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Original Article

The Identification of Aucklandiae Radix, Inulae Radix, Vladimiriae Radix and Aristolochiae Radix, Using Macroscopic, Microscopic and Physicochemical Methods

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Objectives: This study aimed for establishing the discriminative criteria for Aucklandiae Radix, Inulae Radix, Vladimiriae Radix and Aristolochiae Radix, which can be confused as 'Mokhyang', because of similar appearances and synonyms.
Methods: Morphological characteristics of dried herbal medicines were compared by macroscopic observation. To examine microscopic features of 'Mokhyang', paraffin embedding and the staining by using Ju's method were conducted. Physicochemical experiments were performed using HPLC analysis and antioxidant assay.
Results: The types of stem, phyllotaxy and leaf shape were chosen as macroscopic criteria and the size, oil spots, color and root top as well as phloem and central cylinder were compared using microscopic features. The HPLC results showed different amounts of costunolide and dehydrocostuslactone among Aucklandiae Radix, Inulae Radix and Vladimiriae Radix. However, Aristolochiae Radix only contained aristolochic acid. The antioxidant assays also showed that Vladimiriae Radix exhibited strongest antioxidant activity followed by Aucklandiae Radix, Inulae Radix and Aristolochiae Radix.
Conclusions: These results demonstrate that macroscopic, microscopic and physicochemical methods would be useful tools for the discrimination of 4 kinds of 'Mokhyang'.

Key Words : Aucklandiae Radix, Inulae Radix, Vladimiriae Radix, Aristolochiae Radix, Morphology, HPLC, Antioxidant activity

Introduction

'Mokhyang (MH)' is a traditional medicine which is effective for the treatment of hypochondria, accumulation-aggregation, diarrhea, intestinal convulsion and dysentery[1,2]. In The Korean Herbal Pharmacopoeia[3], the dried root of *Aucklandia lappa* Decne (Aucklandiae Radix) and *Inula helenium* L. (Inulae Radix) are listed as genuine of MH. In Chinese Pharmacopoeia[4], besides above two kinds, the dried root of *Vladimiria souliei* (Franch.) Ling (Vladimiriae Radix) is stated as genuine while that of *Aristolochia contorta* Bge. (Aristolochiae Radix) is listed as adulterant of MH[5].

Aucklandiae Radix, known as Unmokhyang (Un-MH) in Korean, is called in other names such as 'Milhyang', 'Chungmokhyang (Chung-MH)' and

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'Ohyang'. Inulae Radix, named Tomokhyang (To-MH) in Korean, is also called various names such as 'Chung-MH' and 'Dokhaengkeun'. Aristolochiae Radix, known as Chung-MH in Korean, has various alternate names such as Maryeongkeun, 'To-MH' and 'Dokhaengkeun'[6]. Because of this similar herbal names and morphological features, the dried roots of 4 kinds have been confused in use and distribution in market.

Furthermore, Aristolochiae Radix is one of Aristolochia herbs containing aristolochic acid (AA) which causes nephropathy. AA-containing herbs have been a global concern after that the first side effects of slimming regimen derived from Aristolochia kinds was reported in early 1990s[7]. Subsequently, the Korean Food and Drug Administration (KFDA) has prohibited the medicinal use of Aristolochiae Radix because it shares the same common name, 'Mokhyang', with other three kinds[5].

Various efforts using macroscopic, microscopic identification[8] and chemical analysis[9] have been performed for the discrimination of Aucklandiae Radix and Inulae Radix. However, the previous studies analyzing three genuine kinds showed only macroscopic morphological differentiation[10] and pattern-recognition analysis by HPLC[11]. Moreover, identification of 4 kinds of MH was rarely published in using macroscopic identification[12] and quantitative determination of costunolide by HPLC[13].

The objective of this study is to provide identification-key of 4 kinds of MH using macroscopic and microscopic methods, and the discrimination of those herbal medicines was also performed by evaluating the amounts of chemical compounds using HPLC analysis and investigating antioxidative activities. To our knowledge, general examination on 4 kinds of MH was firstly carried.

Materials and methods

1. Herbal materials

The roots of *Aucklandia lappa* Decne, *Inula helenium* L., *Vladimiria souliei* (Franch.) Ling and *Aristolochia contorta* Bge. were gathered from various areas such as local market and habitats (Table 1), and ascertained by Prof. Young-Sung Ju, department of herbology, Woosuk University. 14 samples have been deposited in the department of herbology of Woosuk University.

2. Reagents

Costunolide and dehydrocostuslactone[9,14,22] were purchased from ChromaDex (USA), Wako Pure Chemical Co. (Japan). HPLC-grade methanol, water and acetonitrile were purchased from Fisher (USA).

2,2-Diphenyl-1-picrylhydradzyl and 2,4,6-tris (2-pyridyl)-s-triazine were purchased from Sigma Chemical Co. (USA). 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt, potassium peroxodisulfate, and $FeCl_3 \cdot 6H_2O$ were purchased from Kanto Chemical Co. (Japan). Acetic acid was purchased from Junsei Chemical Co. (Japan).

3. Macroscopic and microscopic morphology

Stereoscope (Carl zeiss STEMI2000, Germany) and microscope (Nikon ECLIPSE 80i, Japan) were used to observe the external and internal morphology. Samples of 4 kinds went through fixation, rapid dehydration procedures, paraffin waxing, cutting and modified Ju's triple stain method process in orderly and then examined microscopic features by optical microscope.

4. HPLC analysis

The dried roots of 14 MH samples (coded as AL1-5, IH1-3, VS1-3, and AC1-3) were pulverized

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and extracted with methanol (1 g/10 mL) by ultra-sonication for 2 hours at 30°C. The supernatants were concentrated *in vacuo* to dryness and dissolved with methanol at the concentration of 100 mg/mL. The extracts was diluted to make a final concentration of 1 mg/mL and then passed through a 0.20 μm membrane filter prior to injection.

Each of reference standards were accurately dissolved in HPLC grade methanol to yield a concentration of 100 μg/mL. Working calibration solutions were prepared by serial dilution of the stock solutions with HPLC grade methanol to yield concentration of 1-50 μg/mL for costunolide and 0.5-25 μg/mL for dehydrocostuslactone.

Samples were analyzed by Agilent 1200 series with multiwavelength detector (Agilent, USA). Acquired data was processed using Agilent Chemstation (Agilent 1200 series). The separation was conducted on an Phenomenex Gemini NX C18 (3.0 × 150 mm, 5 μm) on the conditions of flow rate at 0.8 mL/min, column temperature at 35°C and UV wavelength at 225 nm[15]. The mobile phase consisted of deionized water (A) and acetonitrile (B) as a gradient elution: 0min, 60 % B; 29min, 80% B; 30min, 60 % B.

5. Antioxidant activity assays

UV/Vis Spectrophotometer and DU 730 (Beckman Coulter, USA) were used for antioxidant activity tests. The DPPH assay was performed according to the method of Blois[16]. The ABTS assay was modified from the method of Thaipong et al[17]. The $ABTS^+$ solution was diluted with methanol to obtain 0.99 ± 0.05 units at 734 nm. The scavenging activity of DPPH and $ABTS^+$ were calculated as follows: scavenging activity % = $[(A_0 - A_c) / A_0] \times 100$, where A_0 is absorbance of the control and A_c is absorbance of the sample. For Frap assay, the procedure followed the method of Thaipong et al[17] using a trolox (0.05-0.4mg/mL) standard curve. Results were expressed in mM Fe^2/μg free mass.

Results

1. Macroscopic morphology of 4 kinds of 'Mokhyang'

Table 1. The List of Herbal Materials

Code	Herb	Kinds	Collection	
			Place	Date
AL1	Unmokhyang	*Aucklandia lappa Decne*	Kwangmyoungdang, China	2012. 07
AL2	Unmokhyang	*A. lappa Decne*	Omniherb, Yunnan, China	2012. 11
AL3	Unmokhyang	*A. lappa Decne*	Kwangmyoungdang, China	2012. 01
AL4	Unmokhyang	*A. lappa Decne*	Local market, China	2012. 09
AL5	Unmokhyang	*A. lappa Decne*	Yunnan, China	2011. 03
IH1	Tomokhyang	*Inula helenium L.*	Kwangmyoungdang, Korea	2012. 05
IH2	Tomokhyang	*I. helenium L.*	Local market, China	2012. 09
IH3	Tomokhyang	*I. helenium L.*	Korea	-
VS1	Chuanmokhyang	*Vladimiria souliei (Franch.) Ling*	Sichuan, China	2012.07
VS2	Chuanmokhyang	*V. souliei (Franch.) Ling*	Local market, China	2012. 09
VS3	Chuanmokhyang	*V. souliei (Franch.) Ling*	Kwangmyoungdang, China	2012. 07
AC1	Chungmokhyang	*Aristolochia contorta Bge.*	Sichuan, China	2012.07
AC2	Chungmokhyang	*A. contorta Bge.*	Local market, China	2012. 09
AC3	Chungmokhyang	*A. contorta Bge.*	China	-

In original plants, the leaf arrangement of *Aucklandia lappa, Inula helenium* and *Aristolochia contorta* is alternate although it of *Vladimiria souliei* is rosulate. Leaves of 4 kinds were various in the shape. While *Aristolochia contorta* showed climbing stem, racem inflorescence and capsule fruit, other kinds showed straight stem, capitulum inflorescence and achene fruit (Fig.1 and Table 2). On this, macroscopic identification-key of 4 kinds 'Mokhyang' is as follow (Table 3).

In herbal medicine, the roots of 4 kinds were cylindrical shape, however, those were divided in two categories by the thickness and branch of root. The top of Aucklandiae Radix and Inulae Radix were dented, whereas that of Vladimiriae Radix contained black and sticky glue-like substance.

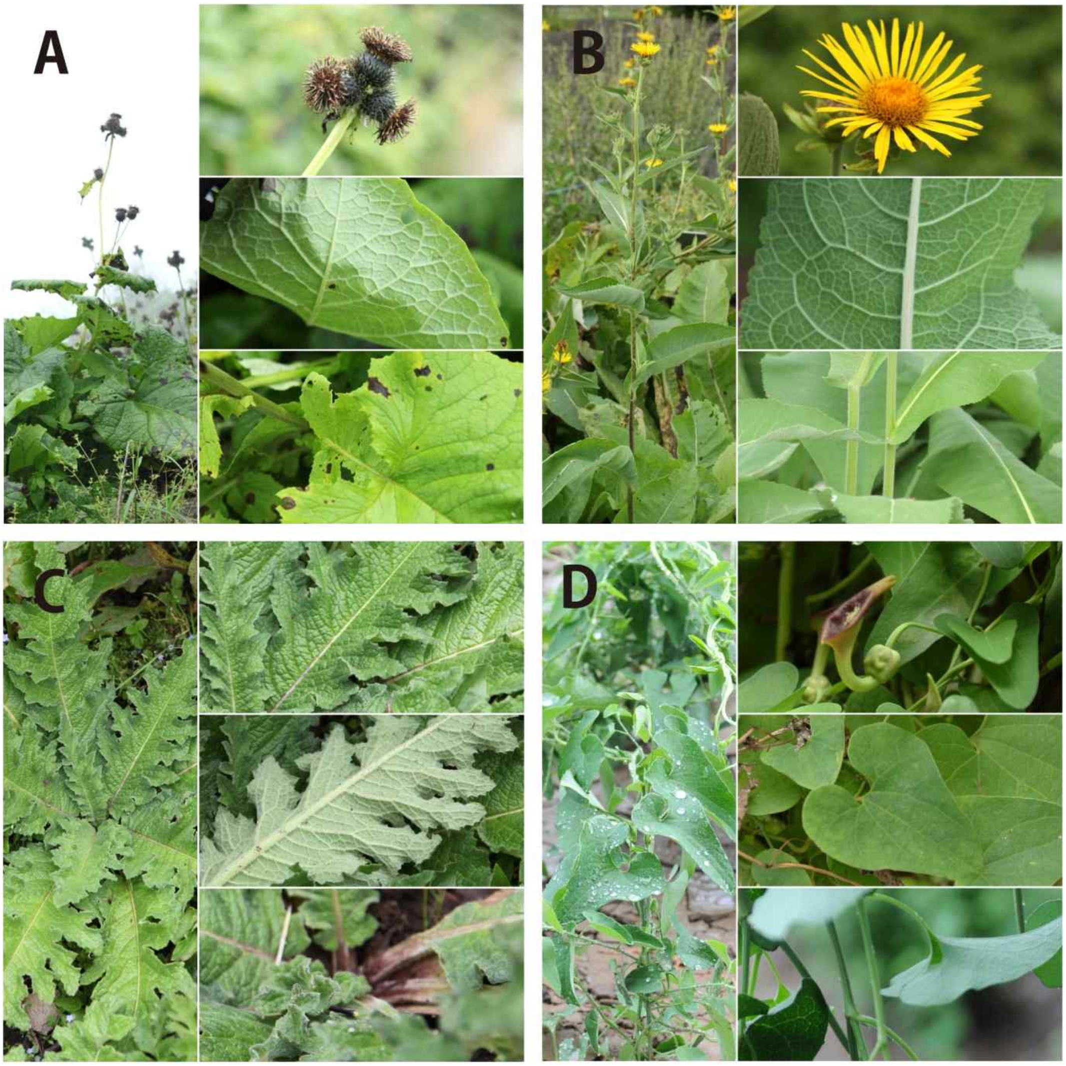

Fig. 1. Macroscopic morphology of original plant of 4 kinds 'Mokhyang'. (A) *Aucklandia lappa*, (B) *Inula helenium*, (C) *Vladimiria souliei*, (D) *Aristolochia contort*a.

Beside Aucklandiae Radix, Inulae Radix and Vladimiriae Radix had dense longitudinal wrinkles and distinct features on outer surface besides, Aristolochiae Radix had fine longitudinal wrinkles and other outer feature was indistinct. On the cross section, 4 kinds were classified through two structures; oil spot and pith (Fig.2, Table 4). On this, macroscopic identification-key of herbal medicine of 4 kinds 'Mokhyang' is as follow (Table 5).

2. Microscopic morphology of 4 kinds of 'Mokhyang'

In common, cork is made up with several rows of cells. In phloem, Aucklandiae Radix and Vladimiriae Radix had denser ray cells than others and both had alternate arrangement of fibre bundles and sieve tube group but differed in the number of arrangement. Ring-shaped cambium was distinct in all 4 kinds. In xylem, depending on kinds, singly scattered or grouped vessels were arranged radially. Aucklandiae

Table 2. Macroscopic Morphology of Original Plants of 4 kinds 'Mokhyang'

Macroscopic morphology		*Aucklandia lappa*	*Inula helenium*	*Vladimiria souliei*	*Aristolochia contorta*
Life cycle		Perennial herb	Perennial herb	Perennial herb	Perennial herb
Root		Cylindrical shape Thick primary root branched slightly	Cylindrical or long cone shape Thick primary root branched slightly	Cylindrical shape Thick primary root No branched	Cylindrical shape Thin primary root No branched
Stem		Straight upright Height 1-2m Sparse short pubescence	Straight upright Height 1-2m Dense short pubescence	Straight upright Extremely short	Climbing Length 4-5m Glabrous
Leaf	Phyllotaxy	Alternate	Alternate	Rosulate	Alternate
	Leaf blade	Triangular-ovate Length 30-100cm Width 15-30cm Thorn at the end of serrate in stem leaf	Wide-elliptical Length 25-50cm Width 10-25cm Amplexicaular in stem leaf	Oblong-lanceolate Length 12-30cm Width 8-20cm	Ovate- cordate Length 4-10cm Width 3.5-8cm 5-7 Leaf venations
	Trichome	Both sides pubescent	Both sides pubescent	Both sides pubescent	Both sides glabrous
	Base	Pinnate	Cordate	Cuneate	Cordate
	Margin	Undulate or irregularly lobed	Irregularly serrate	Serrulate 5-7 paired pinnate clefts	Entire
Flower	Inflorescence	Capitulum 1-3 at terminal or 1 at axillary	Capitulum arranged in corymb At terminal or axillary	Capitulum 2-3 at terminal	Racem 2-8 at axillary
	Petal	Dark purple	Yellow	Purple	Greenish purple
	Corolla	Tubular	Tubular at center Ligulate at border	Campanulate	Trumpet-shaped
	Calyx	Lanceolate About 10 layers	Hemisphere shape 5-10 layers	Bell shaped 4 layers	base bulged middle contractive, top expanded
Fruit		Achene With ridges Linear	Achene 4-5 ridges tetrahedral or pentahedral	Achene 3 ridges Flatly pressed	Capsule 6 ridges Widely or Elliptically ovate

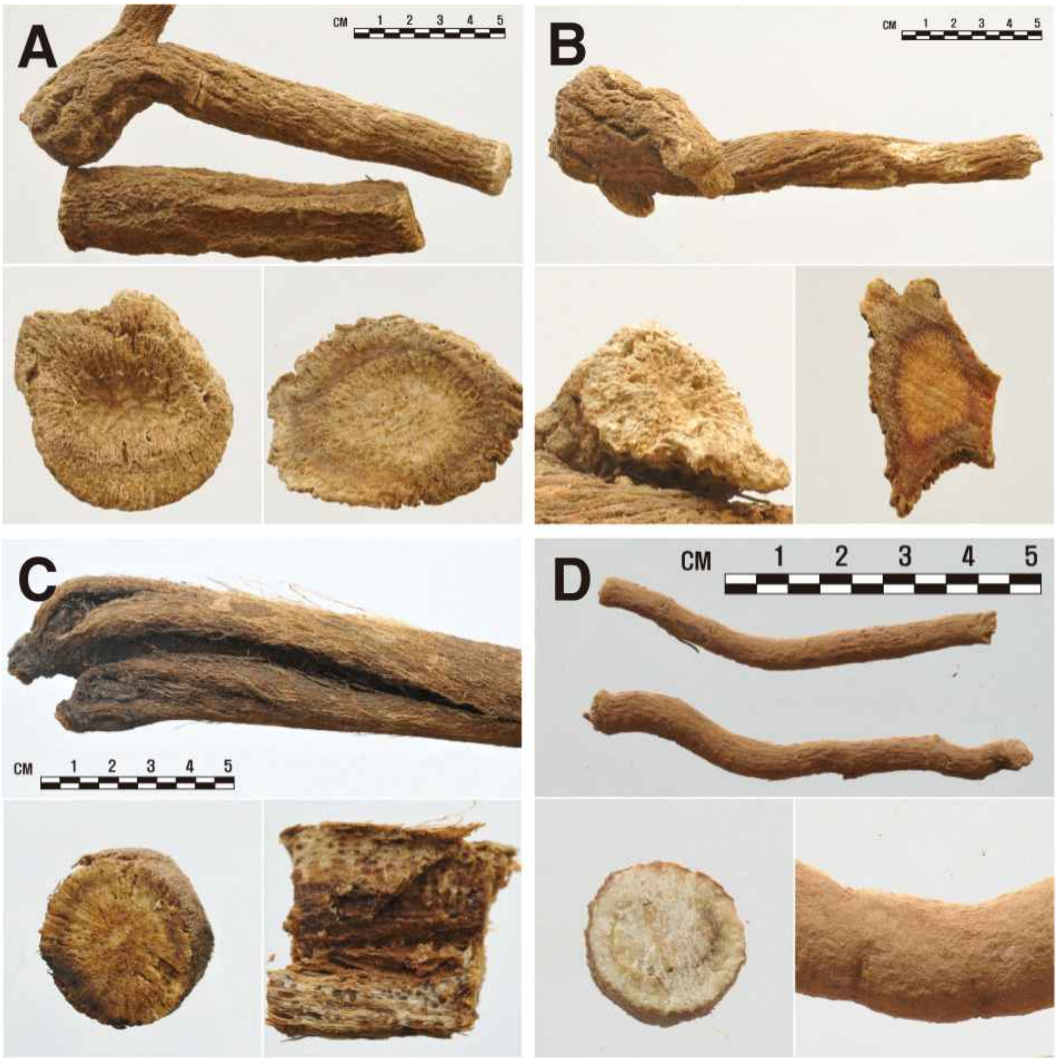

Fig. 2. Macroscopic morphology of herbal medicine of 4 kinds 'Mokhyang'. (A) Aucklandiae Radix, (B) Inulae Radix, (C) Vladimiriae Radix, (D) Aristolochiae Radix.

Table 3. Macroscopic Identification-key of Original Plant of 4 kinds 'Mokhyang'

1. The stem is straight upright or none and both sides of the leaf are pubescent.	
2. The stem is straight upright and the leaf phyllotaxy is alternate.	
3. The leaf base is pinnate and the flower is purple.---	*Aucklandia lappa*
3. The leaf base isn't pinnate and the flower is yellow.--	*Inula helenium*
2. The stem isn't existed and the leaf phyllotaxy is rosulate.--	*Vladimiria souliei*
1. The stem is climbing habit and both sides of the leaf are glabrous.--	*Aristolochia contorta*

Radix and Vladimiriae Radix were tetrarch protoxylem, while Inulae Radix and Aristolochiae Radix were polyarch protoxylem. Only in Aristolochiae Radix, numerous starch granules were presented in

Table 4. Macroscopic Morphology of Herbal Medicines of 4 kinds 'Mokhyang'

Parts		Aucklandiae Radix	Inulae Radix	Vladimiriae Radix	Aristolochiae Radix
Whole		Cylindrical or skeleton-like Bold and thick Hardly broken	Cylindrical or lengthened-conical Bold and thick Hardly broken	Cylindrical or semi-cylindrical Bold and thick Easily broken	Cylindrical or leant-cylindrical Fine and thin Easily broken
Root top		Dented like dry bone	Expanded in cone-shape	With black glue-like material	No significant features
Outer Surface		Yellowish brown, grayish brown Dense longitudinal wrinkles Distinct lateral root scar	Yellowish brown, dark brown Dense longitudinal wrinkles Distinct horizontal rind pores	Yellowish brown, dark brown Dense longitudinal wrinkles Reticulated fibers in scaled off cortex	Yellowish brown, grayish brown Fine longitudinal wrinkles Fine lateral root scar
Size	Length	5-15cm	10-20cm	10-30cm	3-15cm
	Diameter	0.5-5cm	0.5-4cm	1-4cm	0.5-1.5cm
Cross section		Yellowish white or dark brown Yellowish brown Oil spots White pith	Yellowish brown dark brown Oil spots Indistinct pith	Yellowish white or light brown Oil spots White pith	Pale yellow No oil spot Indistinct pith and Wide xylem
Odor		Strong and aromatic	Faint	Faint	Peculiar
Taste		Slight bitter	Slight bitter	Bitter	Bitter

Table 5. Macroscopic Identification-key of Herbal Medicine of 4 kinds 'Mokhyang'

1. The root top hasn't black glue-like material.
  2. Whole root is bold, thick and not easily broken.
    3. The root top is dented like dry bone and it has extinct yellowish brown oil spots on cross section.------------Aucklandiae Radix
    3. The root top is expanded in cone-shape and it sometimes has dark brown oil spots on cross section.-----------------Inulae Radix
  2. Whole root is fine, thin and easily broken and it is yellowish brown on outer surface and has wide xylem on cross section.
  --Aristolochiae Radix
1. The root top has black glue-like material and it has reticulated fibers on outer surface and it is easily broken.
  --Vladimiriae Radix

parenchymatous cells. Oil cavities were scattered in phloem and xylem of all 4 kinds and especially in Vladimiriae Radix it also existed in pith (Fig.3, Table 6). On this, microscopic identification-key of herbal medicine of 4 kinds 'Mokhyang' is as follow (Table 7).

3. HPLC analysis of 4 kinds of 'Mokhyang'

1) Linearity and sensitivity

The coefficient of correlations (r^2) of two standard compounds were > 0.999, suggesting good linear relationship between the peak area (y) and the concentration (x). The limit of quantification (LOQ) was calculated as the concentration of each analyte where the signal/noise(S/N) ratio is ≥ 10, respectively. The LODs and LOQs for each standard compounds were as follows: costunolide, 0.49 and 1.64 μg/mL; dehydrocostuslactone, 0.30 and 1.00 μg/mL, which showed a high sensitivity at the chromatographic condition (Table 8).

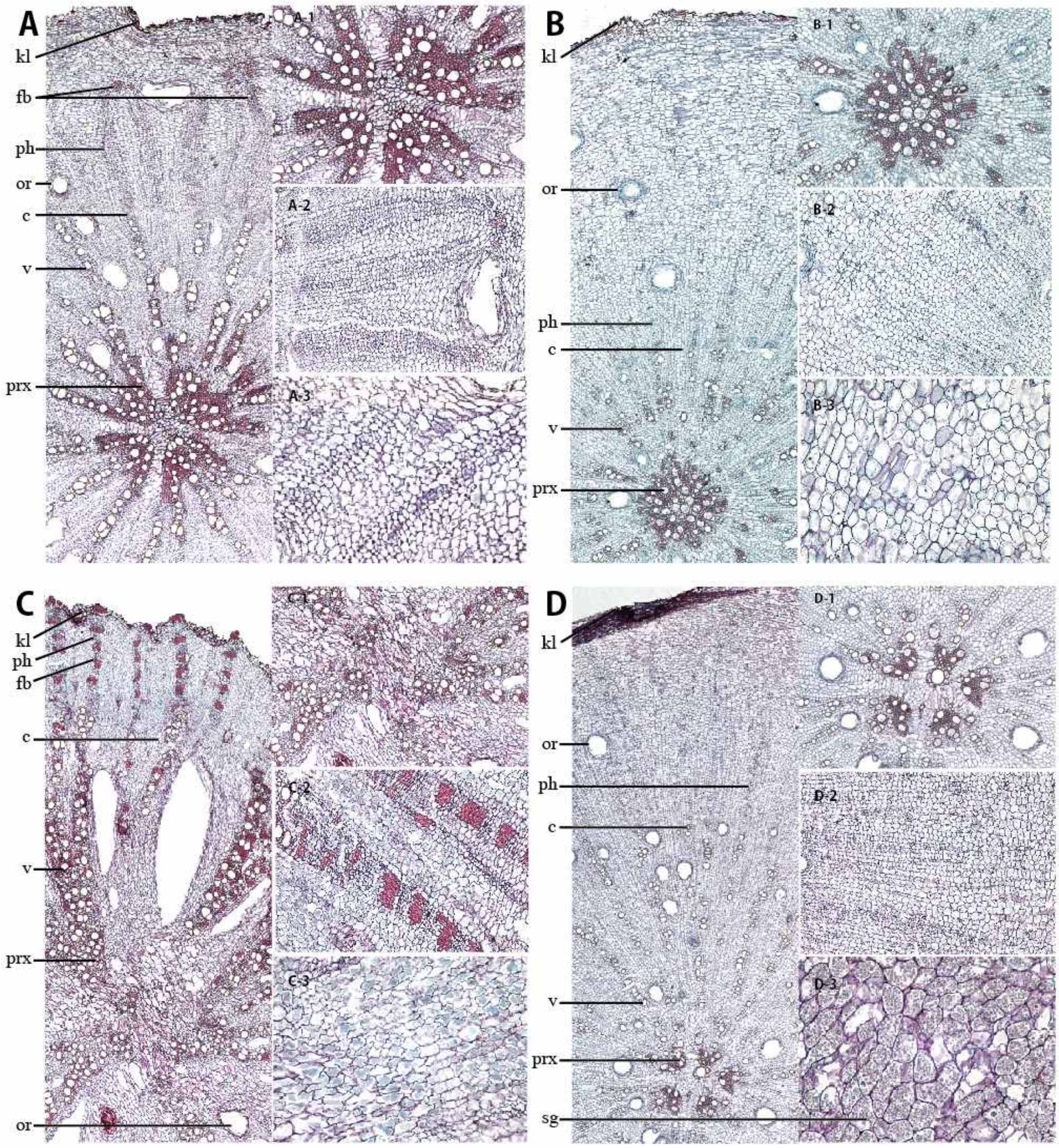

Fig. 3. Microscopic morphology of herbal medicine of 4 kinds 'Mokhyang'. (A) Aucklandiae Radix, (B) Inulae Radix, (C) Vladimiriae Radix, (D) Aristolochiae Radix, c: cambium, fb: fibre bundle, kl: cork layer, or: oil sac, ph: phloem, prx: primary xylem, v: vessel, sg: starch granule.

2) Sample analysis

The representative chromatograms of the samples and standard compound are shown in Fig. 4. Evaluating the phytochemical equivalency of MH using two STD peaks, all 4teen samples were divided into three groups. One (AL1-5, VS1-3) containing costunolide and dehydrocostuslactone both were Aucklandiae Radix and Vladimiriae Radix. The

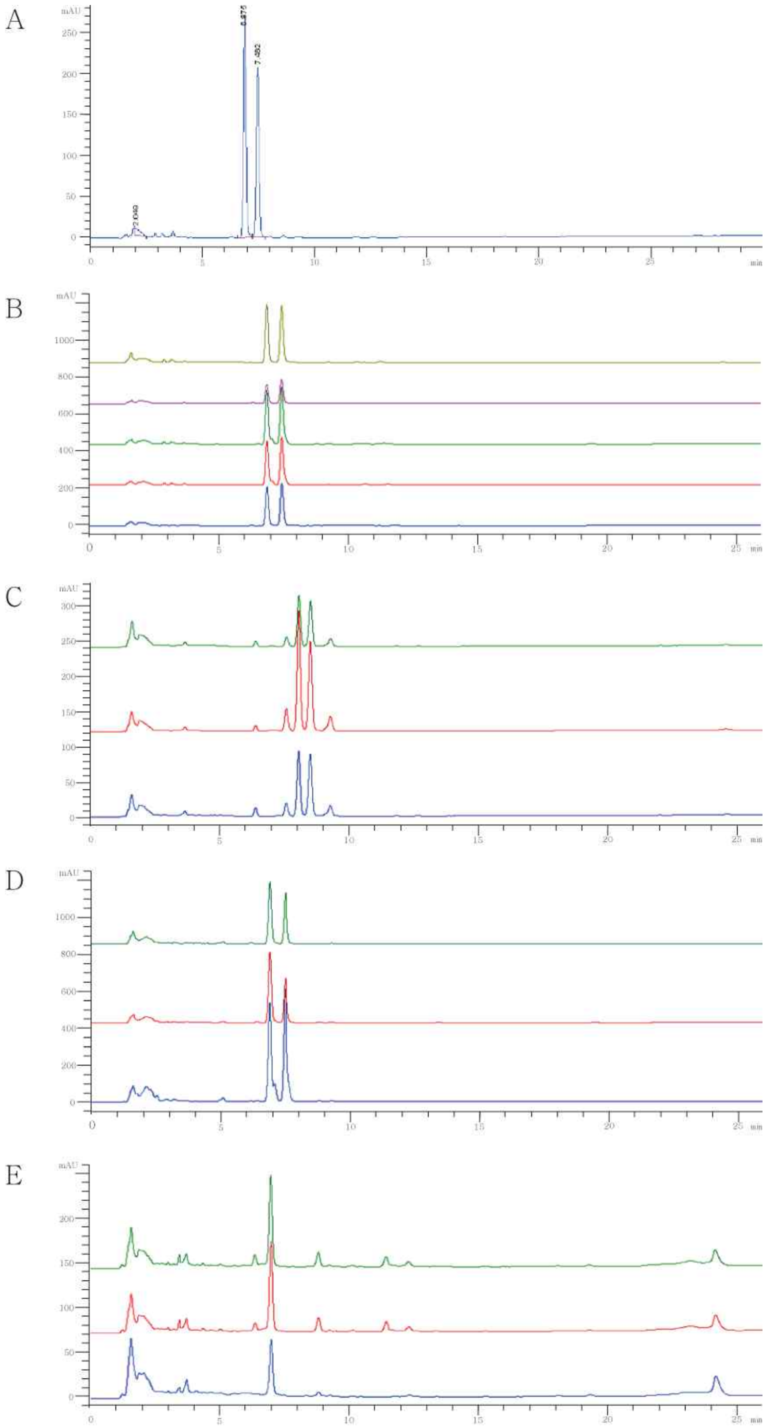

Fig. 4. HPLC Chromatograms (225nm) of (A) standard compound, (B) Aucklandiae Radix; (C) Inulae Radix; (D) Vladimiriae Radix; (E) Aristolochiae Radix; compound 1 : costunolide, compound 2 : dehydrocostuslactone.

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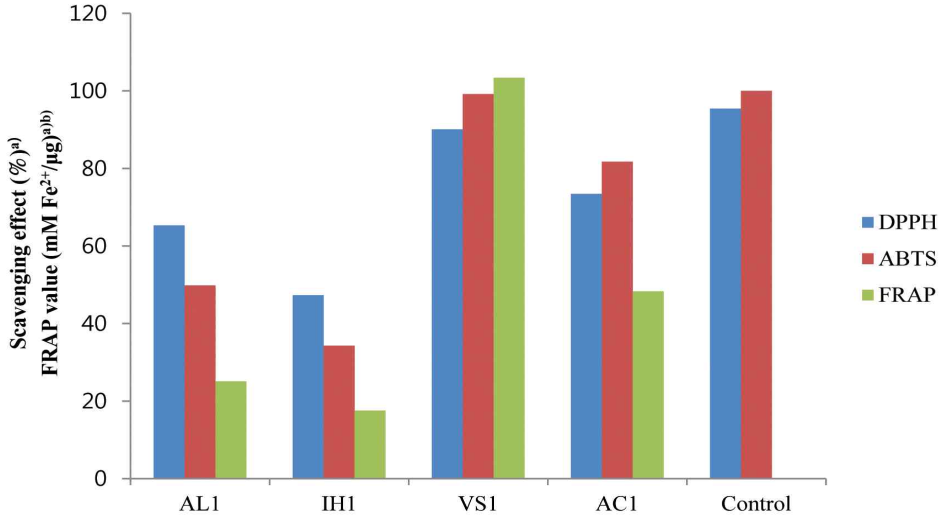

Fig. 5. Antioxidant activity of ethanol extracts of herbal medicine of 4 kinds 'Mokhyang'. a) Each value is presented as mean±SD (n≥3). b) Millimole of total Fe^{2}+/μg of plants based on trolox as standard.

other (IH1-3) containing dehydrocostuslactone only was Inulae Radix. Another (AC1-3) containing none of them was Aristolochiae Radix.

The quantity of each compound present in samples was measured and the results are summarized in Table 9. Among two standard

Table 6. Microscopic Morphology of Herbal Medicines of 4 kind 'Mokhyang'

Parts	Aucklandiae Radix	Inulae Radix	Vladimiriae Radix	Aristolochiae Radix
Cork	3-8 rows of cells	2-4 rows of cells	4-8 rows of cells Usually peeled off	4-9 rows of cells
Phloem	10-12 rows of ray cells Fibre bundles and sieve tube group alternately arranged in 1-3 times	2-3 rows of ray cells No fibre bundle	6-11 rows of ray cells Fibre bundles and sieve tube group alternately arranged in 5-6 times	2-4 rows of ray cells No fibre bundle
Cambium	Distinct ring-shape	Distinct ring-shape	Undulate curved	Distinct ring-shape
Xylem	Vessels singly scattered, arranged radially	Vessels singly scattered, arranged radially	Vessels aggregated in groups, arranged radially	Vessels singly scattered, aggregated in groups, arranged radially
Pith	4 primary pith rays Tetrarch primary xylem	No primary pith rays Polyarch primary xylem	4 primary pith rays Tetrarch primary xylem	5 primary pith rays Polyarch primary xylem
Parenchymatous cell	No starch granule	No starch granule	No starch granule	Filled with numerous starch granule
Oil cavity	Scattered in phloem and xylem Containing yellow secretions	Scattered in phloem and xylem	Scattered in phloem, xylem and pith	Scattered in phloem and xylem

Table 7. Microscopic Identification-key of Herbal Medicine of 4 kinds 'Mokhyang'

1. In phloem, fibre bundles and sieve tube group alternately arranged in less than 5times.
  2. The primary xylem is polyarch.
    3. In parenchymatous cells, there are abundant starch granules. --Aristolochiae Radix
    3. In parenchymatous cells, there is no starch granule.--Inulae Radix
  2. The primary xylem is tetrarch.---Aristolochiae Radix
1. In phloem, fibre bundles and sieve tube group alternately arranged in 5times or more.-------------------------------Vladimiriae Radix

Table 8. Linear Regression data, LOD and LOQ of The Investigated Compounds

Analyte	Regression equation	Test range (μg/mL)	r^2	LOD [a] (μg/mL)	LOQ [b] (μg/mL)
Costunolide (1)	$y= 42.978x - 1.7467$	1-50	0.99944	0.49	1.64
Dehydrocostus lactone (2)	$y= 68.853x - 1.5684$	0.5-25	0.99946	0.30	1.00

a) LOD: limit of detection b)LOQ: limits of quantitation

Table 9. Contents (mg/g) of Costunolide (1) and Dehydrocostus Lactone (2) by HPLC (n=3, mean ± SD).

Sample ID	Acquired results † (mg/g)	
	1	2
AL1	77.91 ± 0.47	56.82 ± 0.17
AL2	91.31 ± 5.35	69.02 ± 1.09
AL3	108.49 ± 0.72	81.99 ± 0.49
AL4	39.45 ± 0.02	32.83 ± 0.04
AL5	116.60 ± 0.94	77.36 ± 0.70
IH1	N.D.[a)]	5.48 ± 0.13
IH2	N.D.	8.69 ± 0.14
IH3	N.D.	3.54 ± 0.03
VS1	200.69 ± 0.57	167.47 ± 0.49
VS2	148.02 ± 3.67	61.24 ± 1.68
VS3	124.63 ± 1.34	67.91 ± 0.76
AC1	N.D.	N.D.
AC2	N.D.	N.D.
AC3	N.D.	N.D.
AL	86.75 ± 30.41	63.60 ± 19.68
IH	N.D	5.91 ± 2.60
VS	157.78 ± 38.96	98 ± 59.50
AC	N.D	N.D

a) Not detected.
AL, *Aucklandia lappa* Decne; IH, *Inula helenium* L.; VS, *Vladimiria souliei* (Franch.) Ling; AC, *Aristolochia contorta* Bge.

compounds, costunolide was the most abundant compound. The content of costunolide in Vladimiriae Radix (157.78 ± 38.96 μg/mL; average value of VS1-3) was higher than that in Aucklandiae Radix (

86.75 ± 30.41 μg/mL; average value of AL1-5),

The content of dehydrocostuslactone of Aucklandiae Radix and Vladimiriae Radix were 63.60 ± 19.68 μg/mL and 98.87 ± 59.50 μg/mL in average, lower than that of costunolide. In Inulae Radix, dehydrocostuslactone (5.91 ± 2.60 μg/mL; average value of IH1-3) showed lowest content among three and costunolide was not detected. Dehydrocostuslactone was detected in all three genuine herbs while costunolide was detected only in Aucklandiae Radix and Vladimiriae Radix. The order of the contents of two compounds was Inulae Radix < Aucklandiae Radix < Vladimiriae Radix. On the other hand, costunolide and dehydrocostuslactone were not detected in Aristolochiae Radix.

4. Antioxidant activity of 4 kinds of 'Mokhyang'

The absorbance profiles of DPPH, ABTS and FRAP assay are shown in Fig.5. In DPPH assay, the scavenging effects was highest in Vladimiriae Radix (VS1, 90.09 ± 0.19%), followed by Aristolochiae Radix (AC1), Aucklandiae Radix (AL1) and Inulae Radix (IH1). In ABTS assay, the scavenging activity of Vladimiriae Radix (99.20 ± 0.22%) was as effective as ascorbic acid (100 ± 0.11%), which was followed by Aristolochiae Radix. While Aucklandiae Radix and Inulae Radix showed low scavenging activity less than 50%. In FRAP, Vladimiriae Radix displayed FRAP value (103.40 ± 2.02 mM Fe^2/μg) 2-fold higher than others and followed by Aristolochiae Radix, Aucklandiae Radix and Inulae Radix.

Discussion

The dried root of 4 species of herbal medicines, *Aucklandia lappa, Inula helenium, Vladimiria souliei* and *Aristolochia contorta*, were called 'Mokhyang (MH)' in the markets, which causes much confusion in using MH. It is because that they share some similar morphological characteristics and have the synonyms of herbal names in various ancient literatures.

Since its first record in 'Shennong's Classic of Materia Medica' at Han dynasty, MH was recorded in various names as 'Milhyang' in 'Myungyuibyulrok' and 'Ohyang' in 'Rakbusizib'. Another alternate name of MH, Chung-MH was first used in 'Bonchogyeong-jipju' saying MH with blue root top was wonderfully efficacious. In Tang dynasty, because of shortage of genuine MH, Aristolochiae Radix known as 'Maduryungkeun' were substituted and called as 'Tochung-MH' which is lately called as Chung-MH. In the result, Chung-MH which was only another name of MH before Myung dynasty became a common herbal name of Aristolochiae Radix after Myung dynasty and used as a substitute of MH[18].

Aristolochic acid (AA)-induced nephropathy was firstly reported in 1991, that is, anemia and kidney disorder caused by AA-containing botanical products[7]. A previous founding on side effects of Aristolochiae Radix reported contained AAs were carcinogenic agents of upper urinary tract[19]. The cytotoxic effects of other components; aristololactam IVa and 7-methoxy-aristololactam IV, in Aristolochiae Radix were demonstrated in human proximal tubular epithelial cell line NK-2 *in vitro*. However, their cytotoxicity was similar or even more toxic than AA[20]. Therefore, KFDA prohibited distribution of Aristolochiae Radix in local market from June first, 2005[5]. UK, Belgium and Taiwan also prohibited *Aristolochia contorta*, and US Food and Drug Administration (FDA) prohibited using all AA-containing botanical medicines[21].

Even though MH is commonly used for treating various problems in digestive system 4 kinds of MH have been used for slightly different medicinal purposes. Aucklandiae Radix primarily helps chest pain. Inulae Radix and Vladimiriae Radix have been used in treatment of dyspepsia, stomach pain, vomiting and diarrhea. Aristolochiae Radix, which is now substituted with Inulae Radix, has been used mainly in hypochondria, dysentery and tenesmus[6]. Because of their differential medicinal efficacy, it is necessary to identify genuine herbs for better beneficial treatment.

Macroscopic identification of original plants was introduced because growers of traditional medicines usually classify kinds based on features of original plants and it is also helpful to consumers when identifying unprocessed herbal medicine. In macroscopic discrimination, 4 kinds of original plants were differentiated by the types of stem, phyllotaxy and leaf shape and 4 kinds of herbal medicines were classified by the size, oil spots, color and root top. To be precise, in microscopic discrimination phloem and central cylinder were used as major features.

In previous studies, chemical analysis of Aucklandiae Radix and Inulae Radix by high performance liquid chromatography (HPLC)[9] have been published. Analyzing three genuine kinds showed pattern-recognition analysis by HPLC[11]. Also, evaluating 4 kinds of MH by HPLC was reported using quantitative contents of costunolide only[13].

A HPLC method was developed and applied to analyze two different standard compounds in 4 kinds of MH simultaneously. The samples from the same kinds showed similar chemical constituents and relative proportions of each compound but discrepancies in contents of each compound were observed. It is presumably due to the difference of agricultural environments. Aristolochiae Radix could be easily differentiated from the genuine kinds of MH by the facts neither of standard compounds were detected and it had low similarity in fingerprint. Auckladiae Radix and Vladimiriae Radix showed high standard compound contents and similar fingerprint and the content of costunolide in Vladimiriae Radix was higher than that in Aucklandiae Radix. Besides Inulae Radix showed low quantities of dehydrocostuslactone and low fingerprint similarity among those three herbs.

In contrast, previous study showed that the content of costunolide in Aucklandiae Radix was higher than that in Vladimiriae Radix[13]. Moreover, both costunolide and dehydrocostuslactone were undetected in Inulae Radix[22]. More samples should be analyzed to precise whether Inulae Radix could be substituted for MH. Even though further researches need to be done, difference in contents of major biologically active compounds in 4 kinds of MH could be reasonable for the variation of therapeutic efficacy.

Comparing 4 kinds of MH on antioxidant activity also showed difference. Vladimiriae Radix was the most effective antioxidant. The antioxidant activity ranking of samples detected by DPPH, ABTS and FRAP assay was strongly positively correlated as shown in Fig.5. The difference between antioxidant capacities of Vladimiriae Radix and other kinds was greater in FRAP assay.

Conclusion

The classical identification of traditional medicines with various original plants has been mainly conducted by comparing morphological features in elucidated form. The newly established identification keys in this study based on macroscopic and microscopic identification, and histochemical and phytochemical methods would provide more practical and accurate authentication of traditional medicine. In addition, this study provides comprehensive view on dicriminating Aucklandiae Radix, Inulae Radix, Vladimiriae Radix and Aristolochiae Radix for the first time. Our data could be helpful to ensure eligible therapeutic use and the safety control in local market by distinguishing 4 kinds of Mokhyang.

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