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ANALYSIS OF NON-SPECIFIC STANDARDIZED PARAMETERS ON KUMIS KUCING LEAVES (*Orthosiphon aristatus* (Blume) Miq.) PURPLE VARIETY

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ABSTRACT

Traditional medicines are used to maintain human health. Indonesia has large biodiversity. Many types of plants in Indonesia are used as medicines. One of the plants in Indonesia that can be used as a medicine is the purple variety of kumis kucing (*Orthosiphon aristatus* (Blume) Miq.). This study aimed to examine the quality and safety of the purple variety of kumis kucing as a raw material for traditional medicine in order to meet the requirements of being free from heavy metal, microbial, and aflatoxin contamination. The results of setting the standardization parameters on the dry material of Kumis kucing leaves obtained drying losses, water content, total ash content, and acid insoluble ash content, respectively 8.45 ± 0.68%; 6.67 ± 1.15 %; 8.70 ± 0.09 %; 1.31 ± 0.16 %. The results of testing for the heavy metals lead (Pb) and mercury (Hg) on dry material and extracts were ≤ 0.0001 mg/kg. The results of testing the levels of microbial contamination in dry material and extracts obtained total bacteria of 1.45x101 and 2.35x101 CFU/gr, respectively. Test results for aflatoxin contamination on the dry material of kumis kucing leaves B1, B2, G1, G2, and total aflatoxin ≤ 0.50 µg/kg and total aflatoxin ≤ 2 µg/kg, respectively. The dried material and leaf extract of the purple variety of kumis kucing tested met the requirements stated in the regulations of the Food and Drug Supervisory Agency number 32 of 2019 regarding traditional medicine safety and quality requirements.

Keywords: *Orthosiphon aristatus* (Blume) Miq, heavy metal contamination, microbial contamination, aflatoxin contamination

INTRODUCTION

Kumis kucing is grouped based on flower morphology into 3 varieties: purple, white-purple, and white (Febjislami et al., 2019). The purple variety of kumis kucing contains more secondary metabolite compounds than the white variety (Faramayuda et al., 2021c). Various pharmacological studies have been carried out, and Kumis kucing plants are known to have anti-inflammatory and antihypertensive activities (Rafi et al., 2015).

Extracts used as medicinal ingredients have constant specific and non-specific properties and are expected to meet the quality requirements of phytopharmaceuticals. Nonspecific parameters include drying loss, specific gravity, water content, total ash content, acid-insoluble ash, metal contamination, pesticide residue contamination, microbial contamination, and aflatoxin contamination (Munim et al., 2009). The toxicity of heavy metals is influenced by several factors, including the level of metal consumed and duration of consumption (Darmono, 1995). Lead (Pb) contamination is a xenobiotic substance that is foreign to the body and can cause various health problems (Ismail et al., 2020). Heavy metal lead can affect the function of the hematopoietic, neurological, endocrine, renal, gastrointestinal, hematological, and reproductive systems. Mercury contamination is a naturally occurring heavy metal. Mercury is found in the air in mineral deposits and industrial areas. Meanwhile, what is found in water and soil comes from natural deposits,

waste disposal, and volcanic activity (Zulharmita et al., 2017). Acute toxicity from inorganic mercury includes symptoms of vomiting, loss of consciousness, abdominal pain, diarrhea accompanied by blood in the feces, albuminuria, anuria, ulceration, and stomatitis (Lubis, 2002).

Biological, chemical, and other contaminants can also harm human health. Pesticide residue and microbial contamination are indicators of product safety. Microbial contamination of plants can be caused by spraying and irrigation processes that are contaminated by microbes as well as by fertilization with animal waste. *Contamination with Escherichia coli, Salmonella, Shigella, and Clostridium* usually originates from human or animal feces (Harsojo & Mellawati, 2009; Faramayuda et al., 2021b). *Escherichia coli (E. coli*) is a pathogenic bacterium that endangers human health.

The purple variety of kumis kucing plants will be used in traditional medicine; therefore, its quality and safety must be guaranteed. To prove that these requirements are met, this research will examine contamination from the heavy metals lead (Pb) and mercury (Hg), microbial contamination, and aflatoxin contamination that may be present in dry material and purple variety kumis kucing leaf extracts. Therefore, to determine whether there is heavy metal lead (Pb) and mercury (Hg) contamination, pesticide residue contamination, and microbial contamination in the leaves of the purple variety kumis kucing (*Orthosiphon aristatus* (Blume) Miq).

Kumis kucing plants have a variety of secondary metabolites such as polyphenols (lipophilic flavonoids and phenolic acids), terpenoids (diterpenes and triterpenes), sterols, caffeic acids (rosmarinic acid, chicoric acid), polymethoxy flavonoids (sinensetin and eupatorin), caffeine acid derivatives, triterpene saponins, diterpene esters, and essential oils. (Tezuka et al., 2000; Olah et al., 2017; Faramayuda et al., 2021a). The kumis kucing plant also contains the polymethoxylated flavonoid compound phenylpropanoids (caffeic acid derivatives). Sinensetin is a flavonoid, the most important phytochemical compound, and a marker compound of kumis kucing plants (Himani et al., 2013; Faramayuda et al., 2021).

RESEARCH METHODS

Equipment and Materials

The tools and materials used were analytical scales (Simadzu, Japan), waterbath, measuring cup, glass stirrer, drying cabinet, glass funnel, aluminum foil, tissue, label paper, glass bottles, HPLC (Shimadzu), C18 column, kumis leaf dry material cat, ethanol 70%, hydrochloric acid, nitric acid, physiological NaCl 0.85%, EMBA Levine Himedia, acetonitrile, methanol, aquabidest, and aflatoxin comparison standard.

Research Procedure

1. Preparation of Simplicia

   The kumis kucing used is a purple variety of kumis kucing. These kumis kucing were obtained from the Unjani Medicinal Plant Garden in Cimahi City at an altitude of ± 685 meters above sea level. The samples were then washed, dried, and chopped for subsequent testing.

2. Extracts Preparation

   Dry powder (250 g) was extracted by maceration using 1000 mL of 70% ethanol. Maceration was performed until the liquid extract became clear. The macerate was then concentrated using a rotary vacuum evaporator, and the extract was evaporated in a water bath until a thick extract was obtained.

3. Heavy Metal Contamination

   A working solution was prepared with concentrations of 0, 1, 2, 3, 4, and 5 mg/L by adding 50 mg/L multi-element standard solution into a volumetric flask and 1 N nitric acid. After that, 100 mL of concentrated HNO_3 was added to 100 mL and covered with a watch glass, and then heated to a temperature of 105–120°C until the volume was ± 5 mL; the walls of the beaker were rinsed and the glass was watched with mineral-free water, 5 mL of concentrated HNO_3 was added to cover the container with a watch glass and store it on an electric heater, increase the temperature of the electric heater until it

boils, and continue heating until no NO_2 gas is formed (generally indicated by the color change to brownish yellow as the reflux process continued). Acid was added as needed if digestion was incomplete (marked by a cloudy test sample). After cooling, 10 mL of 1:1 HCl and 15 mL of mineral-free water were added, reheated for 15 minutes, cooled again, and the walls of the beaker and the glass were washed. The filtrate was washed with mineral free water, filtered, and transferred to a 100 mL volumetric flask. The prepared sample was then injected into the ICP-OES apparatus. Quantitative testing of lead and mercury in the samples was performed by observing the concentration of each sample at selected wavelengths. After obtaining the mercury concentration (µg/L) in the sample, the mercury content (µg/g) was calculated (Wijaya, 2013).

4. Microbial Contamination Test

   Microbial contamination was tested using the Total Plate Count (TPC) method, sterilizing the tools and materials used using an autoclave for 15 minutes at a temperature of 121°C, and then placing into a test tube filled with 0.85% Physiological NaCl, homogenized with a vortex, and then making a series. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} using 0.85% Physiological NaCl, 1 mL of each dilution series was inoculated into a Petri dish, and then 15–20 mL of agar media was added. Homogenize by shaking the Petri dish to form number 8, and do it in triplicate. Incubate at 37°C for 24–48 hours. The number of bacteria growing in the Petri dishes was counted.

5. Aflatoxin Contamination

   A standard stock solution of 5 ppm aflatoxin was prepared, various dilutions of the working standard solution were prepared up to 200 ppb, and a standard series was prepared for HPLC readings. The aflatoxin contamination levels in kumis kucing dry material were determined based on the area with a modified excitation wavelength of 362 nm and emission wavelengths of 425 and 455 nm. The HPLC system used is isocratic elution with the mobile phase being acetonitrile: methanol: aquabidest (16:22:62). A reverse-phase C18 column with a column temperature of 30°C was used. The separation time was 30 minutes using a flow rate of 1 mL/minute.

RESULTS AND DISCUSSION

The dry material leaves of the purple variety kumis kucing were macerated using 70% ethanol. This method was chosen because the maceration method is cold extraction, so it can avoid the decomposition of the active substance by heating. Ethanol is used as a filter solution because it is a universal solvent that easily dissolves active compounds, polar, semi-polar, and non-polar, and is not toxic compared to other organic solvents (Utami et al., 2016; Faramayuda et al., 2021d). The dry material used for extraction was 250 grams in 1000 mL 70% ethanol, resulting in an extract yield of 17.2113% w/w. The extract yield was obtained by dividing the weight of the extract by that of the powder. These results are consistent with the literature, which states that the yield of kumis kucing extract is not less than 8.7% (Ministry of Health of the Republic of Indonesia, 2017).

To determine the metal content of lead (Hg) and mercury (Pb) in dry material and kumis kucing extract, an instrument with high analytical sensitivity, so the instrument used is Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), is required. ICP-OES is a technique for spectrophotometrically measuring metals with a high level of sensitivity. The advantage of using this method is that ICP-OES can be carried out quickly because it measures all metals continuously and directly.

Before analyzing the content of the heavy metals lead (Hg) and mercury (Pb), wet digestion was first carried out using HNO_3 and HCl. The wet digestion method dissolves or changes a sample into a form that can be measured using an acid reagent to analyze the elemental content of the sample (Rusnawati & Alimuddin, 2018).

They tested lead and mercury levels by the wet destruction method in the laboratory using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The reason for using HNO_3 and HCl were used because they can process digestion faster and are strong oxidizers. They can lower the digestion temperature, dissolve metals, and are more stable. The results of testing the levels of heavy metal lead and mercury contamination in dry material and kumis kucing plant extracts can be seen in Table I.

Table I. Results of Determining the Levels of Heavy Metal Contamination of Kumis Kucing Plants

Sample	Concentration (mg/kg)	
	Lead (Hg)	Mercury (Pb)
Kumis Kucing Simplicia	<0.0001	<0.0001
Kumis Kucing extract	<0.0001	<0.0001

According to Food and Drug Supervisory Agency regulation number 32 of 2019 regarding safety and quality requirements for traditional medicines, the permitted heavy metal lead (Pb) contamination is less than 10 mg/kg, while mercury is less than 5 mg/kg. Based on the research results in Table I, the tested samples met the requirements (Food and Drug Supervisory Agency, 2019).

Next, the level of microbial contamination in kumis kucing plants was determined because one of the requirements for a product to be consumed must be to ensure that the product consumed enters the body and is not contaminated with microbial contamination that meets the requirements. The Total Plate Count (TPC) method was used in this study. This method was chosen because TPC is the most sensitive for determining the number of microorganisms. The Total Plate Count (TPC) parameter in food products is very important to pay attention to because it is closely related to the safety of the product for consumption. The results of testing microbial contamination levels in the dry material and kumis kucing plant extracts are shown in Table II.

Table II. Results of Determining Levels of Microbial Contamination of Kumis Kucing Plants

Parameter	Total bacteria in dry material (CFU/mL)	Total bacteria in extract (CFU/g)
Escherichia coli	0	0
Salmonella shigella	0	0
Clostridia	0	0
ALT/Total Bacteria	$1{,}45 \times 10^1$	$2{,}35 \times 10^1$

According to the Food and Drug Supervisory Agency regulation number 32 of 2019 on safety and quality requirements for traditional medicines, the requirement for *Escherichia coli* microbial contamination is less than 10 colonies/g for *Salmonella shigella* and *Clostridia* negative/g, while the total plate count (ALT) is less than 105 colonies/g, which shows that the results of the microbial contamination test on kumis kucing leaf dry material meets the requirements (Food and Drug Supervisory Agency, 2019).

Next, aflatoxin levels were determined in dry material kumis kucing leaves. This research requires an instrument with high analytical sensitivity, because aflatoxin is a toxic and carcinogenic toxin that can cause liver and kidney damage if consumed. Hence, High Performance Liquid Chromatography (HPLC) was used. This tool is used because operating the HPLC tool requires a small number of samples needed are also small. The tests also require a short time to be more efficient and produce the

best results (Fithrul, 2021). The results of testing the contamination levels in kumis kucing plant dry material are shown in Table III.

Table III. Results of Determining Levels of Aflatoxin Contamination of Kumis Kucing Dry Material

Parameter	Results (µg/kg)
Aflatoxin B1	< 0,50
Aflatoxin B2	< 0,50
Aflatoxin G1	< 0,50
Aflatoxin G2	< 0,50
Aflatoxin Total	< 2,00

Based on Food and Drug Supervisory Agency regulation number 32 of 2019 about safety and quality requirements for traditional medicines, the requirement for total aflatoxin contamination (aflatoxin B1, B2, G1, and G2) is ≤ 20 µg/kg with the condition that aflatoxin B1 is ≤ 5 µg/kg. The test results in Table III show that the microbial contamination test on kumis kucing leaf dry material meets these requirements (Food and Drug Supervisory Agency, 2019).

CONCLUSION

The results of the heavy metal contamination levels of lead (Pb) and mercury (Hg) in the dry material and extract showed ≤ 0.0001mg/kg. The results of microbial contamination levels in the dry material and extract did not show the presence of *Escherichia coli*, *Salmonella*, *Shigella*, and *Clostridia*. In contrast, the results for total bacteria were $1{,}45x10^1$ CFU/mL CFU/mL and $2{,}35x10^1$ CFU/gr, respectively. The results of contamination levels of aflatoxins B1, B2, G1, G2, and total aflatoxin were respectively ≤0,50 µg/mL and total aflatoxin ≤2 µg/mL. The test results for heavy metal lead (Pb) and mercury (Hg), microbial contamination, and aflatoxin contamination met the requirements stated in the Food and Drug Supervisory Agency regulation number 32 of 2019 regarding safety and quality requirements for traditional medicines.

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