Determination of Total Flavonoid Content and Standardization

Orthosiphon aristatus Leaves Extracts

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Abstract

Objective: to determine total flavonoids content and standardization of extract of Orthosiphon aristatus (BL) Miq. Methods: Extraction of leaves was conducted with maceration by using 70% ethanol as solvent, followed by the determination of quality extract namely extract specific parameters including organoleptic, soluble ethanol content, extract content and water soluble and non-specific parameters including loss on drying, water content, total ash content, acid not soluble ash. Determination of total flavonoid content used UV-Vis spectrophotometer. BSLT was used in this study for toxicity test. Results: The results of phytochemical screening showed that the powder and extract Orthosiphon aristatus containing flavonoid, triterpenoid, steroid, essential oils, coumarin and saponins. Organoleptic results of the extract showed the extract has a thick consistency, blackish green colour, taste rather chelates and hasn’t a specific aromatic. Loss on drying of 11.73 %, water content of 9.06 %, ash content of 10.92 %, water soluble content of 13.85 %, ethanol soluble content of 7.67 %, acid insoluble ash content of 11.05 %, and water soluble ash content of 5.26 %. The result of BSLT showed the LC₅₀ value of 25.0150 μg/ml. Total flavonoid was 1.7797 %. Accordingly, the extract meets the standardization criteria with DER (Drug Extract Ratio) of 15,2774.

Keywords: Orthosiphon aristatus (BL) Miq, total flavonoid content, determination, standardization.

Introduction

Orthosiphon aristatus (BL) Miq, local name kumis kucing, is a plant in both low and high altitude that grows upright up to 2 meter in high with quadrangular and short -haired or bald stem. The characteristics of leaves are oval, oblong, green and round with a pointed or blunt tip and 2 – 12 cm in length, 1 – 8 cm in wide. The leaves of Orthosiphon aristatus are collected when the flowers are blooming. Indonesia is a country which is rich in Orthosiphon aristatus as traditional medicine believed to act as diuretics and to be useful in the treatment of kidney infections, bladder infections, edema, diabetes mellitus, hypertension and rheumatism. Hence, the role of the plant is very important for the health. Accordingly, in this study standardization of Orthosiphon aristatus leaves was carried out. Standardization of extraction for herbal plants in Indonesia is an important phase in the development of native medicine in Indonesia. The herbal plant extracts can be used as basic ingredients, intermediary ingredients, and finished products.
Methodology

General Experimental Procedure

A number of powder and extracts of kumis kucing leaves were carefully weighed and examined their specific and non-specific parameters, determination of LC$_{50}$ values using BSLT (Brine Shrimp Lethality Test). Total flavonoid assay was performed by using a spectrophotometer UV-Vis with absorption maximum of approximately 433 nm and standardization criteria with DER (Drug Extract Ratio).

Stage Procedure

Determination of Specific and Non-Specific Parameters

1. Determination of loss on drying

Loss on drying is levels of evaporated part. Unless otherwise stated, the determination was performed at the temperature of 105°C. Weigh carefully 1-2 g of the substance in a bottle having been heated previously at the determined temperature for 30 minutes. If the substance is melting at the temperature below the determined temperature, the drying is done at temperatures of 5 and 10°C below the melting temperature for 1 up to 2 hours, then continued at the determined temperature until a stable weight was obtained.

2. Determination of total ash content

Simplicia was finely powdered and weighed as much as 2.5 g in a crucible of porcelain. Afterwards, the powder was ignited in the furnace until all charred matter were removed. Afterwards, allow to cool and weigh it accurately. If the charred matter still remain, the sample was filtered through a ash-free filter paper. The residue on the filter and the filter was put in the same crucible of porcelain and ignited in the furnace. While the filtrate in the different crucible of porcelain was heated in the furnace until constant weight was obtained. Total ash content was calculated to the dry simplicia.

3. Determination of water soluble ash content

Ash obtained in the determination of the ash content was boiled gently with 25 ml of dilute hydrochloric acid for 5 minutes. Collect the matter that are not soluble in the acid and filter through a ash-free filter paper. Wash with hot water and ignite in the furnace for 15 minutes at a temperature of not more than 25°C until the weight remains constant. Calculate the content of water soluble ash based on the weight of the dry sample.

4. Determination of insoluble acid ash content

Ash obtained from the determination of the ash content was boiled gently with 25 ml of dilute sulphuric acid for 5 minutes. Collect the matter insoluble in the acid and filter through a ash-free filter paper. Wash with hot water and ignite until the weight remains constant. Calculate insoluble acid ash content based on the weight of the dry sample.

5. Organoleptic

Consistency and colour of the ethanol extract was visually observed; while the smell was determined using sensory organs, like nose and tongue.

6. Determination of water soluble content

Determination of water soluble content aimed to determine the level of water soluble matter from materials dissolved in the water. Simplicia dry powder as much as 5 gram are macerated for 24 hours using water 100 mL chloroform P (1000: 2.5) in the closed flask repeatedly shaken during the first 6 hours and were then proceed to stand for 18 hours. Afterwards filter it and the 20 mL of resulting filtrate was evaporated to dry in a flat bottom dish which has been tarred. The content was determined by considering the weight of the dry sample.

7. Determination of ethanol soluble content

Determination of ethanol soluble content aimed to determine the level of matter soluble in ethanol. Dry simplicia dry powder in the air, then 5 grams of the powder are macerated for 24 hours using 100 mL of 95% ethanol in the closed flask repeatedly shaken during the first 6 hours and were then proceed to stand for 18 hours. Filter it and 20 mL of resulting filtrate was evaporated to dry in a flat bottom dish which has been tarred. Heat the residue at temperatures up to 105°C. The content was determined by comparing to the weight of the dry sample.

Determination of LC$_{50}$ values using BSLT (Brine Shrimp Lethality Test)

Brine Shrimp Lethality Bioassay was carried out to investigate the citotoxicity of extract of medicinal plants. Artificial sea water was prepared by dissolving sea salt (38.0 g) in distilled water (1 L). The sea water was put in a small tank and teaspoon of brine shrimp egg added to one side of the divided tank which was covered. The other side is not covered so as to allow light that would attract the hatched shrimps. The tank containing the brine shrimp eggs was left at room temperature for 48 hours to allow the eggs to hatch. After hatching, active nauplii free from egg shells were collected and used for the assay. 20 mg of each extract were separately dissolved in 2 ml DMSO, and from these 1000, 100, and 10 μg/ml were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 9 test vial for each extract. Brine shrimp larvae (nauplii, 10) were added to each test vial. The final volume of the solution in each test vial was made up to 5 ml with sea water immediately after adding shrimp larvae. The test vial were maintained
under illumination. Survivor were counted after 24 hours and the percentage death of each dose was determined. The LC50 values were obtained by a plot of percentage of the shrimps killed against the concentrations of the extract and the best fit line was obtained from the data by means of regressions analysis. LC50 was obtained from the best fit line method.

**Determination of total flavonoid content**

The extract equivalent to 200 mg was carefully weighed and put into a round bottom flask. Add 1 ml HMT (Hexamethylen), 20 ml acetone and 2 ml of HCl solution hydrolysis was carried out by reflux for 30 minutes. The mixture was filtered through the cotton. The filtrate obtained was put into 100 ml flask and added with ethyl acetate for 3 times, each time with 15 ml of ethyl acetate. Ethyl acetate phase was collected and added with ethyl acetate to 50 ml. Measure the absorbance using UV-Vis spectrophotometer at maximum wavelength of approximately 433 nm. Percentage of total flavonoid content was using following formula:

\[
\text{%Total flavonoid content} = \frac{C_p(A_s-A_b) \times k}{(\text{weight extract} - \text{Abp})} \times 100
\]

*Cp*: Comparative concentration in percent  
*A*: Absorbance of the sample  
*Ab*: Absorbance of the blank sample  
*Ap*: Absorbance comparison  
*K*: Constant factor (1.25)

**Determination of DER (Drug Extract Ratio)**

A number of 25 grams of the powdered simplicia was extracted with 250 ml of 70% ethanol by stirring at room temperatures. Afterwards, maceration was carried out 3 times in which first one using 100 ml and the second and the third using 75 ml 70% ethanol, respectively 30 minutes. The filtrate obtained was evaporated with a rotary evaporator until thick extract was obtained. Weigh the extract to calculate DER-native was using following formula:

\[
\text{DER-native} = \frac{\text{weight simplicia}}{\text{weight extract}}
\]

**Results and Discussion**

Table 1 present results of phytochemical screening of simplicia powder and extract Orthosiphon leaves. The results of phytochemical screening showed that the powder and extract Orthosiphon aristatus secondary metabolites containing flavonoid, triterpenoid, steroid, essential oils, Coumarin and saponins. Organoleptic results of the extract showed the extract has a thick consistency, blackish green colour, taste rather chelates and hasn’t a specific aromatic odor. Result of the determination of specific and non-specific parameters showed loss on drying of 11.73% and water content of 9.06% in which these results meet the requirement of MMI (Materia Medika Indonesia) that the water content is not more than 10%. Total ash content of the leaves is 10.92% which meets also MMI (not more than 12%). Water soluble content is 13.85% where the value is in agreement with MMI (not less than 11%). Ethanol soluble content is 7.67% whereas the levels in MMI is not less than 4%. Acid insoluble ash content is 11.05% while the value in the MMI is not more than 12%. In addition, water soluble ash content is 5.26%. LC50 values was obtained from the result of BSLT as depicted in Figure 1.

The LC50 values was 25.0150 µg/ml, sample is said to have toxic activity when LC50 values <1000 µg/ml.

Percentage total flavonoid of extract Orthosiphon aristatus was obtained from absorbance of extract Orthosiphon aristatus depicted in Figure 2.

Total flavonoids is 1.7797% in which this level was derived from the class of flavones and flavonolins the extract.

In addition, DER (Drug Extract Ratio), weigh the extract to calculate DER-native was using following formula and value of DER-native was as depicted in Table 2.

**Table 1. Phytochemical screening of simplicia powder and extract Orthosiphon aristatus**
Simplicia powder of Orthosiphon aristatus leaves | Extract Orthosiphon leaves
---|---
1. Alkaloids | -
2. Flavonoids | +
3. Saponins | +
4. Quinone | -
5. Tannin | -
6. Steroid/triterpenoid | +/
7. Essential oils | +
8. Coumarins | +

**Figure 1.** Graphic of Brine Shrimp Lethality Test (BSLT)

**Figure 2.** Absorbance of the sample

**Table 2.** Value of Der-native Orthosiphon Leaves

<table>
<thead>
<tr>
<th>Simplex</th>
<th>Weigh Extract</th>
<th>DER-native</th>
<th>Rendemen t</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1.6364</td>
<td>15.2774</td>
<td>6.5456</td>
</tr>
</tbody>
</table>

**DER-native** is 15.2774 which means that to obtain 1 gram of extract, it needs Kumis kucing leaves as much as 15.2774 gram.

**Conclusions**

1. The results of phytochemical screening showed that the powder and extract orthosiphon aristatus containing flavonoid, triterpenoid, steroid, essential oils, Coumarin and saponins.

2. Organoleptic results of the extract showed the extract has a thick consistency, blackish green colour, taste rather chelates and hasn’t a specific aromatic odor. Loss ondrying of 11.73 %, water content of 9.06 %, total ash content of 10.92 %, water soluble content of 13.85 %, ethanol soluble content of 7.67%, acid insoluble ash content of 11.05 %, and water soluble ash content of 5.26 %. Extract orthosiphon aristatus has a toxic activity with the LC50 value is 25.0150μg/mg

3. Total flavonoid content of orthosiphon aristatus extractis 1.7797 % and DER-native 15.2774

**References**


