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Original Article

QUALITY STANDARDIZATION OF LONGAN SEED EXTRACT (*EUPHORIA LONGAN* STEND)

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ABSTRACT

Objective: Economic development in urban areas is a big attraction that causes urbanization so that the population increases. This has an impact on health, including skin health. One of the skin disorders is acne. Acne can be overcome with the use of traditional medicine. Longan Fruit Seed Extract contains phytochemical compounds that act as antibacterial and antioxidant. The purpose of this study was to obtain scientific data on the standardization of the quality of longan seed extract.

Methods: Longan seeds were made simplicia, extracted by sokhletasi method and tested for standardization of extract quality.

Results: The results of this study for specific parameters obtained extract identity, organoleptic test data form thick (sticky), glossy brown color, has a distinctive odor like longan flesh and sweet taste. The content of water-soluble compounds was 67.02±1.84, ethanol-soluble was 25.62±0.57. The total flavonoid content calculated as quercetin was 0.27±0.041. Non-specific parameters obtained data on water content 27.80 %±0.82, total ash content 1.58%±0.05, acid insoluble ash content 0.066 %±0.04, ALT 64x102 colonies/g, AKK 7x102 colonies/g, 0.0094 ppm Hg, 0.96 ppm As and 0.031 ppm Pb.

Conclusion: Longan seed extract meets the requirements for standardization of extract quality.

Keywords: Extract, Longan fruit seed, Extract quality standardization

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INTRODUCTION

Longan is a symmetrical tree with dense dark green foliage. This plant can grow to over 100 feet (31 m), depending on climate and soil type. The fruit has round to ovoid seeds with a diameter of 22-36 mm, weight 6-19 g. The skin of the fruit is brown or light brown, rough and smoother than lychee. The flesh of the fruit is whitish and translucent, with large seeds. The fruit has 1 seed, round and shiny, brown to dark brown. Flesh does not stick to the seeds, is flavorful, sweet and contains 12–21% dissolved solids [1].

The ethanol extract of longan fruit seeds contains polyphenols (tannins) and flavonoids, with a total polyphenol content equivalent to 17.24% gallic acid and 57.24 ppm IC 50 content. shows active antioxidant power (50–100 ppm) [2]. Longan (Euphoria Semen) seed extract has antibacterial activity that can inhibit the growth of Propionibacterium acnes and Staphylococcus aureus with 2% MIC value and 3% MKC value. This is because the seeds of longan fruit contain phenolic compounds [3].

Standardization in pharmaceuticals is nothing but a series of parameters, procedures and measurement methods, the results of which are elements related to the pharmaceutical quality paradigm, quality in terms of meeting standard requirements (chemistry, biology and pharmacy), including guarantees (limits) of stability as pharmaceutical products in general. Extract quality requirements consist of various general standard parameters and specific standard parameters. The definition of standardization also means the process of ensuring that the final drug product (drug, extract or extract product) has certain parameter values that are constant and predetermined. Given that herbal medicines and various plants have an important role in the health sector, they can even become Indonesia's mainstay products, it is necessary to make efforts to establish quality and safety standards for medicinal plant extracts [4, 5].

MATERIALS AND METHODS

Extract making

Sampling

Seed samples were taken from longan fruit obtained from a fruit shop in Makassar.

Sample processing

Longan fruit that has been washed with running water is peeled and separated from the seeds. Longan seeds are separated from the flesh. Longan seeds are made into coarse powder and then dried by aerating.

Extraction longan fruit seeds

Longan seed simpliciaweighed 100 g put into a lead that has been lined with filter paper, moisten first using 96% ethanol. Enter ethanol 96% approximately 400-500 ml into a round bottom flask and then installed with a condenser. The heating and cooling coats are run and counted for 25 cycles. The extract obtained was evaporated with rotafavor to obtain a thick extract.

Phytochemical screening

Identification of tannins

The test solution is added with 3% iron (III) chloride, if it is positive, it contains tannin, a bluish-green to blackish color will occur.

Identification of flavonoids

The test solution was evaporated to dryness, then 2-3 drops of ethanol were added. Next, magnesium powder and a few drops of 5 M hydrochloric acid are added. If the presence of flavonones, flavonols, flavonols and dihydroflavonols is positive, a red to purple color will appear.

Alakloid identification

The test solution was added 5 ml of 1 N hydrochloric acid, then 3 drops were added to the watch glass, added Meyer's reagent. Note the color of the precipitate formed.

Identification of saponins

The extract was added with 10 ml of water (if necessary, heated briefly on a water bath) to produce a stable foam with the addition of hydrochloric acid.

Determination of standardization parameters

Specific parameters

Extract identity

The identity of the extract includes a description of the nomenclature consisting of the name of the extract (generic, trade, patent), the Latin name of the plant of origin (botanical systematics), the part of the plant used and the Indonesian name of the plant.

Extract organoleptic determination includes shape, color, taste, and smell.

Determination of the concentration of dissolved compounds in a particular solvent.

The concentration of water-soluble compounds

A total of 2.5 grams of the test extract was soaked with 50 ml of a mixture of water and chloroform LP in a cork flask. The immersion was carried out for 24 h and was shaken several times for the first 6 h and then filtered. A total of 10 ml of the filtrate was measured and put into an evaporating dish whose weight was known. The residue was heated at a temperature of 105 °C until a constant weight was obtained. The content of water-soluble compounds was calculated against the weight of the initial extract and expressed in percent (%). The test was carried out 3 times.

Content of compounds soluble in ethanol

A total of 2.5 grams of the test sample was soaked with 50 ml of 95% ethanol for 24 h in a closed flask while being shaken repeatedly for the first 6 h and then left again. After 24 h filtered quickly to avoid evaporation of ethanol. 10 ml of the filtrate was measured put into an evaporation cup of known weight. The residue was heated at 105 °C to a constant weight. Calculated ratein percent of ethanol-soluble compounds to the weight of the initial extract. The test was carried out 3 times.

Analysis of total flavonoid content

Flavonoid qualitative test

The tested extract was added with ethanol, added a little magnesium metal and a few drops of concentrated HCl. Contains flavonoids if a magenta red solution is formed.

Flavonoid quantitative analysis

Preparation of standard stock solution

A total of 10 mg of quercetin was dissolved in 10 ml of ethanol pa; then the volume was made up to 100.0 ml (100 ppm).

Maximum wavelength determination

A series of dilutions were made with a concentration of 30 µg/ml using 10 ml volumetric flask+100 µl Aluminum (III) chloride 10%+100 µlNat. 1 M acetate and then distilled water was added until the volume was sufficient 10.0 ml. The solution was left for 30 min then the absorbance was measured at a wavelength of 400-800 nm.

Quercetin raw curve manufacturing

Dilutions were made in a series of 10, 20, 30, 40, and 50 µg/ml then each dilution was put into a 10 ml volumetric flask. 0.5 ml of each concentration was put into a 10 ml volumetric flask+100 µl Aluminum (III) chloride 10%+100 µllar. 1 M sodium acetate was then added with distilled water until the volume became 10 ml. The solution was left for 30 min then the absorbance was measured using a wavelength of 400-800 nm.

Determination of total plavonoid content

The extract was weighed 0.25 g and dissolved in 50 ml of 70% ethanol (5000 µg/ml). A total of 0.5 ml each cons. put into a 10 ml volumetric flask, then+100 µl Aluminum (III) chloride 10%+100 µlNat solution. acetate 1 M and then made up to 10 ml using distilled water. The solution was left for 30 min then the absorbance was measured at a wavelength of 400-800 nm.

Non-specific parameters

Water content parameters

The covered porcelain crucible was heated at 105 ° for 90 min and weighed. A total of 1 g of the test extract was weighed in the porcelain crucible. The extract was flattened by shaking it to form a layer of 10-15 mm thick. It is then dried at the setting temperature until a constant weight is obtained. Open the lid of the crucible and cool in a desiccator to room temperature. The fixed weight obtained was recorded to calculate the percentage of drying loss. It was replicated 3 times.

Parameters of total ash content

The test extract was carefully weighed as much as 2 g in a porcelain crucible that had been weighed. Slowly incandescent then the temperature was increased gradually until it reached 600±250 °C until carbon-free. Put it in a desiccator until it cools down, then the weight of the ash is weighed. Ash content is calculated in percent by weight of the initial sample. Three times replication.

Ash content which is not soluble in acid

The results obtained in the determination of the ash content were continued for testing the acid insoluble ash content by adding 25 ml of dilute hydrochloric acid is left for 5 min. The acid-insoluble part was then collected by filtering using ash-free filter paper, washed with hot water, filtered and weighed. Acid insoluble ash content is determined in percent of the weight of the initial sample. It was replicated three times.

Determination of total bacteria and total mold

Determination of the total bacterial plate number

Dilutions of 10^{-1} to 10^{-6} were made, 1 ml of each dilution was pipetted and put into sterile petri dishes (made in duplicate). Add 15-20 ml of PCA, homogenized and allowed to solidify. Incubate at 35-37 °C for 24-48 h. Diluent blanks and media were made to determine their sterility.

Determination of mold and yeast numbers

Take 1 ml of the extract from the 10^{-1} dilution using a syringe, put it into a sterile test tube which has been filled with 9 ml of the diluent solution and continue again until a 10^{-6} dilution is obtained. Each dilution was measured 0.5 ml transferred into a petri dish and PDA media was added (made in duplicate). Homogenize and incubate 20-25 °C for 5-7 d. Blanks were also made to test the sterility of the solvents and media used.

Determination of heavy metal limit

Determination of metal limits for Arsenic (As), Mercury (Hg), Lead (Pb) was carried out using Atomic Absorption Spectrophotometer (AAS) in accordance with the working method of the Makassar Health Laboratory Center.

RESULTS AND DISCUSSION

This research is a test of the Independent Technology of Drug Raw Materials, which consists of making extracts which include taking raw materials, processing into simplicial to extractionLongan Seeds [6]. Continuing to standardize the quality of the extract as stated in the [4] that is determination specific parameters (identity assignment, organoleptic determination, levels of water-soluble compounds, levels of ethanol-soluble compounds, and analysis of total flavonoid content). Non-specific parameters (water content parameters, total ash content parameters, acid insoluble ash content, determination of total bacteria and total mold, determination of metal limits (lead, arsenic and mercury).

Longan seeds are separated from the skin and flesh, then cleaned and dried by aerating. The dried simplicia was then coarsely ground and extracted using the Sokhletasi method. The extraction results are obtained as shown in table 1 below.

Table 1: Yield of longan fruit seed extract

No.	Name simplicia	Simplicia weight (g)	Extract weight (g)	Yield (%)
1	Longan Seed Extract	449.86	105.96	23.55

Identification of a compound contained in plants can be done by using a color reaction. Based on the tests that have been carried out, longan seed extract contains tannins, flavonoids and alkaloids (table 2).

Table 2: Phytochemical screening test results

Plant part	Compound group			
	Tannin	Flavonoids	Alkaloids	Saponins
Longan seeds	+	+	+	-

Extracts that will be used as raw materials for the manufacture of traditional medicines must be given an objective identity from the name of the extract, the Latin name, the part of the plant used and the Indonesian name of the plant. The identity of the Longan Seed extract can be seen in the following table.

Table 3: Extract identity

Extract name	Euphoria extractum (Longan extract
Latin names of plants	*Euphoria longana* steud
Part of the plant used	Cement Euphoria
Indonesian names of plants	Longan

Organoleptic tests were carried out to determine the shape, color, smell and taste of the samples. The test is based on direct observation using the five senses. The results of organoleptic testing can be seen in the following table.

Table 4: Organoleptic test results longan seed extract

No.	Organoleptic test	Results
1.	Shape	Thick
2.	Color	Shiny chocolate
3.	Smell	Typical
4.	Flavor	Sweet

Another specific parameter that was tested was the determination of the concentration of compounds soluble in solvents, namely water, and ethanol. In addition, an analysis of the total flavonoid content was also carried out using quercetin as a standard for comparison. The results of these tests can be seen in the following table.

Table 5: Specific parameter test results

No.	Parameter	Results (%)
1.	The concentration of water-soluble compounds	67.02±1.84
2.	Content of compounds soluble in ethanol	25.62±0.57
3.	Total flavonoid content calculated as quercetin	0.27±0.041

The non-specific parameters tested in this study were water content, total ash content and acid-soluble, bacterial and yeast contamination as well as heavy metal testing (As, Hg and Pb).

Table 6: Non-specific parameter test results

No.	Parameter	Results	Condition
1.	Water content	27.80 %±0.82	5-30% [7]
2.	Ash content:		
	- total ash	1.58%±0.05	-
	- Acid insoluble ash	0.066 %±0.04	-
3.	Microbial contamination:		
	- ALT	64 x 10^2 colonies/g	1 x 10^4 colonies/g
	- AKK	7 x 10^2 colonies/g	1 x 10^3 colonies/g [8]
4.	Heavy metal contamination:		
	- Hg	0.0094 ppm	0.5 ppm
	- US	0.96 ppm	5 ppm
	- Pb	0.031 ppm	10 ppm [9]

DISCUSSION

Longan fruit seeds are part of the longan fruit that is not used by the community so that it can be included in the household organic waste group. However, this section still contains phytochemical compounds that have an activity to increase the body's resistance so that it can prevent and treat disease. The ethanol extract of longan fruit seeds contains polyphenols (tannins) and flavonoids and has an active antioxidant activity, namely IC50 57.24 ppm [2]. Due to its phenolic content, this extract has antibacterial activity against Staphylococcus aureus and Propionibacterium acne [3]. Thus, longan seeds have the potential to be developed as traditional medicine.

Before developing plants into traditional medicines, testing must first be carried out to produce standardized extracts so that later stable preparations are obtained both in quality and physically. This test includes non-specific and specific parameters [4].

449.86 g of dried simplicia from Longan seeds were extracted using the sokhletasi method. Sokhletasi is an extraction method which consists of three parts, namely a round bottom flask, a sleeve and a condenser. The extraction process uses two sets of tools that are run simultaneously; then the extraction process is carried out again until all simplicia is used up. according to [10], simplicia in the form of dried seed powder can be extracted using Sokhlet with various solvents. The sample is put into a sleeve, while the solvent is placed in a round bottom flask. The filter used is 96% ethanol. Ethanol is used as a filter because it is more selective, the resulting extract is difficult for microorganisms to grow, non-toxic, neutral, has good absorption, can mix with water in all ratios and requires little heat to concentrate the extract [11]. So that the filter in the round bottom flask can get to the sleeve, it needs heating assistance. Heating causes the liquid to evaporate through the steam pipe and into the back cooler (condenser). The results of the condensation fall into the casing containing the simplicia and extract the chemical components present in the cell. The filter liquid that already contains the compound will return to the round bottom flask through the siphon pipe. The extraction process will take place continuously with a relatively constant amount of solvent [12]. After the extraction process, the extract obtained was then concentrated with an evaporator and continued with evaporation using a water bath to obtain a thick extract. From 449.86 g of dried simplicia, 105.96 g of thick extract was obtained so that the yield of the extract was 23.55 %.

Tannins, flavonoids and alkaloids are secondary metabolites produced by plants. In general, these secondary metabolites play a role in self-defense against other organisms. The content of secondary metabolites depends on the biotic and non-biotic factors of the growing environment. Secondary metabolites produced by plants have pharmacological effects. Identification is based on literature [12]. For the tannin test, the ethanol extract solution, when added with 1% $FeCl_3$ will form a blue-green to blackish color. From the tests carried out, the tannin test of the Longan Seed extract was blue-black so that it was positive for tannin. The flavonoid test was carried out by adding Mg powder to the ethanol extract, then added with 5 M HCl and a red solution was formed, which indicated that the sample contained flavonoids. Test for alkaloids by adding an extract solution with 1 N HCl, plus Meyer's reagent and a white precipitate is formed, which indicates the longan seed extract contains alkaloids. In the saponin test by adding the extract with water and then heating it in a water bath, after shaking it did not form foam, which means the sample did not contain saponins.

Specific parameter testing is carried out to determine the type and content of the compound contained in the extract, whether it is the original compound from the plant or the compound resulting from changes from the original compound. The test results have a direct effect on the pharmacological effects of the plant [4]. Parameters carried out in this study included extract identity, organoleptic test, levels of dissolved compounds in solvents and analysis of total flavonoid content.

The identity of the extract must be given to the extract being tested in the form of objective information, which includes the name of the extract, the Latin name, the part used and the Indonesian name of the plant. The extract used in this study was longan seed extract (Euphoria Extractum) derived from the Euphoria longanaStend plant. In Indonesia, this plant is known as longan (extract identity in table 3).

Longan seed extract, after being extracted with ethanol by sokhletasi and evaporation by rotafavor obtained a thick extract that is brown, shiny and sticky when held. There is a distinctive smell of longan flesh and a sweet taste (table 4).

Determination of the levels of dissolved compounds in the solvent is carried out to determine the amount of solute that is identical to the number of compounds contained in the extract. The test was carried out by the gravimetric method. The determination is made of compounds that are soluble in water and compounds that are soluble in ethanol. The assay was carried out by heating the test extract several times until a constant weight was obtained. The ratio between the weight after heating and the weight of the extract multiplied by one hundred was expressed as the concentration of dissolved compounds in the solvent. In this test, the average levels of compounds dissolved in water were 67.02%±1.84, which means between 65.19%-68.86%. The average concentration of compounds dissolved in ethanol is 25.6%±0.57, so the range of compounds soluble in ethanol is 25.05%–26.19%. Based on these results, it can be stated that the compounds contained in the extract are more polar. The test results can be seen in table 5.

Determination of flavonoid content is carried out to provide information on the content of these compounds in plant samples, the content of these compounds is related to their pharmacological activity. In this test, standard quercetin was used so that the total flavonoid content obtained was quercetin. The test was carried out by replicating 3 times to obtain more valid data. The results obtained were 0.27%±0.041 (between 0.229–0.311 %) total flavonoid content which was calculated as quercetin (table 5).

Standardization of the quality of extracts of non-specific parameters to see the presence or absence of contaminating compounds or compounds resulting from interactions between the contamination compounds and the original compounds or with altered compounds. The results of this test can indirectly affect the pharmacological activity of the plant [4]. The non-specific parameters tested in this study were water content, ash content, microbial contamination and heavy metal contamination.

Determination of the water content in the extract was carried out by the gravimetric method. The aim is to provide a min. or range regarding the amount of water content in the sample. The extract was weighed and ignited several times until a constant weight was obtained. Moisture content can be calculated by subtracting the weight of the extract before annealing by the weight after annealing divided by the weight of the extract which was weighed and then multiplied by one hundred. The average moisture content of Longan Seed extract was 27.80%±0.82 (26.98%–28.62 %). according to [7], the water content for the thick extract is 5–30%, thus the water content obtained meets the requirements.

The ash content test consists of total ash and ash that is not soluble in acid. This test is carried out by igniting the material so that the organic compounds and their derivatives are destroyed and evaporated and the remaining mineral and inorganic elements remain. This parameter will describe the internal and external mineral content from the initial process to the extract. Determination of acid insoluble ash content is carried out by proceeding from the resulting ash from the total ash content. The total ash content of Longan Seed extract was 1.58%±0.051 (1.53%-1.63%) and that which was not soluble in acid was 0.065 %±0.035 (0.030%-0.10%).

Microbial contamination tests carried out were ALT and AKK counts. This parameter is carried out to determine the presence or absence of microbial contamination in the extract so as to provide assurance that the extract does not contain microbial contamination as required because it will interfere with health. according to [8], the maximum limit for total bacterial contamination is 1×10^4 colonies/g and for total yeast, molds is 1×10^3 colonies/g. The ALT results obtained for Longan Seed extract were 64×10^2 colonies/g and AKK was 7×10^2 colonies/g and these results met the requirements [8].

Tests are carried out to provide assurance that the extract does not contain certain heavy metals because it can be toxic. The heavy metals tested were mercury, arsenic and lead; the tests were carried out at BBLK. The maximum levels for Mercury are 0.5 ppm, 5 ppm Arsenic and 10 ppm Lead. The results obtained in this study were 0.0094 ppm, 0.096 ppm and 0.031 ppm, respectively. The results obtained meet the requirements [9].

CONCLUSION

Based on the results obtained, it was concluded that the testing of specific and non-specific parameters of the Longan Fruit Seed extract (Euphoria longanaStend.) met the requirements for standardization of extract quality.

SUGGESTION

It is recommended that the standardization test for the quality of Longan Seed extract is completed in order to complete the scientific data so that it can be used as a standard for the manufacture of traditional medicines.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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