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Standardization of *Polyscias fructicosa* (L.) harms leaf ethanol extract

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Abstract

Polyscias fruticosa (L.) Harms is native to Indonesia and is one of the plants from the Araliaceae family. The purpose of this study was to determine the characterization of ethanol extract of *Polyscias fruticosa* (L.) Harms. Samples were obtained in Soppeng district, South Sulawesi Province, macerated using ethanol 96% as much as 3 liters, then tested for specific and non-specific parameters, specific parameters include extract identity, organoleptic examination, determination of water soluble content and determination of ethanol soluble content, and identification of chemical content, while non-specific parameters include drying shrinkage, determination of specific gravity, determination of water content, determination of total ash content, determination of acid insoluble ash content, microbial contamination, heavy metal contamination and residual solvent. The results showed that ethanol extract of *P.fructicosa* gave results in accordance with the general standard parameters of medicinal plant extracts with specific parameter values as follows: thick texture, blackish green color, distinctive odor; water soluble content of 5.69%; ethanol soluble content of 5.92%; positive for alkaloid, polyphenol, tannin, flavonoid, steroid, and saponin compounds; and non-specific parameter values as follows: drying shrinkage 0.58%; specific gravity 0.817 grams; moisture content 6.28%; total ash content 8.7%; acid insoluble ash content 0.4%; total plate count (TPC) of bacteria < 1.0 x 101 colonies/g, mold number < 1.0 x 101 colonies/g and yeast < 1.0 x 10 colonies/g. 1.0 x 101 colonies/g; Lead (Pb) metal contamination of < 0.00001 µg/g and Cadmium (Cd) of < 0.00001 µg/g; GC-MS analysis showed that the compound with the highest area was Trichloromethane.

Keywords: Extract, *Polyscias fruticosa*, Standardization.

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1. Introduction

Indonesia is one of the countries with the largest medicinal plants in the world. One of the medicinal plants in Indonesia is *Polyscias fruticosa* (L.) Harms, in Indonesia *P.fructicosa* is used as a spice because it can improve taste and organoleptic properties [1], while in traditional Vietnamese medicine, leaves are used as anti-oxidants, anti-stress, improve memory, increase appetite, and as anti-fungal and anti-bacterial [2]. *Polyscias fruticosa* (L.) Harms. contains bioactive components such as alkaloids, glucosamine, saponins, flavonoids, and also tannins [3], In research conducted by Handayani et al., 2020 In the study, three different types of extracts were used, namely n-hexane extract, ethyl acetate extract and 96% ethanol extract, which had a total flavonoid compound content of 0.09345 gQE/g extract or 9.345% *P.fructicosa* leaves had strong antioxidant activity [4].

2. Materials and methods

The tools used in this research are glassware (Pyrex), porcelain cup, petri dish, chamber, dexicator, erlenmeyer with lid, incubator (Memmert), oven (Memmert), pycnometer (Yanaco), rotary evaporator (Ika® RV 10 basic), atomic absorption spectrophotometer (Biobase), furnace (B-ONE), analytical balance (Kern ABJ-NM) and waterbath (Memmert). The materials used in this study are water, distilled water, P. fructicosa leaves, dimethyl sulfoxide (DMSO), 96% ethanol, $FeCl_3$, HCl 2N, H_2SO_4, concentrated HCl, filter paper, GF silica plate254 , NA medium (Nutrient Agar), PDA medium (Potato Dextrose Agar), nitrogen P, Liebermann-Burchard reagent, Mayer reagent and Dragendorff reagent, Mg powder.

2.1. Extract Preparation

Samples of P. fructicosa were taken in Maccile Village, Lalabata District, Soppeng Regency, South Sulawesi Province. *P.fructicosa* leaves that have been obtained are cleaned from the dirt attached by washing with running water then cut into small pieces, after which they are dried in a drying cabinet with a temperature of ± 500 C, then pollinated, and ready for extraction. The dried simplicia that has been pollinated is then weighed as much as 250 grams, then the extraction process is carried out with a maceration process using ethanol 96% for 3 days, then filtered and the liquid extract is evaporated using a rotary vacuum evaporator.

2.2. Extract Identity

The identity parameter of the extract is carried out by providing a naming description that includes the name of the extract, the Latin name of the plant, the plant part used, and the Indonesian name of the plant [5].

2.3. Organoleptic

Organoleptic tests are conducted using the five senses to describe the shape, color, smell, and taste of plant extracts [5].

2.4. Water Soluble Compound Content

Macerate 5.0 grams of extract for 24 hours with 100 mL of LP chloroform water using a stoppered flask while shaking repeatedly for the first 6 hours and then leave for 18 hours. Filter, evaporate 20 mL of the filtrate to dryness in a flat-bottomed cup that has been leveled, heat the residue at 105ºC until the weight remains. Calculate the content in percent of water-soluble compounds calculated against the initial extract [5].

2.5. Ethanol Soluble Compound Level

Macerate 5.0 grams of extract for 24 hours with 100 mL of ethanol (95%) using a stoppered flask while shaking repeatedly for the first 6 hours and then leave for 18 hours. Filter, evaporate 20 mL of the filtrate to dryness in a flat-bottomed, pre-weighed cup, heat the residue at 105o C until the weight remains. Calculate the content in percent of ethanol-soluble compounds (95%) calculated against the initial extract [5].

2.6. Determination of Specific gravity

Use a clean, dry and calibrated pycnometer by determining the weight of the pycnometer and the weight of freshly boiled water at 25°C. Set the temperature of the liquid extract to approximately 20°C, put it into the pycnometer. Set the temperature of the filled pycnometer to 25°C, remove excess liquid extract and weigh. Subtract the weight of the empty pycnometer from the weight of the filled pycnometer. The specific gravity of the liquid extract is the result obtained by dividing the weight of the extract by the weight of water, in a pycnometer at 25°C [5].

2.7. Determination of Moisture Content (Gravimetric Method)

Put approximately 10 grams of extract and weigh carefully in a pre-weighed container. Dry at 105°C for 5 hours and weigh. Continue drying and weighing at 1 hour intervals until the difference between 2 consecutive weighings is not more than 0.25%. Determination of content by this method is not suitable for extracts that have a high essential oil content. In such cases this method is more appropriately called drying shrinkage determination [5].

2.8. Determination of Ash Content

Approximately 2 g to 3 g of the extract that has been crushed and weighed carefully, put into a silicate crust that has been heated and leveled, leveled. Incinerate slowly until the charcoal runs out, cool, weigh. If in this way the charcoal cannot be removed, add hot water, filter through ash-free filter paper. Incinerate the remaining paper and filter paper in the same crucible. Put the filtrate into the crucible, evaporate, incandesce to a fixed weight, weigh. Calculate the ash content against air-dried material [5].

2.9. Determination of Acid Insoluble Ash Content

The ash obtained in the determination of ash content, boil with 25 ml of dilute sulfuric acid P for 5 minutes, collect the part that does not dissolve in acid, filter through a fine glass cruse or ash-free filter paper, wash with hot water, incandesce to a fixed weight, weigh. Calculate the acid-insoluble ash content of the air-dried material [5].

2.10. Chemical content identification

2.10.1. Alkaloid Test

The test solution was put into a test tube and then added with 2N HCl. The first tube is added mayer reagent and the second tube dragendorf reagent. Positive if a white precipitate forms in the first tube and an orange precipitate in the second tube [6].

2.10.2. Flavonoid Test

The test solution is put into a test tube and then Mg powder + concentrated HCl is added. Positive if there is a yellow, orange or red color change [6].

2.10.3. Tannin Test

The test solution is put into a test tube, then a few drops of FeCl solution are added 3. Positive for tannin if a green/blue-black color is formed [6].

2.10.4. Saponin Test

The test solution was put in a test tube and 10 mL of hot distilled water was added. The mixture was shaken until froth appeared and allowed to stand for 1 minute. Next, 2 drops of 2N HCl were added and shaken again until froth was formed. The presence of saponin compounds is characterized by the formation of a stable froth for 10 minutes with a height of 3 cm [6].

2.10.5. Steroid Test

The test solution is put in a test tube and added with Lieberman-Burchard reagent a few drops. Positive if a brownish or violet ring forms on the border of the solution indicating the presence of terpenoids. While a blue ring appears if it indicates the presence of steroids [6].

2.11. Residual Solvent

2.11.1. Gas-Liquid Chromatography Method

The gas chromatography apparatus is equipped with a flame ionization detector and a 1.8 m X 4 mm glass column containing an S3 stationary phase with a particle size of 100 mesh to 120 mesh. Use nitrogen P or helium P as carrier gas. Before use condition the column overnight at 235°C with the carrier gas flowing at a slow flow rate. Adjust the carrier gas flow and temperature (approximately 120°C) so that the internal standard acetonitrile eluates within 5 minutes to 10 minutes [5].

2.12. Microbial contamination

Weighed 1 gram of extract was dissolved in 1 mL of dimethyl sulfoxide (DMSO), then shaken until homogeneous. Prepared 3 brown bottles and each bottle is inserted 9 mL of distilled water. Pipette 1 mL of extract that has been dissolved into the first bottle, shaken until homogeneous so that a

dilution of 10-1 is obtained. Then, pipetted 1 mL of solution from dilution 10 into the second brown bottle so as to obtain dilution 10-2 and continued with dilution 10 and each dilution was replicated twice [7].

2.12.1. Total Plate Count (ALT)

1 mL of each dilution was taken and put into a Petri dish using a different syringe. Then, in each petri dish, 10 mL of melted Nutrient Agar (NA) medium was poured and the petri dish was homogenized. Petri dishes were placed in an inverted position and then placed in an incubator at 37°C for 24 hours. Observe and count the number of colonies that grow [7].

2.12.2. Total Yeast and Mold Count (TYMC)

Taken from each dilution, as much as 1 mL was put into a petri dish using a different syringe. Then, each petri dish was poured with 10 mL of melted Potato Dextrose Agar (PDA) medium and the petri dish was homogenized. Petri dishes were placed in an inverted position and then placed in an incubator at 25°C for 3 days. Observe and count the number of colonies that grow [7].

2.12.3. Heavy Metal Contaminants (Pd and cd)

Weighed 2 grams of extract into a porcelain cup and then fumigated using a furnace for ± 2 hours at 600°C. Then the ash was dissolved using HNO3, filtered, and then the volume was added to 25 mL. Samples were analyzed using an atomic absorption spectrophotometer with a wavelength of 217 nm for Pb (lead) and 228.8 nm for Cd (cadmium) [7].

3. Results and Discussions

3.1. Results

The results of the specific and non-specific parameters can be seen in table 1.

3.2. Discussions

Examination of the identity of the extract aims to provide an objective identity of the specific name of the plant used. Organoleptic examination aims as an initial recognition of an extract using the five senses [5].

3.2.1. Organoleptic

Organoleptic examination includes shape, color, odor and taste. Organoleptic examination aims as an initial introduction to an extract using the five senses [5]. The results of organoleptic testing can be seen in table 1.

3.2.2. Dissolved Compound Content in a Specific Solvent

Testing of compounds dissolved in certain solvents, namely water and ethanol, aims to provide an overview of the number of compounds contained in *P.fructicosa* leaves [5]. Parameters of water-soluble and ethanol-soluble compound levels are carried out by dissolving simplicia with water or ethanol solvents to determine the description of compound levels based on their polarity where polar compounds will dissolve in water and compounds that are semi-polar to non-polar will dissolve in ethanol. From table 1 it can be seen that water-soluble compounds amounted to ...% and those soluble in ethanol amounted to %. It can be concluded that more extracts are dissolved in ethanol compared to water where there are more compounds that are semi-polar to non-polar compared to polar compounds.

3.2.3. Specific gravity determination

Specific gravity is the mass per unit volume at a certain room temperature (25°C) determined with a special pycnometer or other device that aims to provide limits on the amount of mass per unit volume which is a special parameter of liquid extracts to concentrated extracts (viscous) which can still be poured to provide an overview of the dissolved chemical content [5]. The result obtained in the determination of specific gravity is 0.81 grams and for this parameter does not have an allowable value or range [5].

3.2.4. Determination of Moisture Content

The determination of water content aims to provide a minimum limit or range about the amount of water content in an extract by heating at 105° C for 5 hours [5]. According to the literature the water content in the extract should not be more than 10% [5]. The greater the amount of water content in the sample, the easier it is for the sample to grow mold fungi. This can cause a decrease in the biological activity of the sample. According to quality requirements, the moisture content of the extract must be less than 10% [8]. The results of the moisture content obtained amounted to 6.28%, which means it meets the requirements set out in the literature.

3.2.4. Total ash content

The total ash content parameter aims to provide an overview of the content of internal minerals or physiological ash and external minerals or non-physiological ash derived from the initial process of making extracts until the end of the process. In this test, the extract is heated so that organic compounds and their derivatives are deconstructed and evaporated until only mineral and inorganic elements remain [5]. In the determination of total ash content, a result of 8.70% was obtained and this parameter does not have an allowable value or range.

3.2.5. Acid Insoluble Ash Content

Determination of the acid-insoluble ash content parameter illustrates the presence of acid-insoluble mineral or metal contamination in a sample. This parameter does not have an allowable value or range. The greater the acid insoluble ash content indicates the presence of silicates derived from soil and metallic elements of silver, lead and mercury which are toxic to the human body if the accumulation of heavy metals in the body in a long enough period of time [9]. The result obtained from the determination of acid insoluble ash content was 0.40%.

3.2.6. Identification of Chemical Content of Extracts

Color Testing

This test is carried out with the aim of providing an overview of the chemical content contained in *P.fructicosa* leaf extract [5]. In the alkaloid test, HCl is added before the reagent is added because alkaloids are alkaline so they are extracted with solvents containing acids. Positive results in the Meyer test are characterized by the formation of a white precipitate, while positive results for alkaloids in the Wagner test are characterized by the formation of an orange precipitate [6].

Table 1: Data on specific and non-specific parameters of standardization of ethanol extract of *Polyscias fruticosa* (L.) Harms

SPECIFIC PARAMETERS					
No.	Parameters			Results	Terms
1.	Extract identity	Extract name		Ethanol extract of *Polyscias fruticosa* (L.) Harms leaves	-
		Plant parts used		Leaves	-
		Latin name of the plant		*Polyscias fruticosa* (L.) Harms	-
		Indonesian Name		Kedondong laut	-
2.	Organoleptic	Shape		Viscous	-
		Color		Blackish green	-
		Smell		Typical	-
		Taste			-
3.	Dissolved Compound Contentin a Specific Solvent	Water-soluble compound content		5,69 %	-
		Ethanol-soluble compound content		5,92%	-
4.	Identification of Chemical Contentof Extracts	Alkaloids	Mayer Reagent	White precipitate (+)	A white precipitate formed.
			Wagner's Reagent	Orange precipitate (+)	An orange precipitate formed.
		Tannin		Blue-black (+)	Green or blue-black
		Flavanoids		Orange (+)	Red or orange
		Steroids		Brown ring (+)	Brown or violet ring at the tube test surface
		Saponins		Foam (+)	The permanent foam did not disappear after the addition of concentrated HCl.
NON-SPECIFIC PARAMETERS					
No.	Parameters			Results	Terms
1.	Drying shrinkage			0,58%	-
2.	Specific gravity			0.81 grams	-
3.	Water content			6,28%	≤ 10%
4.	Total ash content			8,70%	-
5.	Acid insoluble ash content			0,40%	-
6.	Microbial Contamination	Total Plate Number		< 1.0 x 10^1 colonies/g	< 10 colonies/g
		Mold count		< 1.0 x 10^1 colonies/g	< 10 colonies/g
		Yeast count		< 1.0 x 10^1 colonies/g	< 10 colonies/g
7.	Heavy Metal Contamination	Cadmium (Cd)		< 0.00001 µg/g	≤ 10 mg/Kg
		Lead (Pb)		< 0.00001 µg/g	≤ 0.3 mg/Kg
8.	Residual solvent	Ethanol (-OH)			No residual solvent left

A positive test for the presence of tannins is the formation of a green or blue-black color after the addition of $FeCl_3$ 1%. This occurs when $FeCl_3$ reacts with hydroxyl groups present in tannin compounds [6]. In flavonoid testing using concentrated Mg + HCl powder, Positive if there is a yellow or orange red color change. Magnesium and hydrochloric acid in the wilstater test react to form bubbles which are gas H_2 , while the metal Mg and concentrated HCl in this test serves to reduce the benzopyrone nucleus contained in the flavonoid structure so that the color becomes red, yellow or orange [6]. Steroid testing using Liebermann-Burchard reagent. Positive results are characterized by the formation of brown or violet rings at the boundary of the solution when added with H_2SO_4 . The color change occurs due to the formation of double bonds CH_3COOH and H_2SO_4 with the sample. Saponin is a glycoside form of sapogenin so it is polar [6]. Saponins are compounds that are surface active and can cause foam if shaken in water. The emergence of foam in the saponin test indicates the presence of glycosides that have the ability to form foam in water that is hydrolyzed into other glucose and the addition of 2 N HCl aims to observe the durability of the foam. Positive test for the presence of saponins is evidenced by the formation of foam and permanent and does not disappear in the addition of a few drops of concentrated HCl [6].

3.2.7. Content Analysis using GCMS Spectrophotometer

Gas Chromatography Mass Spectrometry (GC-MS) is a gas chromatography technique used in conjunction with mass spectrometry. The use of gas chromatography is done to find compounds that are volatile under high vacuum conditions and low pressure when heated. While mass spectrometry to determine molecular weight, molecular formula, and produce charged molecules [10]. The results of the analysis showed that there were 52 *peak* compounds contained in ethanol extract of *P.fructicosa* leaves. The largest compound component in the ethanol extract of *P.fructicosa* leaves is located at *peak* 42 with an area of 8.29%, which is Trichloromethane.

3.2.8. Microbial Contamination

Microbial contamination examination is a non-specific parameter that is also needed in the quality standardization of extracts. The examination aims to provide assurance that the sample extract does not contain microbes in the form of bacteria and mold fungi yeast exceeding the maximum limit set for quality standardization. Microbial contamination that exceeds the maximum limit can cause health problems in the body. According to the standardization of natural medicinal materials states that the requirements for the results of the examination of microbiological parameters for TPC (Total Plate Numbers) of bacteria are < 10 colonies/g and for mold yeast numbers are < 10 colonies/g. The results of the examination of microbial contamination of sample extracts obtained bacterial ALT is < 1.0 x 101 colonies/g and yeast mold numbers are < 1.0 x 101 colonies/g.

3.2.9. Heavy Metal Contaminants (Pd and cd)

Examination of metal contamination carried out in the form of examination of metal contamination of lead (Pb) and cadmium (Cd). The metal contamination examination aims to ensure that the sample extract under study does not exceed the maximum limit set as quality standardization. According to BPOM RI, 2021, the maximum limit of lead metal contamination (Pb) is <0.3 µg/g. The results of the examination of lead metal contamination (Pb) obtained are <0.00001 µg/g and cadmium metal contamination (Cd) is <0.00001 µg/g, the results of both do not exceed the maximum limit of standardization requirements that have been set.

4. Conclusions

The results showed that the ethanol extract of *Polyscias fruticosa* (L.) Harms gave results in accordance with the general standard parameters of medicinal plant extracts.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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