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Standardization of Simplicia and Extracts of Arabic Bidara (Ziziphus spina-christi (L.) Desf.) And Tree Saga (Adenanthera pavonina L.) Leaves

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ABSTRACT

The purpose of this study was to standardize simplicia and extracts of Arabic bidara (Ziziphus spina-christi L.) leaves and tree saga (Adenanthera pavonina L.) leaves. The standardization of the simplicia includes determination of water soluble essence content, ethanol soluble essence content, water content, drying shrinkage, total ash content, heavy metal contamination (Pb, Cd, Hg, and As), total aflatoxin, total plate number, and yeast mold number. The standardization of extract includes determination of water soluble essence content, ethanol soluble essence content, drying shrinkage, total ash content, total plate number, yeast mold number, phytochemical screening, and total phenol levels. The results of simplicia standardization showed that the simplicia of Ziziphus spina-christi L. leaves and Adenanthera pavonina L. leaves had water soluble essence content of 40 ± 0% and 46.67 ± 11.547%, ethanol soluble essence content of 33.33 ± 11.547% and 20 ± 0%, water content of 0.1259 ± 0.01296 % and 0.2106 ± 0.0278%, drying shrinkage of 2.33 ± 0.2929 % and 2.26 ± 0.2778%, total ash content of 7.4% and 8.64%, Pb of 2.613 ± 0.097 mg/L and 2.483 ± 0.097%, As of 0.051 mg/L and 0.116 mg/L, Cd of 0% and 0%, metal Hg of 0% and 0%, total aflatoxin of 0 mg/L and 0 mg/L, total plate number of 0.27 x 107 colonies/mL and 0.5 x 107 colonies/mL, and yeast mold number of 2.3 x 107 colonies/mL and 0.4 x 107 colonies/mL. Both ethanol extracts contain polyphenolic compounds, tannins, flavonoids, alkaloids, saponins, and anthraquinones. Total phenol levels of ethanol extracts of Ziziphus spina-christi L. leaves and Adenanthera pavonina L. of 426.98 ± 0.50 mgGAE/g and 574.47 ± 0.50 mgGAE/g, respectively. From all standardization parameters, simplicia and extracts of Ziziphus spina-christi L. and Adenanthera pavonina L. were suitable with the Ministry of Health or BPOM Indonesia standards.

Introduction

Simplicia and extract are one of the raw materials of traditional medicine that are often used today. Traditional medicine raw materials should meet product or ingredient quality standards, so that efficacy and safety are guaranteed. One of the efforts that must be done is to standardize the raw materials of the drug. Standardization is a stage of fulfilling requirements as raw materials for traditional medicine that aims to guarantee and maintain safety, uniformity of quality, and efficacy. One example of standardization is by determining specific and nonspecific parameters in simplicia and extracts in plants that have potential as herbal drugs.

Indonesian medicinal plants that have the potential to be researched and developed into antihyperlipidemic drugs are Arabic bidara (Ziziphus spina-christi L.). The leaves of Ziziphus spina-christi L. contain phenols, flavonoids, tannins [1], total saponins [2] and total triterpenoids [3]. Phytochemical compounds contained in the leaves of Ziziphus spina-christi L. include: 22a-Acetoxy-christinin A, christinin A1, christinin A2, Lotoside III, 15-Acetoxy-lotoside IV, cidrigenin 3-O-a-L-rhamnopyranosyl-(1/4)-a-L- rhamnopyranosyl-(1/2)-b-D-glucopyranoside, conarigenin 3-O-a-L-rhamnopyranosyl-(1/4)-a-L- rhamnopyranosyl-(1/2)-b-D-glucopyranoside, Siconigenin 3-O-a-L-rhamnopyranosyl-(1/4)-a-L- rhamnopyranosyl-(1/2)-b-D-glucopyranoside, and quercetin 3-O-(4-O-trans-p-coumaroyl)-a-L- rhamnopyranosyl-(1/2)-[a-L-

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rhamnopyranosyl-(1/6)]-b-D- galactopyranoside [4]. Various pharmacological activities of Ziziphus spina-christi L. leaves include: as anticancer [5] [6], anti-inflammatory [6] [7] [8] [9], antibacterial [10], antinociceptive [11], antidiabetic [12], antifungal [13] [14],antioxidant [15] [16] [17] [18] [19] [20] [21] [22], antimalarials [23] [24] [25], antidiabetics [26] [27] [28], antiobesity [29] [30], anticancer [31] [32] [33], and antihyperglycemic [34].

Another plant that has the potential to be researched and developed into antihyperlipidemic drugs is the tree saga (Adenanthera pavonina L.) which has been widely used in traditional medicine to treat various diseases, including: hypertension, diarrhea, gout, rheumatism, and cancer [35]. Phytochemical screening of Adenanthera pavonina L. leaves identifies the presence of alkaloids, carbohydrates, proteins, flavonoids, glycosides, saponins, steroids, tannins, and resins [36]. Phytochemical compounds contained in the leaves of Adenanthera pavonina L. include: quercetin 3-o-α-dirhamnopyranosyl-(1‴→2″,1⁗→6″)-β-glucopyranoside-4′-methoxy, kaempferol-3-o-α-dirhamnopyranosyl-(1‴→2″,1⁗→6″)-β-glucopyranoside, isovitexin, quercetin-3-o rhamnopyranosyl(1″ ′→4″)-β-glucopyranoside, quercetin-3-o-β-glucopranoside-4′-o-rhamnopyranoside, kaempferol-3-o-α-rhamnopyranosyl(1‴→2")-β-glucopyranoside, quercetin-3-o- rhamnopyranosyl(1‴→2″)-β-glucopyranoside, quercetin-3-o-β-glucopyranoside, kaempferol, quercetin [37], pavonine [38], squalene, n-hentriacontane, phytol, 2,2,2-diethoxy ethanamine [39], 1-trichosanol, α-6-od-tetraglucoside [40], aridanine, 3-[(2acetamido-2-deoxy-beta-d-glucopyranosyl)oxy]-16alpha acid -hydroxyolean-12-en-28-oat, (+)-pinitol, sucrose, (-)-butin, apigenin, isoliquiritigenin 4-methyl ether, oleanolic acid, daucosterol [41], octacosanol, dulcitol, glycoside beta-sitosterol, and stigmasterol [42]. Various pharmacological activities of A. pavonina L. leaves include: as an antioxidant [43] [44], antinociceptive [45], anticancer [46], antibacterial [47] [48] [49], antiemetic [50], and anti-inflammatory [51] [52] [53].

Standardized herbal medicines are natural medicinal preparations that have been scientifically proven safe and efficacy with preclinical trials and standardized raw materials. To improve the herbal status of a mixture of Ziziphus spina-christi L. and Adenanthera pavonina L. as standardized herbal medicines, the preparation must be made in standardized extracts.

Based on the description above, it is necessary to carry out a standardization process for plants to standardize simplicia and extracts from the Ziziphus spina-christi L. and Adenanthera pavonina L leaves. This study is expected to provide information about the standardization of simplicia and extract of Ziziphus spina-christi L. and Ade*nanthera pavonina* L. leaves that are useful for later research.

RESEARCH METHOD

Experimental

Material

The material of the leaves of the Ziziphus spina-christi L. and Adenanthera pavonina L was obtained from the Situbondo. Chemicals included absolute chloroform (Merck), absolute ethanol (Merck), aquadest, Mg powder (Merck), HCI (Merck), Dragendorf reagent, Mayer reagent, Wagner reagent, absolute ethanol (Merck), FeCl3 (Merck), acid anhydride (Merck), H2SO4 (Merck), and reagen Folin-Ciocalteu's Phenol.

Tool

The tools used included glassware, oven, *Rotary evaporator*, UV-Vis spectrophotometers, and Atomic Absorption Spectroscopy (SSA).

Procedure

1. Plant Determination

Determination was done by matching the suitability of parts of the leaves of the *Ziziphus spina-christi* (L.) Desf. and the leaves of *Adenanthera pavonina* L. with its morphological features to establish truths relating to the leaves of the *Ziziphus spina-christi* (L.) Desf. and the leaves of the *Adenanthera pavonina* L. The determination was carried out at the Biology Service Unit of the Faculty of Science and Technology, Universitas Airlangga.

2. Standardization of Simplicia of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. leaves

Simplicia standardization was carried out using the Indonesian Materia Medika method and the Indonesian Herbal Pharmacopoeia, which included: the determination of water soluble essence content, ethanol soluble essence content, water content, drying shrinkage, total ash content, heavy metal contamination (Pb, Cd, Hg, and As), total aflatoxin, total plate number, and yeast mold number.

Determination of Water Soluble Essence Content

Sample of 5 grams was macerated for 24 hours using 100 mL mixture of chloroform LP water using a corked flask while repeatedly then left for 18 hours. The total of 20 mL of filtrate was filtered and evaporated to dry

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in a shallow flat-bottomed intermediate dish, and the residue was heated at 105 ºC to a fixed weight. The percent content of water-soluble compounds is calculated against the initial simplicia weight (Director General of POM RI, 2000).

Determination of Ethanol Soluble Essence Content

Sample of 5 grams was macerated for 24 hours using 100 mL ethanol (95%) using a corked flask while repeatedly shaking for the first 6 hours then left for 18 hours. The total of 20 mL of filtrate was filtered and then evaporated to dry in a shallow dish of intermediate flat-bottom, the residue heated at 105 ºC to a fixed weight. The percent content of ethanol-soluble compounds is calculated against the initial simplicia weight (DIRJEN POM RI, 2000).

Determination of Water Content

The aluminum dish was heated in oven at 105 °C for 1 hour and cooled in exicator for ± 15 minutes. Next, the weight of the empty aluminum cup was weighed. The simplicia sample was weighed of ± 2 g in aluminum dishes of known weight. Next**,** it was dried in the oven at 100 – 105 °C for 6 hours. Then, it was cooled in excitator and then was weighed. Next**,** it was heated in the oven at temperature of 100 – 105 °C for ± 1 hour, cooled in excitator for ± 15 minutes, and weighed. This treatment was repeated until constant weight was reached (weight difference ± 0.0002 g). Then, it was calculated moisture content [54].

Determination of Drying Shrinkage

Simplicia was weighed of 1 g and was weighed thoroughly and was put into porcelain crutches that had been heated at 105 °C for 30 minutes. The simplicia was flattened in crutches and was put into the oven, opened and closed the crutches, heated at 105 ºC to a fixed weight.

Determination of Total Ash Content

Simplicia powder of 2 grams was weighed and placed into previously incandescent silica crutches and then intermediated. Incandescent simplicia powder in the crutches until the charcoal runs out, then cooled and then weighed until it gets a fixed weight. The total ash content was calculated against the initial powder weight in %w/b.

Measurement of Heavy Metal Contamination (Pb, Cd, Hg, As)

Determination of heavy metal levels including Lead (Pb), Cadmium (Cd), Mercury **(**Hg**),** and Arsenic (As) using the Atomic Absorption Spectroscopy instrument.

Measurement of Total Aflatoxin

Determination of aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2 using the 5.4/IK/2/1/6.3 (LC-MS/MS) method.

Determination of Total Plate Number

1 mL of each dilution was piped into sterile petri dish (triplo) using different sterile pipette for each dilution. Into each petri dish pour 15 mL of NA (Nutrient Agar) media that has been melted, then the petri dish was shaken so that the suspension was mixed evenly. After the substrate solidifies, the petri dish was incubated at 37 ºC for 24 hours in the inverted position.

Determination of Yeast Mold Number

The solution was put 1 ml into the test tube and then added with 9 mL aquadest. The solution was made as 5 replications. Then, it was taken the 5th replication to mix with PDA media. The solution and agar were immediately poured into the petri dish and spread on the petri dish evenly and sufficiently. Control tests (blanks) were made to determine the sterility of the media and diluent. Petri dishes were incubated at 20 – 25 °C for 3 – 5 days in the inverted position after which the medium has solidified. The number of growing colonies of the fungus was observed and calculated after 3 days of incubation.

3. Extraction and Standardization of Ethanol Extract of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. Leaves

Extraction

The dry powder of simplicia material was extracted separately with 96% ethanol using the maceration method. The filtrate obtained was evaporated with a *rotary evaporator* until the thick extract of *Ziziphus spina-christi* (L.) Desf. and of *Adenanthera pavonina* L. Leaves was obtained.

Standardization

Standardization of ethanol extracts of *Ziziphus spina-christi* (L.) Desf. and of *Adenanthera pavonina* L. Leaves was carried out using the method of Materia Medika Indonesia and the Indonesian Herbal Pharmacopoeia, which included: determination of water soluble essence content, ethanol soluble essence content, drying shrinkage, total ash content, total plate number, yeast mold number, phytochemical screening, and total phenol levels.

Phytochemical Screening [55]

Polyphenols and Tannins

A total of 1 gram of sample was extracted with 15 mL aquadest of heat and then cooled. After that 5 drops of 10% NaCl and filtered and the filtrate. The filtered

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filtrate was added with 3 drops of FeC_{l3}.

Flavonoids

Total of 3 mL of sample was evaporated, and washed with hexane until clear. The residue was dissolved in 20 mL of ethanol and then filtered. The filtrate was added 0.5 mL of concentrated HCl and then heated in the water bath. If there was dark red to purple discoloration showed positive results (Bate Smith-Metchalf method).

Alkaloids

Total of 5 grams of plant material was extracted with ammoniated chloroform and then filtered. Then, 1 mL of 2N sulfuric acid was added to the filtrate. Then, was shaked until 2 layers were formed. Next, a layer of acid (top layer) was taken and then was added by 3 drops of Wagner reagent. The formation of a precipitate of reddish-brown color indicated the presence of alkaloids.

Saponins, Triterpenoids and Steroids

-Froth Test:

Extract as much as 0.3 grams was inserted test tube, then was added using 10 ml distilled water, and was shaked vigorously for about 30 seconds. The positive foam test contains saponins when there was stable froth for more than 30 minutes with height of 3 cm above the liquid surface.

-Color reaction:

0.3 grams of extract was dissolved in 15 ml ethanol, then was taken of 5 ml. The total of 5 mL plus 3 drops of anhydrous acetic acid and 1 drop of concentrated H2SO4, then shaken gently and observed color change. The occurrence of blue-green indicated the presence of steroid saponins, red-purple indicates the presence of steroid triterpenes and light yellow indicated the presence of saturated saponins (Liebermann-Burchard test).

Anthraquinone

Extract of 0.3 grams was extracted with 10 mL of distilled water, and was filtered, then the filtrate was extracted with 3 ml of toluene in the separate funnel. Extraction was carried out twice. Then, the toluene phase was collected and divided into two parts, referred to as VA and VB solutions. VA solution as blanks. VB solution plus ammonia and shaken. The red color indicates the presence of anthraquinone compounds (Borntrager test).

Glycosides

The test was conducted using Salkowski's method. The extract was taken by 1 gram dissolved with ethanol 5 mL. The extract was added 2 ml of chloroform, then was added H $2SO_4$. Samples containing glycosides will appear as red-brownish rings.

Determination of Total Phenol Levels [56] [57]

Total of 2 mL of the sample solution was pipetted and was added with 0.4 mL of Folin-Ciocalteau reagen. Then, was shaken left for 4 – 8 minutes and was added by 4.0 mL of Na_2CO_3 7% solution and was beaten until homogeneous. Then, with aquabidestillata up to 10 mL and let stand for 2 hours at room temperature. The absorption was measured at a wavelength of 748 nm. Next, 5 repetitions were carried out so that the phenol levels obtained were obtained as mg equivalent to gallic acid/g extract

RESULTS AND DISCUSSION

***Determination of Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. Leaves**

Before being used as a research site, the leaves of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. were tested for plant determination. The determination test of both of the leaves was carried out at the Biological Services Unit of the Faculty of Science and Technology, Universitas Airlangga. The importance of the determination test is to clearly identify the type of plant being studied. Plant determination helps to avoid errors in the collection of the main ingredients and scientific analysis. By knowing exactly the type of plant being studied, research can be more accurate. The correctness of the type of plant to be studied is ascertained by comparing the characteristics of the plant with existing botanical references to confirm its identity.

Based on the results of plant identification tests conducted by the Biological Services Unit of the Faculty of Science and Technology, Universitas Airlangga stated that the plant samples used in this study were species Ziziphus spina-christi (L.) Desf. and Adenanthera pavonina L. The result of the determination of the Ziziphus spina-christi (L.) Desf. leaf samples was single leaves, incomplete leaves consisting of petioles (petiolus) and leaf blades (lamina). The shape of the leaves is ovalis/ellipticus. Leaf length 3.3 cm – 4.7 cm; Leaf width 1.7 cm – 3.0 cm. The edges of the leaves are flat/ integer. Pinnate leaf reinforcement/penninervis. Blunt leaf tip/obtusus. The base of the leaf is blunt/obtusus. The upper surface of the leaves is dark green, the lower surface of the leaves is light green. Seated leaves alternate. Thin tender/herbaceous leaf flesh. On the other hand, the result of determination from the Adenanthera pavonina L. leaf sample was even-pinnate compound leaves, the

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shape of ovalis/ellipticus leaves. Leaf length 3.7 cm – 4.8 cm; Leaf width 2.0 cm – 2.3 cm. The edges of the leaves are flat/ integer. The upper leaf bone is pinnate/penninervis; The lower part is fingered/palminervis**. Blunt leaf tip/obtusus. The base of the leaf is blunt/obtusus. The upper surface of the leaves is green, the lower surface of the leaves is slightly white (cream). Petiole length 0.7 mm – 0.8 mm. Thin tender leaf flesh/ herbaceus.**

***Standardization of Leaves Simplicia of Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L.**

Ziziphus spina-christi (L.) Desf. and *Adenanthera pavonina* L. leaves have potential as antihyperlidemia herbal medicines so it is necessary to standardize the raw materials of simplicia and extracts of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. leaves. The determination of this standardization value needs reference that indicates that these simplicia and extract meet the requirements that have been set. The official standardization reference for the leaves of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. itself has not been listed in the publication of the Ministry of Health or from other sources so that as a reference in this study is to use general requirements.

The purpose of standardization itself is to ensure the quality and safety standards of medicinal plant extracts. The determination of quality standards carried out includes specific and non-specific parameters. Standardization is carried out physically, chemically and biologically. Standardization of simplicia is an important step in maintaining the quality, safety, and consistency of herbal products and promoting safe and effective use. Standardization of simplicia aims to ensure the quality and safety of medicinal plant raw materials. By setting strict quality standards, the resulting products have a consistent quality, which is important for the efficacy and safety of the use of herbal medicines. Standardization of Simplicia also involves testing for contaminants such as heavy metals and other contaminants. This is important to ensure that herbal products are safe to use without risk to the health of consumers. In the standardization of simplicia of the leaves of the *Ziziphus spina-christi* (L.) Desf. and the leaves of the *Adenanthera pavonina* L. several tests were carried out, namely: the determination of water soluble essence content, ethanol soluble essence content, water content, drying shrinkage, total ash content, heavy metal contamination (Pb, Cd, Hg, and As), total aflatoxin, total plate number, and yeast mold number.

Table 1. Standardization of leaves simplicia of *Ziziphus spina-christi* (L.) and *Adenanthera pavonina* L.

Paramaters	Leaves simplicia of *Ziziphus spina-christi* (L.)	Leaves simplicia of *Adenanthera pavonina* L.
Water Soluble Essence Content (%)	40 ± 0	46.67 ± 11.547
Ethanol Soluble Essence Content (%)	33.33 ± 11,547	20 ± 0
Water Content (%)	0.1259 ± 0.01296	0.2106 ± 0.0278
Drying Shrinkage (%)	2.33 ± 0.2929	2.26 ± 0.2778
Total ash content (%)	7.4	8.64

Table 1 showed the test results of the water soluble essence content, ethanol soluble essence content, water content, drying shrinkage, total ash content of simplicia of *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. leaves. The determination of the water and ethanol soluble essence content aims to provide an initial picture of the amount of dissolved content in a particular solvent. The results of dissolved compound parameters using ethanol and water solvents have met the requirements set by the Ministry of Health of the Republic of Indonesia (2000). The determination of dissolved compound levels is not related to pharmacological effects but can be used to estimate compounds that are polar (soluble in water) and compounds that are semi-polar (soluble in ethanol). The results of the determination of the water soluble essence content of leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. were 40 ± 0% and 46.67 ± 11.547 % while the determination of the ethanol soluble essence content of leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. were 33.33 ± 11.547 % and

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20 ± 0%. Based on the data obtained, it was proven that polar compounds (soluble in water) are more than compounds that are semi-polar (soluble in ethanol).

According to the Indonesian Herbal Pharmacopoeia (2009), generally, the required water content is < 10% (Ministry of Health RI, 2009). The water content in leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. obtained was 0.1259 ± 0.01296 % and 0.2106 ± 0.0278 % so that it can be concluded that it meets the requirements that have been set because it does not exceed the permissible limit. The higher the moisture content, the easier it is for the simplicia to overgrow mushrooms and mold. This can decrease the biological activity of simplicia during storage [58].

The determination of simplicia drying shrinkage aims to provide a maximum limit (range) regarding the number of compounds lost during the drying process (Ministry of Health RI, 2000). The method used in this drying shrinkage is the gravimetric method. The results showed that the compounds lost in the drying process of leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. amounted to 2.33 ± 0.2929% and 2.26 ± 0.2778%. Drying shrinkage is often identified with water content, but the difference in the water content is only to find out the maximum limit of water in the extract while drying shrinkage not only includes water but also compounds that have a lower boiling point than water that will also evaporate in the drying process.

Total ash content gives an description of mineral content both external and internal. The determination of ash content was measured by inserting simplicia into furnace with temperature of 450 ^{0}C until ash was formed. Simplicia was heated until the organic compounds and derivatives were destructed, leaving only inorganic compounds. The total ash content of leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. were 7.4 % and 8.64 %, respectively. Based on Kepmenkes RI Number 261/MENKES /SK/IV/2009, the ash content of the extract should not be more than 10.2%. So, the total ash content value obtained was suitable with the standard [59].

Table 2 Heavy metal contamination levels of leaves simplicia of *Ziziphus spina-christi* (L.) and *Adenanthera pavonina* L.

Parameters	Leaves simplicia of *Ziziphus spina-christi* (L.)	Leaves simplicia of *Adenanthera pavonina* L.
Pb (mg/L)	2.613 ± 0.097	2.483 ± 0,097
Cd (mg/L)	0 ± 0	0 ± 0
Hg (mg/L)	0 ± 0	0 ± 0
As (mg/L)	0.051	0.116

Testing of heavy metal contamination on leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. aim to assure that simplicia does not contain heavy metals Pb, Cd, Hg, and As or does not exceed predetermined values so as not to have toxic effects on health. According to the Ministry of Health of the Republic of Indonesia in 2017, the maximum limit of Pb, Cd, Hg, and As levels that are allowed is 10 mg/L; 0.3 mg/L, 5 mg/L, 0.5 mg/L and 0.5 mg/L, respectively.

In this study, the levels of lead (Pb) heavy metal contamination in leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. were 2,613 ± 0,097 mg/L and 2,483 ± 0,097 mg/L. Besides that, the levels of arsenic (As) heavy metal contamination in leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. were 0.051 ± 0 mg/L and 0.116 ± 0 mg/L. Furthermore, heavy metals Cd and Hg were not found in either leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. nor *Adenanthera pavonina* L. as shown in **Table 2**. These results showed that the Pb, Cd, Hg and As heavy metal contamination does not exceed the maximum limit set by the National Standardization Center. Lead is heavy metal that is toxic even at low concentrations. Medicinal products that contain medicinal materials with excessive amounts of heavy metals, of course, are feared to cause heavy metal poisoning in consumers.

Table 3. Total aflatoxin contamination levels of leaves simplicia of *Ziziphus spina-christi* (L.) and *Adenanthera pavonina*

Parameters	**Leaves simplicia of *Ziziphus spina-christi* (L.)**	**Leaves simplicia of *Adenanthera pavonina* L.**
Aflatoxin B1 (mg/L) Aflatoxin B2	0 ± 0	0 ± 0

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(mg/L)	0 ± 0	0 ± 0
Aflatoxin G1 (mg/L)	0 ± 0	0 ± 0
Aflatoxin G2 (mg/L)	0 ± 0	0 ± 0

Aflatoxin is mycotoxin produced by *the molds Aspergillus flavus* and *A. parasiticus* (IARC 2002). The mold can contaminate simplicia and extracts that will produce toxins in the form of aflatoxin B1, B2, G1, and G2 (Syarief et al. 2003). Aflatoxin test is performed to ensure that the leaves simplicia of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. do not contain aflatoxin contamination that can cause toxicity. Aflatoxin contamination can cause mutagenic (gene mutations), teratogenic (inhibiting fetal growth) and carcinogenic (causing cancer in tissues). The presence of aflatoxin contamination in the extract can endanger health [60].

Based on the aflatoxin test results shown in **Table 3**, leaves simplicia of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. proven not to be contaminated by four main compounds of aflatoxin (AfB1, AfG1, AfB2, AfG2) so it is safe to be used as raw material for drugs. Analysis of aflatoxin contamination in this study, using the Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) tool conducted at the PT. Angeler BioChemlab. Sample storage conditions can also be one of the trigger factors for aflatoxin contamination, for example samples stored in airtight containers so that an environment with low oxygen content is formed and can cause fungi to produce aflatoxins.

Table 4. Microba contamination of leaves simplicia of *Ziziphus spina-christi* (L.) and *Adenanthera pavonina* L

Parameters	Leaves simplicia of *Ziziphus spina-christi* (L.)	Leaves simplicia of *Adenanthera pavonina* L.
Total Plate Number	0.27 x 10^7 colonies/mL	0.5 x 10^7 colonies/mL
Yeast mold numbers	2.3 x 10^7 colonies/mL	0.4 x 10^7 colonies/mL

Microbial contamination is contamination in food derived from microbes that can harm and harm human health. A medicinal product that contains natural ingredients should not contain contaminated microorganisms. The maximum limit of microorganism contamination required depends on the dosage form and this is determined by determining the total plate number and yeast mold number. According to theBPOM regulation on traditional medicine quality requirements for microbial contamination, the total plate number must be ≤ 104 colonies/g and the yeast mold number must be ≤ 103 colonies/g [61].

The results of microbial contamination tests were obtained by determining the total plate number and yeast mold number on the leaves simplicia of *Ziziphus spina-christi* (L.) Desf. were 0.27 x 10^7 colonies/mL and 2.3 x 10^7 colonies/mL. On the other hand, the determination of total plate number and yeast mold number in leaves simplicia of the *Adenanthera pavonina* L. is 2.3 x 10^7 colonies/mL and 0.4 x 10^7 colonies/mL. Thus, it can be concluded that the total plate number of microbial contamination in leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. is ≤ 104 colonies/g while the contamination of yeast mold numbers in leaves simplicia of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L.) is ≤ 103 colonies/g.

Standardization of Ethanol Extract of Ziziphus spina-christi* (L.) Desf. *and Adenanthera pavonina* L. *Leaves

In this study, the preparation of extracts was carried out using dry powder simplicia material extracted separately with 96% ethanol using the maceration method. Ethanol can extract compounds with wide polarity ranging from nonpolar compounds to polar compounds. The advantages of using ethanol solvents are that they are non-toxic, neutral, and have a low boiling point. The filtrate obtained is evaporated with a *rotary evaporator* until a thick extract of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. is obtained. Then, standardization of extracts is carried out.

In the standardization of extracts of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. leaves, several tests were carried out, namely: determination of total ash content, water soluble essence content, ethanol soluble essence content, drying shrinkage, total plate number, and yeast mold number. In addition, extracts of the *Ziziphus spina-christi* (L.)

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Desf. and *Adenanthera pavonina* L. leaves were also carried out to phytochemical screening and total phenol level testing.

Table 5 Results of extract standardization

Paramaters	Extract of *Ziziphus spina-christi* (L.) Desf.	Extract of *Adenanthera pavonina* L.
Water Soluble Essence Content (%)	27 ± 11.54	20 ± 0
Ethanol Soluble Essence Content (%)	27 ± 11.54	20 ± 0
Drying Shrinkage (%)	3.25 ± 2.9006	4.6 ± 4.0835
Total ash content (%)	0.97	1.36

Table 5 shows the test results of water soluble essence content, ethanol soluble essence content, drying shrinkage, and total ash content of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. extract. 'The results of determination of the water soluble essence content of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. were 27 ± 11.54% and 20 ± 0% while the determination of ethanol soluble essence content of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. was 27 ± 11.54 % and 20 ± 0%.

In determining the drying shrinkage, the results showed that the drying shrinkage rate of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. was 3.25 ± 2.9006 % and 4.6 ± 4.0835 %. The total ash content of the *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. was 0.97% and 1.36%. Thus, the total ash content value of the two extracts obtained is suitable with the standard.

Table 6 Microbial contamination of *Ziziphus spina-christi* (L.) Desf. and *Adenanthera pavonina* L. extract

Parameters	Extract of *Ziziphus spina-christi* (L.) Desf.	Extract of *Adenanthera pavonina* L.
Total Plate Number	0.47 x 10^7 colonies/mL	0.83 x 10^7 colonies/mL
Yeast mold numbers	3.6 x 10^7 colonies/mL	0.83 x 10^7 colonies/mL

The results of microbial contamination tests (**Table 6**) were obtained by determining the total plate number and yeast mold number on the leaf extract of the *Ziziphus spina-christi* (L.) Desf. were 0.47 x 10^7 colonies/mL and 3.6 x 10^7 colonies/mL. On the other hand, the determination of the total plate number and yeast mold number in leaf extract of *Adenanthera pavonina* L. was 0.83 x 10^7 colonies/mL and 0.83 x 10^7 colonies/mL. Thus, it can be concluded that the total plate number of microbial contamination in the leaf extract of the *Ziziphus spina-christi* (L.) Desf. and the leaf extract of the *Adenanthera pavonina* L. is ≤ 104 colonies/g while the contamination of the yeast mold number in the leaf extract of the *Ziziphus spina-christi* (L.) Desf. and the leaf extract of the *Adenanthera pavonina* L. is ≤ 103 colonies/g.

Table 7 Phytochemical screening of extracts

Parameters	Extract of *Ziziphus spina-christi* (L.) Desf.	Extract of *Adenanthera pavonina* L.
Polyphenols & Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Saponins (Froth Test)	+	+
Saponins (Color Test)	+	+
Anthraquinone	+	+
Glycosides	-	-

Phytochemical screening aims to determine the presence of a class of secondary metabolite compounds contained in the extract. In addition, it is also a qualitative description of the extracted content. The

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chemical content test aims to provide an initial picture of the composition of the chemical content. Chemical content tests were carried out on ethanol extract of the *Ziziphus spina-christi* (L.) Desf. and the *Adenanthera pavonina* L. leaves. The results obtained showed that both extracts contained classes of polyphenolic compounds, tannins, flavonoids, alkaloids, saponins, and anthraquinone (**Table 7**).

Table 8 Total phenol extract content

Sample type	Total Phenol Levels (mgGAE/g)
Extract of *Ziziphus spina-christi* (L.) Desf.	426.98 ± 0.50
Extract of *Adenanthera pavonina* L.	574.47 ± 0.50

The determination of total phenol levels was carried out spectrophotometrically. In the total phenol test using Follin Ciocalteu and Na_2CO_3 reagents. The hydroxyl group in the phenolic compound will react with the Folin Ciocalteau reagent to form a blue complex. From the results of the study, the total phenol content in the ethanol extract of the *Ziziphus spina-christi* (L.) Desf. was 426.98 ± 0.50 mgGAE/g, and in ethanol extract of the *Adenanthera pavonina* L. is 574.47 ± 0.50 mgGAE/g.

CONCLUSION

Leaves simplicia of the Ziziphus spina-christi (L.) Desf. have water soluble essence content of 40 ± 0%, ethanol soluble essence content of 33.33 ± 11.547 %, the water content of 0.1259 ± 0.01296 %, drying shrinkage of 2.33 ± 0.2929 %, total ash content of 7.4 %, Pb of 2.613 ± 0.097 mg/L, Cd of 0 mg/L, Hg of 0 mg/L, As of 0.051 mg/L, total plate number of 0.27 x 107 colonies/ mL and yeast mold number of 2.3 x 107 colonies/mL while leaves simplicia of the Adenanthera pavonina L. have water soluble essence content of 46.67 ± 11.547 %, ethanol soluble essence content of 20 ± 0 %, total ash content of 0.2106 ± 0.0278 %, drying shrinkage of 2.26 ± 0.2778 %, total ash content of 8.64 %, Pb of 2.483 ± 0.097 mg/L, Cd of mg/L, Hg of 0 mg/L, As of 0.116 mg/L, total plate number of 0.5 x 107 colonies/mL and yeast mold number of 0.4 x 107 colonies/mL.

Ethanol extract of the *Ziziphus spina-christi* (L.) Desf. has water soluble essence content of 27 ± 11.54%, soluble ethanol essence content of 27 ± 11.54 %, drying shrinkage of 3.25 ± 2.9006 %, total ash content of 0.97 %, total plate number of 0.47 x 10^7 colonies/mL, yeast mold number of 3.6 x 10^7 colonies/mL, contains polyphenolic compounds, tannins, flavonoids, alkaloids, saponins, anthraquinones, and has total phenol content of 426.98 ± 0.50 mgGAE/g while ethanol extract of the *Adenanthera pavonina* L. has water soluble essence content of 20 ± 0 %, ethanol soluble essence content of 20 ± 0 %, drying shrinkage of 4.6 ± 4.0835 %, total ash content of 1.36 %, total plate number of 0.83 x 10^7 colonies/mL, yeast mold number of 0.83 x 10^7 colonies/mL, contains polyphenolic compounds, tannins, flavonoids, alkaloids, saponins, anthraquinones, and have total phenol content of 574.47 ± 0.50 mgGAE g.

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