If it produces a brown precipitate with the addition of Wagner's reagent, it is positive that it contains alkaloids. An orange precipitate is formed when Dragendroph's reagent is added.
5. **Identification of Flavonoids[9]**
   The extract was dissolved in 70% ethanol as a solvent. This mixture is then added with magnesium powder and concentrated HCl. If it produces a yellow, red or orange color precipitate, it means that it contains flavonoids (flavones, chalcones, and aurons).
6. **Identification of saponins[9]**
   A number of extracts were dissolved in hot water, then shaken vigorously for 10 seconds. If positive contains saponins, it will produce foam as high as 1-10 cm which does not disappear for 10 minutes and if 1 drop of 2 N HCl is added, the foam does not disappear.
7. **Identification of Terpenoids and Steroids[10,11]**
   A number of extracts were dissolved in ether. A layer of ether was added to the drip plate and allowed to dry. The filtrate was dried drip with two drops of acetic acid anhydride and 1 drop of H_2SO_4 concentrated. If positive for terpenoids, it will produce orange, red or yellow colors. But if it is positive for steroids it will form a green color.
8. **Identification of Tannins[10,11]**
   The extract was shaken with hot water in a test tube, then $FeCl_3$ reagent was added . If it is positive for the presence of pyrogallol tannins, it will form a characteristic blue-blue-black color. If it produces a green or blue-green color and a precipitate, it means it contains catechol tannins.

b. **Non-specific parameters[12]**
   1. **Ash Content**
      Determination of Total Ash Content .
      Into the empty weighted exchange rate, 1.5 grams of ethanol extract of Dengen stem bark was carefully added. Then the sample was put into the furnace until it glowed until a constant weight was obtained.
      The ash content is obtained by the equation below.

$$Ash\ content = \frac{(\text{container weight} + \text{residue}) - \text{container weight}}{\text{Extract Weight}} x\ 100\%$$

      Determination of Acid Insoluble Ash Content :
      As a result of the ash content determination, 5 mL of dilute hydrochloric acid was added. This solution was then boiled for 5 minutes. The part that is not soluble in acid is collected by filtering it using ash-free filter paper that has been weighed previously. The resulting residue was rinsed using hot water, then filtered and put back into the furnace until a constant weight was obtained.
      Acid insoluble ash content is obtained by the following equation:

$$\textbf{Acid Insoluble Ash Content} = \frac{(\text{container weight} + \text{residue}) - \text{container weight}}{\text{Extract Weight}} x\ 100\%$$

2. **Density**

Extract concentrations of 5% and 10% were made. Each concentration was measured density. The pycnometer used is one that has been cleaned with water and ethanol, is dry and has been calibrated. Then weighed as an empty pycnometer. The pycnometer inserted into the extract solution made, to peak at a temperature of 25 °C and weighed. The same is done for the water sample. The density of each extract and water sample was calculated, then the specific gravity was calculated using the equation below.

$$Density = \frac{\text{Extract Density}}{\text{Water Density}}$$

3. **Water content**

Yag porcelain into the exchange rate has been calibrated previously included as much as 2 g extract carefully, and heated in an oven at 105 °C to leave the ashes. The exchange rate is then cooled in a desiccator and then weighed. The above work is done repeatedly until a constant weight is obtained. The percentage of water content is obtained by the following equation

$$Water\ Content = \frac{(\text{container weight} + \text{residue}) - \text{container weight}}{\text{Extract Weight}} x\ 100\%$$

4. **Microbial contamination**

The ethanol extract of the Dengen stem bark was weighed, then dissolved in 10 mL of sodium CMC suspension as a dilution of 10^{-1}. From this dilution the next 5 concentrations were made. That is 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}.

a. Determination of Bacterial Contamination (Total Plate Number (ALT))

From each dilution, 1 ml was pipetted, then put into a previously sterilized petri dish. After that, 15 mL of nutrient agar (NA) media was added. Kemudin samples incubated in an incubator at 37 °C for 24 hours.

Observations were made by counting the number of colonies that grew and multiplying by the dilution factor. The work was carried out three times.

b. Determination of Mold/Yeast Contamination

Into a sterile petri dish, 1 mL of each dilution was added. Then 15 ml of Potato Dextrose Agar (PDA) media was added until homogeneous.

Samples were incubated at room temperature for 3 days. Observations were made by counting the number of colonies that grew and multiplying by the dilution factor. Done three times.

5. **Heavy Metal Content**

Put about 10 g of the substance into a crucible, and carefully incandescent at low temperature until charred. During annealing, the crucible should not be tightly closed. On the part that has been charred add 2 ml of concentrated nitric acid, heat carefully until white smoke is no longer formed. Incandescent at a temperature of 500 °C to 600 °C until the charcoal burns out. Then cooled and dissolved in HNO_3 1% in a 25 ml flask, filtered with ash-free filter paper. Observed by AAS (Atomic Absorption Spectroscopy). Calculate the content of the initial extract.

RESULTS

Table 1. Extract yield

Dry simplicia weight (g)	Extract Weight (g)	Extract Yield (%)
500 g	70.18	14,036

Table 2. Specific Parameters

Specific Parameter		Results
Organoleptic	Color	Brownish red
	Smell	Typical

	Texture	Thick
	Flavor	Sepat
Content of Water Soluble Compounds		6.16 ± 0.125%
Content of Compounds Soluble in Ethanol		44.61 ± 0.15%
Identification of Active Compounds	Alkaloids	(-)
	Flavonoids	(+)
	Saponins	(+)
	Terpenoids and Steroids	(-)
	Tannins	(+)

Table 3. Non-Specific Parameters

Parameter	Score
Ash Level	4.17 ± 0.0285%
Water content	21.08 ± 0.209%
Acid Insoluble Ash Content	0.024 ± 0.03%
Specific Gravity	0.8256 ± 0.002 (5%) 0.8248 ± 0.005 (10%)
Total Bacterial Contamination	< 1.0 x 10^{1}
Total Mold Contamination	< 1.0 x 10^{1}
Mold/Yeast Culture	Negative
Heavy Metal Content (Mercury-Hg)	0.3335 g/g
Heavy Metal Content (Copper-Cu)	< 0.01 g/g
Heavy Metal Content (Lead-Pb)	0.094 g/g

DISCUSSION

Extract standardization is a requirement for extracts to meet the requirements listed in the official monograph published by the Ministry of Health[13]. Standardization of this extract aims to ensure uniformity of active components, safety, quality and benefits of the ingredients in question. Extract standardization includes two parameters, namely specific parameters and non-specific parameters.

Dengen bark ethanol extract was obtained by maceration extraction method. This activity was carried out until the simplicia did not produce color anymore, in this case the Dengen stem bark was macerated for 10 days with 5 times changing the solvent. This maceration method was chosen because it wanted the active compound to be produced to remain stable without any damage due to high heating[11]. In this extraction using ethanol solvent, where ethanol solvent is a solvent that is semipolar so that it can attract non-polar compounds and polar compounds so that it is expected that the number of compounds that are attracted can be qualitative and quantitative.

A. Specific Parameter

Specific parameters in this study include organoleptic, water soluble compounds content, levels of soluble compounds in ethanol and qualitative examination of active compounds including alkaloids, flavonoids, saponins, terpenoids and steroids, and tannins.

Organoleptic examination describes how the shape (texture), smell, color and taste. Determination of the organoleptic parameters of this extract aims to provide an objective and simple visual description of the extract using the five senses. Dengen bark ethanol extract is a thick extract with a hard texture and has a brownish red color. This extract has a distinctive smell and astringent taste. This is most likely due to the high content of tannins in this extract.

The next examination is the levels of water-soluble and ethanol-soluble compounds. This examination aims to determine the lowest quantity of soluble compounds in the two solvents, in this case where the water solvent is a polar solvent group while the ethanol solvent is a semipolar solvent. The results obtained in this study were compounds that were soluble in water by 6.16 ± 0.125% and those soluble in ethanol were 44.61 ± 0.15%. This indicates that the content of soluble ethanol extract is greater than that of water soluble. This is because the solvent used in extracting simplicia is ethanol solvent, so the compounds that have been attracted will mostly dissolve back into the ethanol solvent compared to the water solvent.

Qualitative examination of active compounds including alkaloids, flavonoids, saponins, terpenoids and steroids, and tannins. This examination aims to identify the presence or absence of these compounds in the ethanol extract of Dengen stem bark. This qualitative identification uses compound group reagents to produce color and or precipitate if these compounds are contained in the extract. The identification results showed that the ethanolic extract of the bark of Dengen contained flavonoids, saponins and tannins. These compounds will later

be responsible for providing pharmacological effects. Flavonoid compounds and tannins are compounds belonging to the polyphenol group which generally act as antioxidants[14]. The presence of polyphenolic compounds has been determined quantitatively in the Sinala S (2019)[15] study showing a yield of 445.02 mg/g gallic acid with an antioxidant effect at an IC_{50} value of 48.33 ppm.

B. Non Specific Parameters

Examination of non-specific parameters included water content, ash content, acid insoluble ash content, heavy metal content, specific gravity and total bacterial and mold contamination.

The water content in an extract gives an idea of the amount of water contained in an extract. This content is related to the purity of a chemical content. Large amounts of water can increase the growth rate of fungi and bacteria. This condition makes it easy for the hydrolysis process of chemical metabolites in the extract. According to anonymous, the water content in a simplicia or extract should be less than 10%. If the water content exceeds this limit, an enzymatic process will occur by enzymes that destroy chemical metabolites in the extract, such as hydrolase, oxidase and polymerase[16]. In the long storage process, simplicia and extracts can be damaged due to the enzymatic process where these enzymes can convert chemical metabolites with pharmacological effects into other compounds which most likely do not provide pharmacological effects like the original compounds. This causes the quality of an extract to decrease due to contamination which will eventually damage the extract[18]. Based on this, the removal of water content up to a certain amount is useful for extending the durability of the material during storage[19]. because the damage mentioned above will not occur if the dried material has a low moisture content.

The results obtained showed that the Dengen bark extract contained 21.08 ± 0.209% water. This result is in accordance with the requirements for the water content in a thick extract, which is between 5-30%. The water content of the extract of this dengen stem bark shows that the drying process in simplicia is not optimal, so it requires a maximum level of drying.

Determination of ash content aims to determine the content of mineral compounds that are internal and external which are obtained during the simplicia growth period. The ash obtained from the annealing process shows that inorganic compounds or mineral salts are non-volatile in nature, while the organic compounds contained in the extract by annealing process at high temperatures will evaporate. The ash content indicates the level of purity of a material (extract) where the lower the value of the ash content of a material, the higher the purity of the material so that the better the quality [19]. The high and low ash content of a material is caused, among other things, by the different mineral content in the source of the raw material and can also be influenced by the demineralization process at the time of manufacture. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing[12].

In this study, the total ash content obtained was 4.17 ± 0.0285% and the acid insoluble ash content was 0.024 ± 0.03%. These results have met the requirements where the ash content is not more than 8% and but the acid insoluble ash content has not met the requirements, namely not less than 1%.

Parameters of heavy metal content were determined using the atomic absorption spectrophotometer (AAS) method. Determination of the levels of heavy metals in an extract is carried out to ensure that the extract does not contain harmful metals that exceed the limits set by the government. Excess heavy metal content can cause toxicity to the human body, causing disease. Heavy metals will be into red blood cells. Although, they are eliminated by the urine but some of heavy metal like lead accumulated in the skeleton, and are released only slowly from this body compartment[20]. The results of the examination showed that the examination of heavy metal levels included mercury (0.3335 g/g), copper (< 0.01 g/g) and lead (0.094 g/g). These results have met the requirements for the maximum limit of lead metal contamination in spices according to the Decree of the Director General of POM No.03725/B/SK/VII/89, where the maximum limit of Hg metal contamination is a maximum of 0.5 ppm and Pb metal is equal to or equal to 10 mg /kg.

Determination of specific gravity aims to determine the purity of an extract where the extract is not contaminated or mixed with other ingredients. Specific gravity was determined using the pycnometer method. This determination uses two extract concentrations, namely 5% and 10% with the aim of comparing the results to be obtained. If the results of both are in the same range, then the specific gravity data is valid. The results obtained from the measurement of specific gravity with 5% extract are 0.8256 ± 0.002 while 10% extract is 0.8248 ± 0.005.

Microbial contamination testing includes bacterial and mold/yeast contaminants. This test is one of the tests for the purity requirements of the extract. This test is carried out to determine the amount of microorganism contamination contained in an extract and to indicate the presence or absence of certain bacterial growth in the extract. The number of microbes present in an extract indicates the quality of the material, because

the amount of bacterial or mold/yeast contamination that exceeds the limit can decompose the active substances in the extract through hydrolysis or enzymatic processes so that it will damage the extract. According to the book Medicinal Plant Extracts Monograph stating that the maximum limit microbial contamination that has been set is 10^4 colony / g and to mold which is 10^3 colonies/g [21].

The test results obtained showed the number of molds/yeasts and bacterial ALT with both values <1.0 x 10^1 colony/g, and there was no mold/yeast culture.

CONCLUSION

Based on the results of the study, the conclusion that can be drawn from this study is that the ethanolic extract of the stem bark of dengen (*Dillenia serrata*) has met the requirements for standardization of applicable extracts which include specific and non-specific parameters as medicinal raw materials.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7