

PHARMACOGNOSTIC SPECIFICATION OF *VETIVERIA ZIZANIOIDES* ROOTS IN THAILAND

Somchai Issaravanich¹ Chanida Palanuvej¹ Tanasorn Tunsaringkarn¹ Anusorn Rungsiyothin¹
Niran Vipunngeun¹ Anchalee Chuthaputti² Nijsiri Ruangrungsi^{1,3,*}

¹College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand

²Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health, Nonthaburi 11000, Thailand

³Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT: Dried roots of *Vetiveria zizanioides* Nash (commonly known in Thai as 'PHAEK-HOM') were collected from 15 Thai traditional drug stores in 13 provinces located at 4 regions of Thailand. Crude drugs were evaluated by World Health Organization (WHO) quality control methods for medicinal plant materials. Cross section of the root and powdered drug were studied for microscopic characters. The mean contents of pharmacognostic specifications of *Vetiveria zizanioides* roots were described as follow: foreign matter 0.30% w/w, acid-insoluble ash 9.10% w/w, total ash 10.63% w/w, ethanol-soluble extractive 4.98% w/w, water-soluble extractive 48.03% w/w, loss on drying 9.34% w/w, volatile oil content 0.23% w/w and water content 8.48% w/w. The roots of *V. zizanioides* contained numerous starch grains which varied in size distributed in reserved parenchyma and the radial arrangement with fibre-parenchyma was found. TLC and GC fingerprint of volatile oil of *V. zizanioides* roots were demonstrated.

Keywords: *Vetiveria zizanioides* root, pharmacognostic specification

INTRODUCTION: Vetiver is a common name of the grass in the genus *Vetiveria* (Family Gramineae), which consists of 11 species widely distributed in tropical regions of Asia, Africa, Pacific Islands, and Australia. In Thailand, the two species occur in open areas; *Vetiveria zizanioides* Nash ('Phaek-Hom' in Thai) mostly in moist places, while *V. nemoralis* A. Camus ('Phaek-Don' in Thai) tends to be in drier area. The dominant vetiver grass species grown in Thailand is *Vetiveria zizanioides*²⁾. Roots of *V. zizanioides* is used in Thai traditional medicine as a medicament for carminative, antipyretic, diuretic and also used for hair care and tonic bath³⁾. The previous studies of biological activity of its crude methanol extract showed antimicrobial activities⁴⁾. This study aimed to examine the pharmacognostic specification of *V. zizanioides* roots, based on the crude drug samples currently available in Thailand.

MATERIALS: The dried crude drugs were purchased from 15 Thai traditional drug stores located at 4 regions of Thailand as follow : Bangkok (3 stores), Chon Buri, Nakorn Pathom, Kamphaeng Phet, Pichit, Nakorn Sawan, Chiang Mai, Nakorn Ratchasima, Ubon Ratchathani, Roi et, Songkhla, Trang and Nakorn Si Thammarat. The samples were identified as *V. zizanioides* Nash by one of the authors (N.R.). The voucher and numbers of specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand.

METHODS: Macroscopic, microscopic and constant numbers due to quality of crude drug were examined by World Health Organization (WHO) guideline standard methods⁵⁾.

Macroscopic and Microscopic Examination

Size, colour and other visual inspections of crude drugs were examined. Anatomical and histological

*To whom correspondence should be addressed.
E-mail: nijsiri.r@chula.ac.th, Tel. 0 2218 8201

characters were determined as follow: transverse sections and powdered samples (ground and sifted through a 250 micron sieve) were inspected respectively under microscope (Olympus BX41) with a magnification of 4x, 10x and 40x and compared the scale with the 0.01 mm micrometer.

Determination of Foreign Matter

Fifty grams of crude drugs were spread in a thin layer and sorted the foreign matter by visual inspection. The remainders of the samples were sifted through a 250 micron sieve. The portions of the sorted foreign matter were then weighed and calculated the content in grams per 100 grams of crude drug.

Determination of Total Ash

Three grams of ground crude drug were accurately weighed and placed in a previously ignited and tared crucible. The samples were spread in an even layer and ignited by gradually increasing the heat to 500-600°C until white then cooled in a desiccator and weighed without delay.

Determination of Acid-Insoluble Ash

The crucible containing the total ash was added with 25.0 ml of hydrochloric acid (70 g/l), covered with a watch-glass and boiled gently for 5 minutes. Then rinsed the watch-glass with 5 ml of hot water and added this liquid to the crucible. The insoluble matters were collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matters was transferred to the original crucible, dried on a hot plate and ignited again. The crucible was then cooled in a desiccator and weighed without delay.

Determination of Ethanol-Soluble Extractive

Five grams of powdered crude drug were macerated with 100.0 ml of absolute ethanol in a closed conical flask for 6 hours in shaking bath and allowed to stand for 18 hours. They were filtered rapidly to avoid loss of ethanol. Twenty milliliter of the filtrate were evaporated to dryness in a tared small beaker and dried with heat to constantly weight.

Determination of Water-Soluble Extractive

Five Grams of ground crude drug were acerated with 100.0 ml. of distilled water in shaking bath and allowed to stand for 18 hours. They were filtered and 20.0 ml of the filtrate were evaporated to dryness in a tared small beaker and dried at 105°C to constant weight.

Determination of Loss on Drying

Five grams of ground crude drug were weighed in a tared small beaker and dried with heat at 105°C to constantly weight.

Determination of Moisture

Fifty grams of ground crude drug were added with 200.0 ml of water-saturated toluene and distilled by Azeotropic distillation. As soon as water was completely distilled, rinsed the inside of the condenser tube with toluene and continued the distillation for 5 more minutes. Allowed the receiving tube cooled to room temperature. When water and toluene layers were separated, read off the volume of water.

Determination of Volatile Oil Content

Hundred grams of ground crude drug were added with 600.0 ml of water and distilled by Clevenger apparatus. When volatile oil was completely distilled, the inside of the condenser tube was rinsed with water. Allowed the receiving tube cooled to room temperature. When volatile oil and water layers were separated, read off the volume of volatile oil.

Chemical Identification of Volatile oil

Volatile oil was investigated by capillary column gas chromatography with mass spectrometer as detector (GC/MS). GC/MS qualitative and quantitative analyses were carried out using a Finnigan Trace GC ultra with Finnigan PolarisQ ion trap detector and BPX5 fused silica column (30 m x 0.25 mm, 0.25 µm film thickness). The injector temperature was 180°C. Sample, 1 µl of the oil, was injected by splitter (1:100) into capillary column. The oven temperature was 60°C for 1 min., then ramp to 240°C with the rate of 3.3°C/min. Helium was used as carrier gas (flow rate 1 ml/min). MS was performed by EI positive mode at 70 eV

ionization voltages. The constituents of the oil were identified by matching their mass spectra and retention indices with NIST02 MS library and Percentage composition was computed from GC peak areas.

Thin-Layer Chromatographic Identification

One gram of powdered crude drug was refluxed with 20 ml of methanol. The solvent extract was filtered and evaporated to dryness. Dissolved the residue in 0.5 ml of methanol, applied 10 μ l to the thin-layer plastic plate coated with silica gel GF254 (Polygram[®] SIL G/UV254, 0.25 mm thickness, 20 cm x 20 cm), developed the chromatogram in the chamber with the specified solvent. Removed the plate, allowed it to dry in air and observed the produced spots in daylight,

under short-wave and long-wave ultraviolet light, sprayed the spots with the specified reagent.

RESULTS AND DISCUSSION:

Vetiveria zizanioides is a densely tufted perennial grass, with rootstock branching and spongy aromatic roots⁶⁾. Figure 1 showed *V. zizanioides* whole plant with panicle inflorescence. Figure 2 showed the dried roots as crude drug of *V. zizanioides*. The anatomical and histological investigation of the dried roots revealed the isolated and aggregated starch grains and raphide crystals, which varied in size scattered in parenchyma cells. The single hair cells (Trichomes), sclerenchyma fiber cells, sclereid cells and many vessels were also present (Figure 3 and 4).

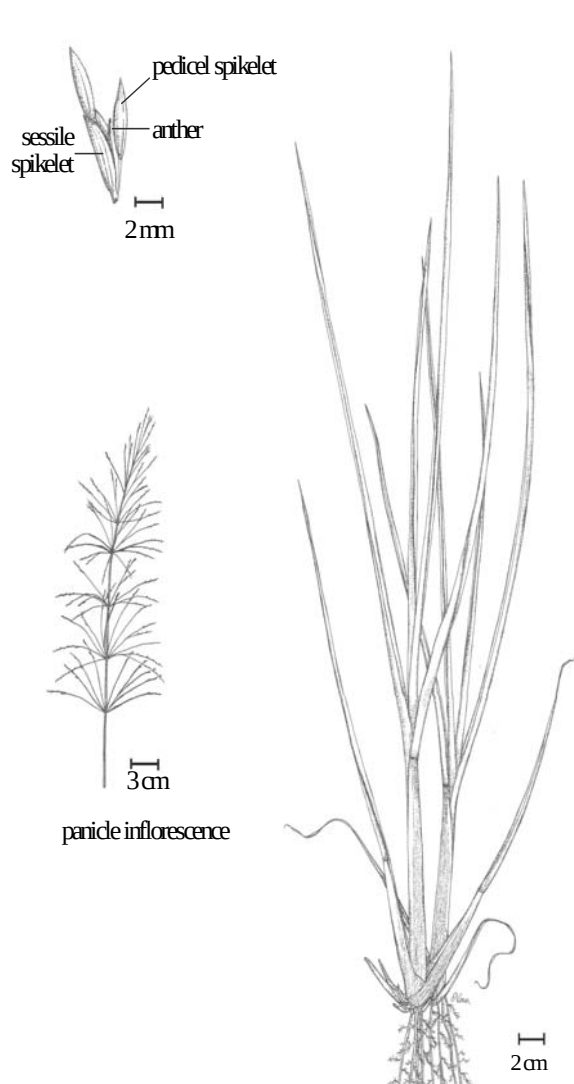
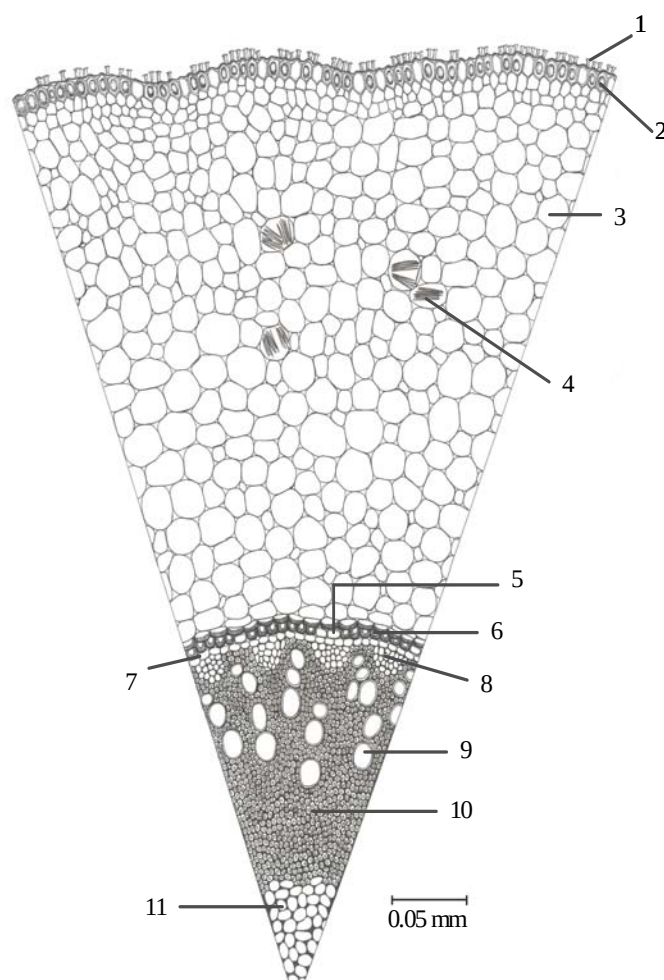
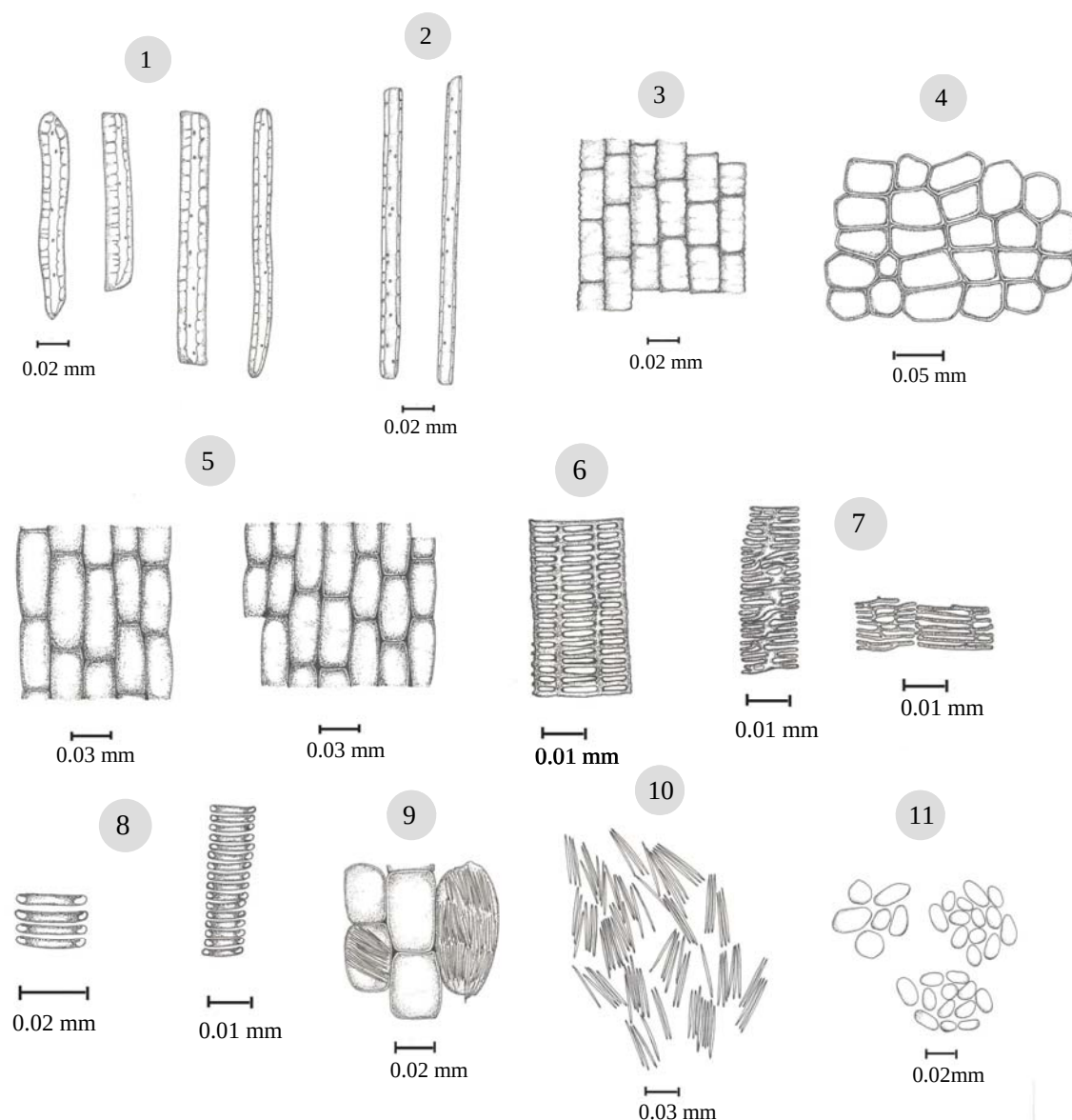


Figure 1 The whole plant of *Vetiveria zizanioides* Nash



1. trichome, 2. starch grain, 3. parenchyma of cortex, 4. crystal in reserved parenchyma, 5. passage cell, 6. endodermis, 7. pericycle, 8. phloem, 9. xylem vessel, 10. sclerenchyma fiber, 11. pith

Figure 3 Transverse section of *V. zizanioides* Nash dried roots



1.sclerenchyma sclereid, 2. sclerenchyma fiber, 3. epidermal cell (longitudinal view), 4.parenchyma (transverse view), 5.parenchyma (longitudinal view), 6.scalariform vessel, 7.reticulate vessel, 8.annular vessel, 9.parenchyma containing raphide crystals, 10.raphide crystals in acicular bundle, 11.starch grain

Figure 4 Histological character (powdered) of *V. zizanioides* Nash dried roots

Table 1 showed the pharmacognostic properties of *V. zizanioides* roots. The contents of foreign matter, acid-soluble ash, total ash, moisture and volatile oil content were found to be 0.30, 9.10, 10.63, 8.48 and 0.23% of dry weight respectively. Whereas the determination of ethanol - soluble extractive, water - soluble extractive and loss on drying were found to be 4.98, 48.03 and 9.34% of dry weight. TLC fingerprints of methanolic extracts and volatile oil GC/MS chromatogram and its chemical

composition were shown in Figure 5, 6 and table 2. Cameroon-7- α -ol and silphiperfol-6-ene were found as major compositions of *V. zizanioides* Nash volatile oil. These components are different from the previous report⁷. A possible explanation for this discrepancy is that samples from different sources may contain different oil components. The samples in this study were crude drugs obtained from the traditional drug stores instead of the crop samples in the previous study.

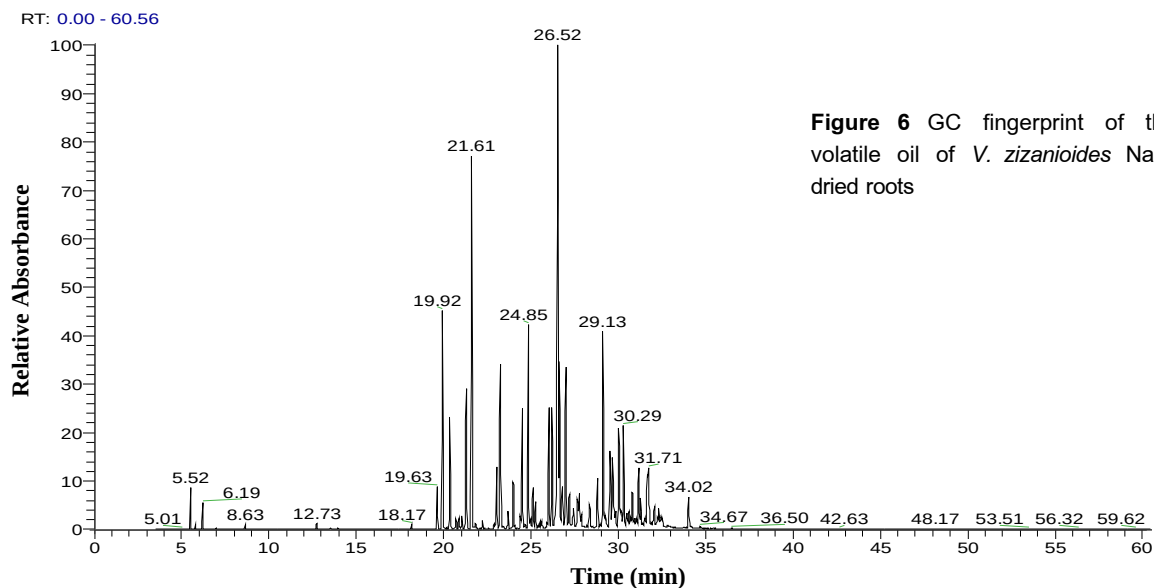


Figure 6 GC fingerprint of the volatile oil of *V. zizanioides* Nash dried roots

Table 1 The constant numbers (as % dry weight) due to the quality of *V. zizanioides* Nash dried roots

Specification	Mean $\pm$ SD	Min - Max	n
Foreign matter	0.30 $\pm$ 0.20	0.05 – 0.78	29
Acid-insoluble ash	9.10 $\pm$ 3.41	1.99 – 17.85	28
Total ash	10.63 $\pm$ 3.68	3.25 – 19.68	28
Ethanol-soluble extractive	4.98 $\pm$ 3.15	0.50 – 14.30	45
Water-soluble extractive	48.03 $\pm$ 13.29	4.70 – 62.90	45
Loss on drying	9.34 $\pm$ 1.69	4.20 – 11.73	45
Volatile oil content	0.23 $\pm$ 0.28	0.00 – 0.95	30
Water content	8.48 $\pm$ 1.17	5.40 – 10.04	30

ACKNOWLEDGEMENT: Thanks are due to the Department for Thai Development of Traditional and Alternative Medicine, Ministry of Public Health for partial financial support and College of Public Health Sciences, Chulalongkorn University for providing the facilities.

REFERENCES:

1. STID.1993. Vetiver grass – a thin green line against erosion, National Research Council, National Academy press. Washington, DC.
2. Chomchalow N. 2000. Manual of the International Training Course on the Vetiver System, ORAPB, Bangkok, Thailand.
3. Joy PP, Thomas J, Mathew S and Skaria BP. 2001. Medical Plants. Tropical Horticulture Vol.2, Naya Prokash, Calcutta. pp. 449-632.

Table 2 Chemical constituents of the volatile oil of *V. zizanioides* Nash dried roots

RT	Chemical Composition	Kovat's Index ⁸⁾	Area%
5.52	Tricyclene	926	0.66
6.19	Camphene	953	0.35
12.73	Camphor	1143	0.11
18.17	Bornyl acetate	1285	0.11
19.63	Silphiperfol-5-ene	1328	0.85
19.94	Presilphiperfol-7-ene	1336	5.00
20.37	Silphiperfol-5-ene<7-epi>	1348	2.76
20.88	Silphiperfol-4,7(14)-diene	1360	0.15
21.61	Silphiperfol-6-ene	1379	8.39
22.22	Cadina-1(10),6,8-triene	-	0.17
23.04	Cedrene<beta>	1420	1.37
23.24	Caryophyllene(E-)	1417	4.65
23.69	Copaene<beta>	1432	0.40
23.99	9-Methyl-S-octahydroanthracene	-	1.14
24.50	Humulene<alpha>	1454	3.22
24.85	Cadina-1(6),4-diene<cis>	1463	4.76
25.26	Neocallitropsene<alpha>	1476	0.63
25.52	Cuparene	1504	0.39
26.03	3-Methyl-2-pent-2-enyl-cyclopent-2-enone	-	3.15
26.52	Cameroonan-7-alpha-ol	1511	13.57
26.99	Humulane-1,6-dien-3-ol	-	4.22
27.76	Silphiperfolan-6-beta-ol	-	1.24
28.35	Guaia-3,9-diene	-	0.81
28.80	Prenopsan-8-ol	1577	1.24
29.13	Caryophyllene oxide	1583	5.17
29.68	Alloaromadendrene oxide-(2)	-	2.67
30.04	Humulene epoxide II	1608	3.07
30.29	Humulene<beta>	-	3.03
30.49	Silphiperfol-6-en-5-one	1625	0.65
30.80	Acorenol<alpha>	1633	1.47
31.69	Vatirenene<beta>	-	3.31
32.06	Bisabolol<epi-beta>	1671	0.71
32.30	Himachal-4-en-1-beta-ol<11-alphaH>	1699	1.26
34.04	1,4-Methanoazulen-7(1H)-one, octahydro-4,8,8,9-tetramethyl-, (+)-	-	0.82

4. Putiyanan S, Nantachit K, Bunchoo M, Khantava B and Khamwan C. 2005. Pharmacognostic identification and antimicrobial activity of *Vetiver zizanioides* (L.) Nash root. Chiang Mai Med Bull 44(3): 85-90.
5. World Health Organization. 1998. Quality control methods for medicinal plant materials, ISBN 92 415 45100 (NLM Classification QV 766).
6. Kirtikar KR, Basu BD and An ICS. 1981. Indian Medicinal Plants Vol.4, Second Reprint, Taj Offset Press, Delhi, India. pp. 2671-3.
7. Adams RP, Nguyen S, Johnston DA, Park S, Provin TL and Habte M. 2007. Comparison of vetiver root essential oils from cleansed (bacteria-and fungus - free) vs. non-cleansed (normal) vetiver plants. Biochem Syst Ecol 36 (3): 177-182.
8. Adams RP. 1995. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publishing Corporation, Illinois, USA.

ข้อกำหนดคุณสมบัติทางเภสัชเวชของรากแฝกหอมในประเทศไทย

สมชาย อิศระวานิชย์¹ ชนิตา พลาหเวช¹ ธนสร ตันตยงฆาร¹ อนุสรณ์ รังสิโยธิน¹ นรินทร์ วิพันธุ์เงิน¹

อัญชลี จูทะพุทธิ² นิจศิริ เรืองรังษี^{1,3,*}

¹วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย ²กรมพัฒนาการแพทย์แผนไทยและแพทย์ทางเลือก กระทรวงสาธารณสุข
นนทบุรี 11000 ³ภาควิชาเภสัชเวช คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพมหานคร 10330

บทคัดย่อ: สุ่มตัวอย่างรากแฝกหอม (*Vetiveria zizanioides* Nash) จากร้านยา 15 แห่ง ใน 13 จังหวัด ทั้ง 4 ภาคของประเทศไทย มาวิเคราะห์มาตรฐานเครื่องยาตามวิธีควบคุมมาตรฐานขององค์การอนามัยโลก และ ศึกษาภาคตัดขวางของราก และผงของรากแฝกหอมด้วยตาเปล่า และกล้องจุลทรรศน์ ผลการศึกษาพบว่าค่าเฉลี่ย (คิดเป็นร้อยละของน้ำหนักตัวอย่างแห้ง) ขององค์ประกอบทางเภสัชเวชของรากแฝกหอมมีดังนี้ คือ สารปนเปื้อนร้อยละ 0.30 เก้าที่ไม่ละลายในกรดร้อยละ 9.10 แก้วรวมร้อยละ 10.63 ปริมาณสารสกัดด้วยเอทานอลร้อยละ 4.98 ปริมาณสารสกัดด้วยน้ำร้อยละ 48.03 น้ำหนักที่หายไปเมื่อทำให้แห้งร้อยละ 9.34 น้ำมันหอมระเหยร้อยละ 0.23 และความชื้นร้อยละ 8.48 จากการศึกษาภาคตัดขวางของรากแฝกหอมด้วยกล้องจุลทรรศน์ พบว่ามีเม็ดแป้งขนาดต่าง ๆ กันกระจายสะสมในชั้นพาเรนไคมา และพบการเกาะกลุ่มเป็นทรงกลมของเส้นใยพาเรนไคมา การศึกษาทางโครมาโตกราฟีแสดง ที่แอลซี และจีซี ฟิงเกอร์ปรีนซ์ของน้ำมันระเหยของรากแฝกหอม

คำสำคัญ: รากแฝกหอม คุณสมบัติทางเภสัชเวช

ติดต่อได้ที่ njsiri.r@chula.ac.th โทรศัพท์ 0 2218 8201 โทรสาร 0 2255 2177

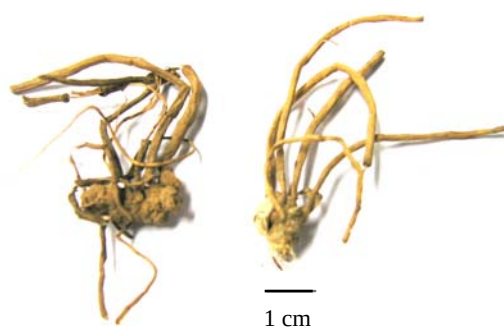
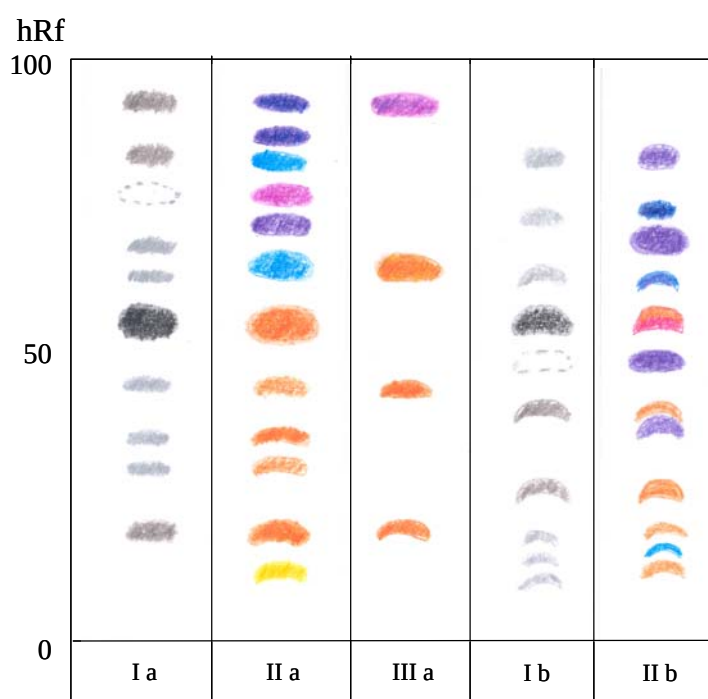


Figure 2 The dried roots of *Vetiveria zizanioides* Nash



Solvent system

a = Hexane : Ethyl acetate 70 : 30

b = Chloroform

I = detection under UV light (254 nm); dot line (366 nm)

II = detection with vanillin-sulfuric acid

III = detection with Ehrlich's reagent

Figure 6 TLC fingerprint of methanolic extract of *V. zizanioides* Nash dried roots