

Chemical Specification of *Orthosiphon aristatus* (Blume) Miq.

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ABSTRACT Although *Orthosiphon aristatus* (Blume) Miq. (Lamiaceae) is widely used in Thailand, it has not been included in Thai Herbal Pharmacopoeia. The main objective of this study is to provide scientific information on quality control to facilitate its appropriate chemical specification. Chemical evaluation of 23 samples of aerial part and 6 samples of leaves of *Orthosiphon aristatus* (Blume) Miq. revealed that the appropriate chemical specifications of dried aerial part and leaves could be established respectively i.e. water content not more than 9, 10%w/w, ash content not more than 6, 13%w/w, acid insoluble ash content not more than 1%w/w of each, water soluble extractive not less than 12, 16%w/w, ethanol soluble extractive not less than 5, 11%w/w, 50% ethanol soluble extractive not less than 13, 23%w/w, potassium content not less than 0.7, 2% w/w, pH value not less than 5.0 on both. Furthermore, chemical identification by means of color test and thin layer chromatographic analysis were determined.

Key word : *Orthosiphon aristatus* (Blume) Miq., Lamiaceae, chemical specification

INTRODUCTION

The use of natural medicine is a persistent aspect of present-day health care. Although modern medicine may be available throughout the world, many people have begun to turn to alternative or complementary therapies, including medicinal herbs. *Orthosiphon aristatus* (Blume) Miq. is widely distributed in Thailand. It has been scientifically evaluated for possible medical preparation. Safety and efficacy data are available. *Orthosiphon aristatus* (Blume) Miq. is commonly used in primary health care program. Therefore, quality assurance of this herbal medicine has now become a key issue.

Orthosiphon aristatus (Blume) Miq. belongs to the family Lamiaceae. It is occasionally planted throughout Thailand for medicinal and ornamental purpose. It has local names in Thai, Yaa-nuat-maeo is more well known than Phayap-mek, Bangrak-pa⁽¹⁾. It is a perennial herb, 25 - 100 cm tall, with quadrangular stem. Leaves opposite, ovate to rhomboid, 3 - 7 cm long and 2 - 5 cm wide. Inflorescence terminal, many white or pale-lilac flowers which long-protruding stamens^(2, 3). The sketch of *Orthosiphon aristatus* (Blume) Miq. has shown in Figure 1.

2. Confirmatory test (Thin layer chromatographic analysis)

Test solution :

Refluxed 2 g of powdered drug with 50 ml of water for 30 minutes, filtered, and then evaporated the filtrate to dry to get water extract. Refluxed the extract with 25 ml of methanol for 10 minutes, filtered and evaporated the filtrate until dryness. Methanol extract of water extract could be obtained.

The test solution was prepared by redissolving methanol extract with methanol. The final volume of the test solution was adjusted to concentration of 10 mg/ml based on the weight of methanol extract of water extract.

Test solution : methanol extract of water extract of

1. aerial part of *Orthosiphon aristatus* (Blume) Miq.
2. leaves of *Orthosiphon aristatus* (Blume) Miq.

Standard solutions :

Dissolved reference standard with methanol and then adjusted the volume to concentration of as follow:

- | | |
|--------------------|-----------|
| 1. rosmarinic acid | 0.5 mg/ml |
| 2. caffeic acid | 0.2 mg/ml |
| 3. ursolic acid | 0.5 mg/ml |

Adsorbent :

Silica gel GF₂₅₄ precoated plate

Developing solvent :

Toluene-ethyl acetate-formic acid 9 : 9 : 1

Developing distance : 10 cm.

Spotting amount :

5 µl each of test solution and 3 µl each of standard solution

Detection :

The chromatographic analysis was developed in the same manner on two TLC plates. After removal of the plates, allowed them to dry in room temperature, then examined the plates with the following detections.

I. UV 254 nm

II. Natural products-polyethyleneglycol reagent (NP/PEG reagent)⁽²⁰⁾

NP reagent = 1% Diphenylboric acid-2-aminoethyl ester in methanol

PEG reagent = 5% Polyethyleneglycol 4000 in ethanol

One chromatographic plate was heated at 80°C at least 10 min, sprayed with excess amount of NP reagent and followed by PEG reagent. Then, the chromatogram was observed in long wavelength UV light (UV₃₆₆).

III. Vanillin-phosphoric acid reagent⁽²⁰⁾

Preparation of reagent, dissolved 1 g of vanillin in 25 ml of ethanol, then added 25 ml of water and 35 ml of 85% ortho-phosphoric acid.

Another chromatographic plate was sprayed with this reagent, then heated at 120°C until spots distinctly appeared. Observed chromatogram under UV₃₆₆ light.

B. Quality Evaluation

1. Loss on drying

Loss on drying was obtained from a Memmert hot air oven by following the methods in Thai Herbal Pharmacopoeia⁽²¹⁾



Figure 1 *Orthosiphon aristatus* (Blume) Miq.

From previous phytochemical studies, three main groups of chemical constituents of *Orthosiphon aristatus* (Blume) Miq. are flavonoids, organic acids and terpenoids. For instance, flavonoids compounds are eupatorin, sinensetin, salvigenin, ladanein, vomifoliol, 5-hydroxy-6, 7, 3', 4'-tetramethoxyflavone, 6-hydroxy-5, 7, 4'-trimethoxyflavone, 7, 3', 4'-tri-O-methyluteolin, tetramethylscutellarein and scutellarein tetramethylether^(4, 5, 6, 7). Caffeic acid and its derivatives including rosmarinic

acid^(6, 7) and 2, 3-decaffeoyltartaric acid⁽⁸⁾ predominate over the flavones in aqueous extract. In the class of terpenoids, diterpene constituents could be isolated, for example, orthosiphols A, B, D-N^(6, 7, 9, 10) orthosiphonones A-B^(11, 12) staminols A-B^(7, 10) norstaminol A^(7, 13) staminolactones A-B^(7, 13) norstaminone A⁽⁹⁾ nororthosiphols A-B⁽¹¹⁾ and neoorthosiphols A-B^(14, 15) etc. Together with diterpenoids, triterpenoids and steroids such as ursolic acid, hederagenin and β -sitosterol were elucidated⁽⁷⁾. Further-

more, high potassium content in this plant has also been reported. In the search for pharmacological activity, methylripariochromene A from leaves has antihypertensive effect, some lipophilic flavonoids possess radical scavenging activity, alcoholic extract has diuretic activity, etc^(4,5,11,16,17,18,19). Pharmacologically active compounds in *Orthosiphon aristatus*

(Blume) Miq. that are responsible for the diuretic properties and for antibacterial properties, the predominating caffeic acid derivatives should be taken into consideration⁽⁸⁾. Chemical structures of some constituents are shown in Figure 2

Orthosiphon aristatus (Blume) Miq. featured in pharmacopoeias of some countries.

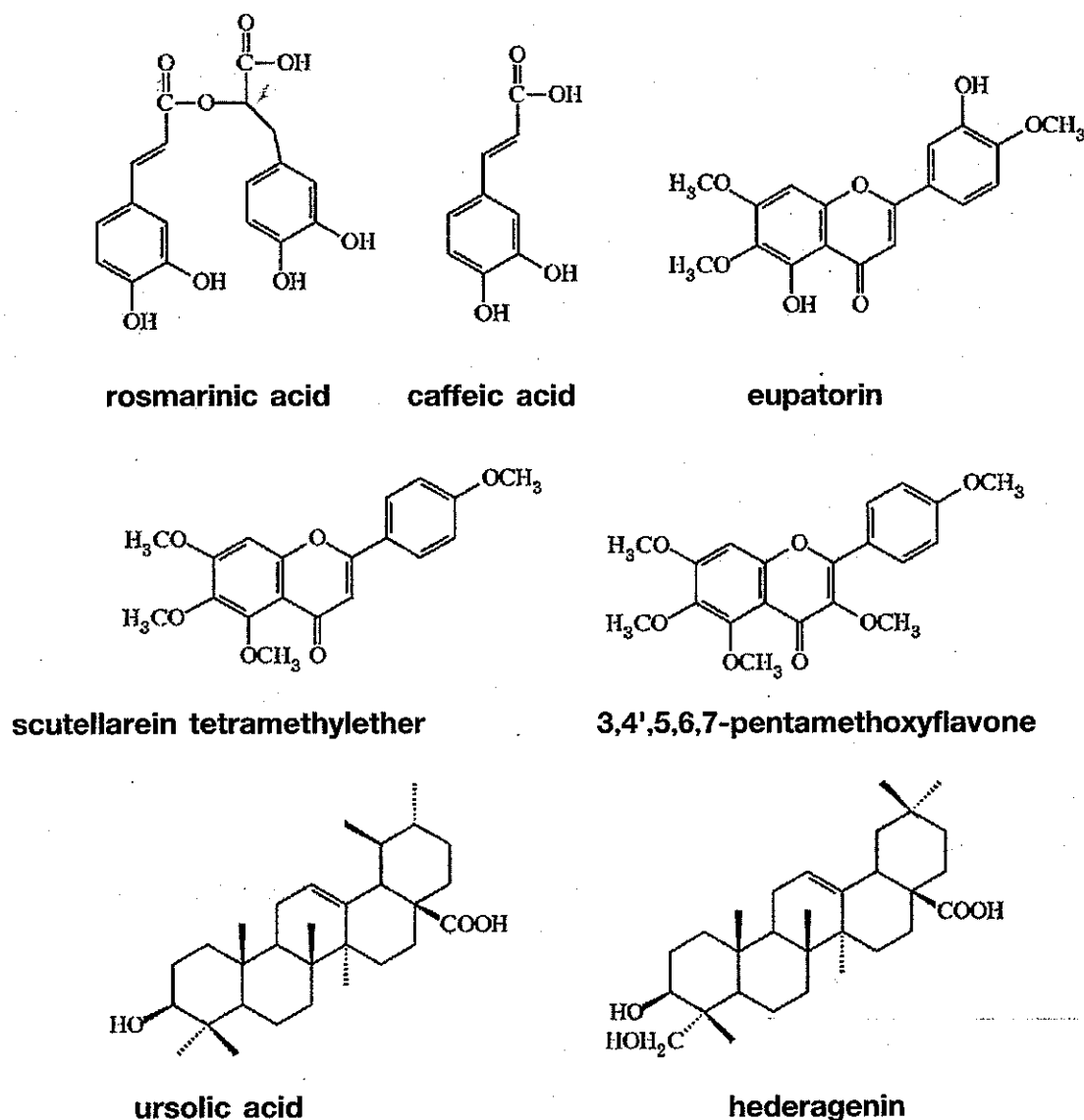


Figure 2 Chemical constituents of *Orthosiphon aristatus* (Blume) Miq.

Although it is widely used in Thailand, it has not been included in Thai Herbal Pharmacopoeia. The objective of this investigation is to provide scientific information on quality control/quality assurance of *Orthosiphon aristatus* (Blume) Miq. to facilitate its appropriate chemical specification in Thailand.

MATERIALS AND METHODS

Materials

1. Plant materials

Fresh aerial part and leaves of *Orthosiphon aristatus* (Blume) Miq. (Family Lamiaceae) were collected from many parts of Thailand. They were identified^(2, 3) by Botanical laboratory, Medicinal Plant Research Institute at Department of Medical Sciences, where the voucher specimens (Bansiddhi 97-01 to 97-05) are deposited. 23 samples of aerial part and 6 samples of leaves were washed thoroughly, dried in an oven at 45°C for 48 hours. The dried samples were ground to powder, then passed through a sieve with mesh no.180 and kept in well-closed containers.

2. Adsorbent

Silica gel GF₂₅₄ precoated plate 20 × 20 cm. Layer thickness 0.25 mm. (E. Merck)

3. Solvents and chemicals

Analytical grade

4. Reference standards

4.1 rosmarinic acid (Chromadex)

4.2 caffeic acid (Sigma)

4.3 ursolic acid (Sigma)

5. Retsch AS200 Basic sieves

6. Memmert hot air oven

7. Satorius analytical balances

8. UV cabinet

9. CAMAG TLC Plate Heater

10. Thermolyne Type 6000 furnace

11. Perkin Elmer 3030 Atomic adsorption spectrophotometer

12. Microwave digestion CEM Corporation MDS2100

13. Eyela NE-1 rotary evaporator

14. Hanna pH meter

Methods

A. Chemical identifications

1. Preliminary test

Test solution :

2 g of powdered drug was refluxed with 50 ml of water for 30 minutes, filtered and then evaporated the filtrate to 25 ml.

Reagent :

(1) Potassium Permanganate (KMnO₄) TS
3.3 g of potassium permanganate was dissolved in 1000 ml of water, the solution was boiled for 15 min. Stopped the flask, allowed it to stand for at least 2 days and filtered.

(2) Ferric Chloride (FeCl₃) TS

1 g of ferric chloride hexahydrate was dissolved in sufficient water to produce 100 ml of solution.

(3) 6 M Hydrochloric acid

Procedure :

1. To 2 ml of test solution, added a few drops of Potassium permanganate TS, shaken well. The color of Potassium permanganate TS was noted.

2. To 1 ml of test solution, added a few drops of Ferric chloride TS, observed the color of the solution.

3. 0.5 ml of test solution was evaporated until dryness, moistened the residue with one drop of 6 M hydrochloric acid, observed the color of the flame (from bunsen lamp) through the cobalt-glass.

2. Ash content

Total ash content and acid-insoluble ash content were carried out on Thermolyn Type 6000 Furnace using the methods in Thai Herbal Pharmacopoeia⁽²¹⁾.

3. Extractives content

Water soluble extractive, ethanol soluble extractive, 50% ethanol soluble extractive contents were determined as described in Thai Herbal Pharmacopoeia⁽²¹⁾.

4. Determination of potassium content

Potassium content was performed with a Perkin Elmer 3030 Atomic Absorption (AA) Spectrophotometer.

Analytical procedure was followed the method of United State Pharmacopoeia⁽²²⁾.

Potassium content was determined by means of AA analysis, 0.3 g of powdered drug, accurately weighed, added 2.0 ml of nitric acid, digested by using Microwave digestion CEM Corporation MDS2100. Sample solution was transferred to a 25 ml volumetric flask and

adjusted to volume with water. One ml of solution was further diluted to 50.0 ml, and then potassium content analysis was performed.

5. Determination of pH

Analytical procedure was followed the method of British Pharmacopoeia⁽²³⁾.

RESULTS

A. Chemical identifications

The results of preliminary test and confirmatory test of *Orthosiphon aristatus* (Blume) Miq. aerial part and leaves have been presented in Table 1, 2 and Figure 3.

TLC patterns of aerial part and leaves of *Orthosiphon aristatus* (Blume) Miq. are in a similar way. The R_f values of components of both parts are concluded in Table 2.

B. Quality evaluation

All the samples were collected from various sources, unsuitable collection or possible deterioration due to incorrect or extended storage might effected the content

Table 1 Chemical identification of *Orthosiphon aristatus* (Blume) Miq.

Sample	Identification test			
	Preliminary test			Confirmatory test (TLC analysis)
	KMnO ₄ TS	FeCl ₃ TS	Flame test	
Aerial part	Color of KMnO ₄ TS was disappeared in all the samples	All the samples gave greyish-green color	Reddish-violet flame was observed in all the samples	All the samples showed rosmarinic acid, caffeic acid, ursolic acid and other unidentified compounds
Leaves	Color of KMnO ₄ TS was disappeared in all the samples	All the samples gave greyish-green color	Reddish-violet flame was observed in all the samples	All the samples showed rosmarinic acid, caffeic acid, ursolic acid and other unidentified compounds

Table 2 hR_f values of components of *Orthosiphon aristatus* (Blume) Miq.

Spot	hR_f (color)	Detection with		
		NP/PEG, UV ₃₆₆ (color)	UV ₂₅₄	Vanillin - Phosphoric acid TS (Color)
1	2-8	moss green	-	-
2	15-20	moss green	-	light orange
3	30-33	moss green	quenching	-
4	36-44	moss green	quenching	orange
5	48-53	pale blue	-	pale yellow
6	53-57	pale blue	-	pale yellow
7	58-61	blue	-	moss green
8	61-64	greenish blue	quenching	blue
9	64-67	pale blue	-	pale blue
10	81-86	-	-	orange red

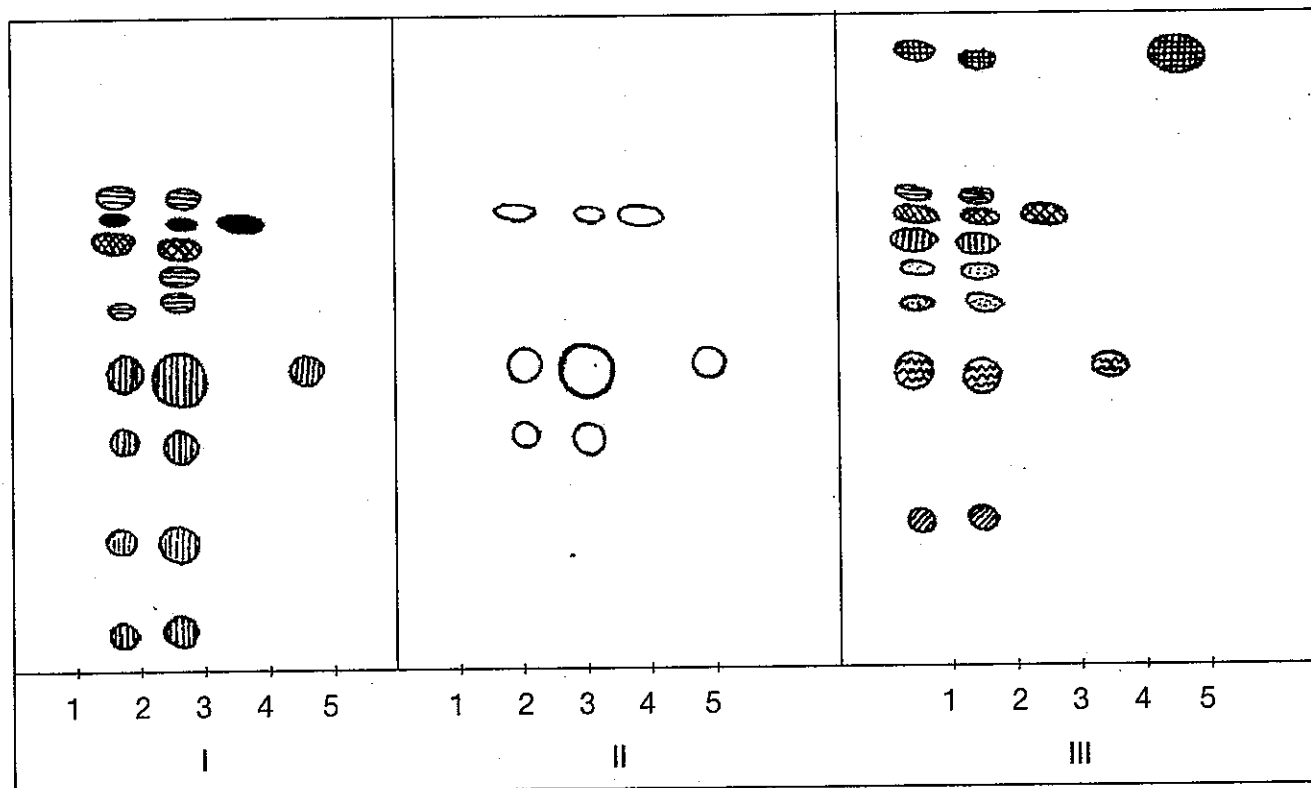


Figure 3 TLC chromatograms of *Orthosiphon aristatus* (Blume) Miq.

Remark

I = detection with NP/PEG reagent, UV ₃₆₆	II = detection with UV ₂₅₄
III = detection with Vanillin-phosphoric acid reagent, UV ₃₆₆	
1 = test solution of aerial part	2 = test solution of leaves
3 = caffeic acid	4 = rosmarinic acid
5 = ursolic acid	
⊖ = moss green	⊕ = pale blue
● = greenish blue	⊗ = light orange
⊘ = pale yellow	⊙ = orange
	⊚ = orange red
	○ = quenching

of constituents in all the samples. To assess the value of *Orthosiphon aristatus* (Blume) Miq., their quality evaluations were determined in 5 main categories; i.e. loss on drying, ash, extractive, potassium and pH. The results of quality evaluation of aerial parts and leaves have been showed in Table 3 and 4, respectively.

Table 3 Quality evaluation of *Orthosiphon aristatus* (Blume) Miq. aerial part

Determination	Range ($\bar{X} \pm 10\%$)	$\bar{X} \pm SD$
Loss on drying	7.24 - 8.84	8.04 \pm 1.16
Total ash	4.73 - 5.79	5.26 \pm 1.61
Acid insoluble ash	0.14 - 0.18	0.16 \pm 0.10
Water extractive	11.92 - 14.56	13.24 \pm 3.86
Ethanol extractive	4.98 - 6.08	5.53 \pm 1.24
50% Ethanol extractive	12.80 - 15.64	14.22 \pm 4.48
Potassium content	0.75 - 0.91	0.83 \pm 0.63
pH	5.00 - 6.12	5.56 \pm 0.24

Table 4 Quality evaluation of *Orthosiphon aristatus* (Blume) Miq. leaves

Determination	Range ($\bar{X} \pm 10\%$)	$\bar{X} \pm SD$
Loss on drying	7.79 - 9.53	8.66 \pm 1.68
Total ash	10.18 - 12.44	11.31 \pm 1.21
Acid insoluble ash	0.36 - 0.44	0.40 \pm 0.12
Water extractive	26.99 - 32.99	29.99 \pm 6.53
Ethanol extractive	11.02 - 13.48	12.25 \pm 2.88
50% Ethanol extractive	23.84 - 29.14	26.49 \pm 3.41
Potassium content	2.46 - 3.00	2.73 \pm 0.47
pH	5.81 - 7.11	6.46 \pm 0.18

DISCUSSIONS

The chemical identification of 23 samples of aerial part and 6 samples of leaves of *Orthosiphon aristatus* (Blume) Miq. were performed. Since the constituents of this medicinal plant consist of phenolic compounds, the preliminary tests were focus on detection of phenolic constituents by color reaction with FeCl_3 TS and KMnO_4 TS. Moreover, the presence of potassium salt in this plant could be investigated by flame test. All of these reactions could be as the characteristic of *Orthosiphon aristatus* (Blume) Miq. Thin layer chromatographic analysis of phenolic acid, flavonoids and terpenoids was obtained on silica gel using a mixture of Toluene-Ethyl acetate-Formic acid (9 : 9 : 1) as a developing solvent and spraying with NP/PEG reagent. Rosmarinic acid, a major component, gave moss green color with the Rf value of about 0.40. A greenish blue color of caffeic acid could be also observed with Rf value of about 0.62. Instead of NP/PEG reagent, spraying with vanillin-phosphoric acid TS and observed under UV₂₅₄, the orange-red spot of ursolic acid occurred. This system gave a good spread of components and displayed high reproducibility of Rf value.

From the result of quality evaluation, the appropriate chemical specifications of *Orthosiphon aristatus* (Blume) Miq. could be set up. When \bar{X} is the arithmetic mean of the results, the maximum limits $\bar{X} + 10\%$ (if the results are not integers, they will be rounded to the next higher integers) are stated for the limited amount of loss on drying, total ash and acid-insoluble ash contents and the term "not

more than" are expressed for their specifications. Besides these, the required limits of extractives, potassium content, pH value are stated for the maximum limits $\bar{X} - 10\%$ and the term "not less than" is used for their specifications. The data on quality evaluation of *Orthosiphon aristatus* (Blume) Miq. are considered from the experimental results of *Orthosiphon aristatus* (Blume) Miq. aerial part (Table 3), water content by loss on drying, ash content, acid-insoluble ash content should be not more than 9%, 6% and 1%, respectively. Water soluble extractive, ethanol soluble extractive, 50% ethanol soluble extractive should not less than 12%, 5% and 13%, respectively. Potassium content should not less than 0.7%. pH value should not less than 5. From quality evaluation results of *Orthosiphon aristatus* (Blume) Miq. leaves in Table 4 water content by loss on drying, ash content, acid insoluble ash content should not more than 10%, 13% and 1%, respectively. The value of extractive content: water soluble extractive, ethanol soluble extractive, 50% ethanol soluble extractive should not less than 26%, 11% and 23%, respectively. Potassium content should not less than 2%. pH value should not less than 5.

CONCLUSIONS

The appropriate chemical quality specification for the dried aerial part and leaves of *Orthosiphon aristatus* (Blume) Miq. could be respectively as follow:

Loss on drying : not more than 9,
10% w/w

Ash content : not more than 6,
13% w/w
Acid insoluble ash : not more than 1,
1% w/w
Water soluble extractive : not less than
12, 26% w/w
Ethanol soluble extractive : not less than
5, 11% w/w
50 % ethanol soluble extractive : not less
than 13, 23% w/w
Potassium content : not less than 0.7,
2% w/w
pH : not less than 5.0, 5.0

The chemical identification of *Orthosiphon aristatus* (Blume) Miq. by means of color test and chromatographic analysis can be provided.

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ข้อกำหนดทางเคมีของหญ้าหนวดแมว

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บทคัดย่อ หญ้าหนวดแมว (*Orthosiphon aristatus* (Blume) Miq. วงศ์ Lamiaceae) เป็นสมุนไพรที่มีการใช้อย่างแพร่หลาย แต่ยังไม่มีการบรรจุข้อมูลของสมุนไพรชนิดนี้เข้าในตำรามาตรฐานยาสมุนไพรไทย การศึกษานี้มีวัตถุประสงค์เพื่อจัดทำข้อกำหนดทางเคมี ซึ่งจะเป็ประโยชน์ในการจัดทำมาตรฐานสำหรับการควบคุมคุณภาพสมุนไพรนี้ต่อไป ข้อมูลจากการศึกษาส่วนเหนือดินของสมุนไพรหญ้าหนวดแมว 23 ตัวอย่าง และเฉพาะส่วนใบอีก 6 ตัวอย่าง นำมาจัดทำข้อกำหนดทางเคมีตามลำดับ ดังนี้ ปริมาณความชื้น ไม่เกินร้อยละ 9, 10 โดยน้ำหนัก, ปริมาณเถ้า ไม่เกินร้อยละ 6, 13 โดยน้ำหนัก, ทั้งส่วนเหนือดินและใบมีปริมาณเถ้าที่ไม่ละลายในกรด ไม่เกินร้อยละ 1 โดยน้ำหนัก, ปริมาณสิ่งสกั้ดด้วยน้ำ ไม่น้อยกว่าร้อยละ 12, 26 โดยน้ำหนัก, ปริมาณสิ่งสกั้ดด้วยแอลกอฮอล์ ไม่น้อยกว่าร้อยละ 5, 11 โดยน้ำหนัก, ปริมาณสิ่งสกั้ดด้วยแอลกอฮอล์ 50% ไม่น้อยกว่าร้อยละ 13, 23 โดยน้ำหนัก, ปริมาณโพแทสเซียม ไม่น้อยกว่าร้อยละ 0.7, 2 โดยน้ำหนัก, ทั้งส่วนเหนือดินและใบมีความเป็นกรด-เบส ไม่น้อยกว่า 5.0 นอกจากนี้ยังได้ศึกษาเอกลักษณ์ทางเคมีทั้งการทดสอบการเกิดสีและการวิเคราะห์คุณภาพด้วยรงคเลขพิวบางด้วย