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Studies on the chemical compositions of *Hyptis suaveolens* (L.) Poit.

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Abstract: This study aimed at identifying the chemical compounds isolated from the aerial parts and roots of *Hyptis suaveolens* (L.) Poit. The compounds were isolated and purified by a combination of various chromatographic techniques including silica gel column chromatography, silica gel reverse phase column chromatography, Sephadex LH-20 gel column chromatography, semi-preparative HPLC and recrystallization. The chemical structures were analyzed and elucidated based on physiochemical properties and spectroscopic analysis. From the aerial parts of *H. suaveolens*, eight compounds were isolated and identified as quercetin 3-*O*- β -D-glucopyranoside (**1**), apigenin (**2**), sorbifolin (**3**), quercetin (**4**), kaempferol (**5**), genkwanin (**6**), rosmarinic acid (**7**) and methyl rosmarinate (**8**). Two compounds were isolated from the roots of *H. suaveolens* and identified as podophyllotoxin (**9**) and picropodophyllotoxin (**10**). Compounds **2–6** were isolated from *H. suaveolens* for the first time while compound **10** was isolated from the genus of *Hyptis* for the first time. The results of this study provided a scientific basis for the development of the medicinal value of *H. suaveolens* and have important theoretical significance for the chemical utilization of *H. suaveolens* resources.

Keywords: structural identification; quercetin 3-*O*- β -D-glucopyranoside; sorbifolin.

INTRODUCTION

Hyptis suaveolens (L.) Poit, locally known in China as Maolaohu belongs to the family Lamiaceae, covers important horticultural crops having economical value and is an important medicinal plant that is distributed in dense clumps along roadsides, in over-grazed pastures and around stockyards throughout the tropics and subtropics. *H. suaveolens* has a number of synonyms, such as *Ballota suaveolens* L., *Bystropogon graveolens* Blume, *Bystropogon suaveolens* (L.) L'Hér., *Gnoteris cordata* Raf., *Gnoteris villosa* Raf., *Hyptis congesta* Leonard, *Hyptis*

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ebracteata R.Br., *Hyptis graveolens* Schrank, *Hyptis plumieri* Poit., *Marrubium indicum* Blanco, *Mesosphaerum suaveolens* (L.) Kuntze, *Schaueria graveolens* (Blume) Hassk. or *Schaueria suaveolens* (L.) Hassk. *H. suaveolens* is a fast-growing perennial and aromatic herb, 0.4–2 m tall with hairy stems and leaves, having branches and usually woody at the base. The leaves are weak, oval in outline, tip broadly pointed.^{1,2} The plant is native to the tropics of America and now considered as a weed worldwide. There are also many distributions on the southern coast of China. The medicinal use of the plant has been reported in 22 countries, which emphasizes its potential values of application in modern pharmacy. In traditional medicine, almost all parts of this plant are used to treat numerous ailments, such as respiratory and gastrointestinal infections, as antirheumatic and antisuppurific baths, to treat indigestion, colic, stomachache, colds, fever, burns, wounds, cramps and various skin complaints.^{3–6} *H. suaveolens* has both medicinal individuality as well as insecticidal properties. In several countries, it is commonly used as an insect repellent by incinerating or placing it naturally.^{7–10} Among these traditional medicinal uses, the leaves are used most frequently, followed by seeds and thirdly the whole plants. In addition, the decoction of the roots was reported to contain urosolic acid, a natural HIV integrase inhibitor, and was highlighted for the preparation of an appetizer.¹¹ The chemical components of the essential oils obtained from the leaves of *H. suaveolens* were intensively investigated in previous studies. Many new chemical compounds, such as monoterpenes and sesquiterpenoids, obtained from the essential oils of *H. suaveolens* leaves were isolated and their structures elucidated, and their antibacterial, antifungal and anti-*Aspergillus* activity confirmed.^{12–16} In addition, it was reported that the leaves had anticancer and antifertility properties, while their aqueous extract showed an antinociceptive effect and acute toxicity, especially for the essential oil.^{17,18} However, little attention has been given to the extraction, separation and structural identification of chemical compositions from the entire plant, leading to a lack of a systematic study. Therefore, information concerning the chemical composition of *H. suaveolens* is still incomplete. As the drug is endowed with huge exploitation and utilization value, it is medicinally important to know precisely and comprehensively its chemical composition. In view of the importance of this, the present investigation is concerned with the extraction, separation, purification and structural identification of the aerial parts and roots of *H. suaveolens* using physicochemical and chromatographic methods to help in the development of the utilization and pharmacological activity of this drug.

MATERIALS AND METHODS

General procedures

All reagents used for the analyses were of analytical grade and were not subjected to further purification. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Haiyang Chemical Works, China), ODS (50–70 μm , Tianjin Jianhexiang Chromato-

graphic Works, China) and Sephadex LH-20 (Beijing Ruida Henghui Scientific and Technical Corporation, China). Semi-preparative HPLC was realized on a Gilson 322 instrument, using a Shim-pack GIS C18 column (3.0 cm×250 mm, 5 μm, Shimadzu, Japan). The ESI-MS spectra were recorded on a Waters ACQUITY UPLC/Q-TOF micro LC-MS spectrometer (Waters, USA). The NMR spectra were obtained on an AVANCE 500 spectrometer (Bruker, Germany).

Collection, identification and preparation of the plant materials

Fresh whole plants of *H. suaveolens* growing on the grounds of Guangdong Pharmaceutical University, Guangzhou Higher Education Mega Center were randomly collected, taxonomically identified and authenticated by Prof. Shengguo Ji, School of Traditional Chinese Medicine, Guangdong Pharmaceutical University. They were cleaned to remove physical impurities, air-dried in the shade, mechanically powdered, sieved using 10 meshes and stored in hermetic containers under dry air for further studies. This research was conducted in the Key Laboratory of the State Administration of Traditional Chinese Medicine for the Production & Development of Cantonese Medicinal Materials, School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, China, during 2016–2017.

Extraction and purification of samples

The coarse powder materials of aerial parts (8.67 kg) and roots (1 kg) of *H. suaveolens* were subjected to ultrasonic extraction three times with ethanol (90 % and 2×70 %, the ratio of solid (powder) to liquid (ethanol) was 1:10), and these extracts were concentrated in a rotary evaporator under reduced pressure and controlled temperature (< 45 °C). They were then extracted separately and successively with petroleum ether, ethyl acetate and *n*-butanol. (b.p. 60–90 °C, 10 L). The ethyl acetate extracts were placed in an airtight container and stored in a refrigerator. Purification of the samples were performed using silica gel column chromatography, silica gel reverse phase column chromatography, Sephadex LH-20 gel column chromatography, Gilson semi-preparative chromatography and recrystallization.

Identification of constituents

The constituents of the extracts were determined by a combination of physicochemical properties, UV spectrometry and nuclear magnetic resonance spectrometry (NMR), and identified by comparing their mass spectral patterns with the data for original samples and by matching those cited in the literature.^{19–28}

RESULTS AND DISCUSSION

The chemical compounds of the aerial parts and roots of *H. suaveolens* were determined and are listed in Table I. Their structures were identified by comparison of physicochemical analysis and their spectral data (ESI-MS, ¹H-NMR and ¹³C-NMR data) with literature values. The characteristics, solubility and melting points (m.p.) of compounds are also given in Table I, which were used to deduce the functional group. By comparing with the UV absorption and ESI-MS data, given in Table II, the molecular formula of each compound could be obtained. The ¹³C-NMR and ¹H-NMR spectra data are presented in Tables S-I and S-II, respectively, of the Supplementary material to this paper. From these data, detailed structural information of compounds, shown in Fig. 1, were obtained.

The results revealed that the identified compounds from the aerial parts of *H. suaveolens* included quercetin 3-*O*-β-D-glucopyranoside (**1**), apigenin (**2**), sorbi-

folin (**3**), quercetin (**4**), kaempferol (**5**), genkwanin (**6**), rosmarinic acid (**7**) and methyl rosmarinate (**8**), while the identified compounds from the roots of this plant included podophyllotoxin (**9**) and picropodophyllotoxin (**10**). Compounds **2–6** were found in *H. suaveolens* for the first time, while compound **10** was isolated for the first time from the genus of *Hyptis*. Compound **1**, also called isoquercetin, was found in this plant in a previous study,²⁹ which was identified in course of LC-MS measurements, but it was not isolated. Compounds **7–9** were also obtained from the LC-MS in this plant according to previous literature.^{30,31}

TABLE I. Components detected in the extracts of *H. suaveolens* with solubility and melting point data

Compound	Characteristics	Solubility	M.p. / °C	Functional group classes
Quercetin 3- <i>O</i> - β -D-glucopyranoside	Yellow powder	Methanol-soluble; water- and ethyl acetate-insoluble	–	Flavonoid glycoside
Apigenin	Yellow powder	Methanol-soluble; water-insoluble	347–348	Flavone
Sorbifolin	Yellowish white needle crystals	Methanol-soluble; water-insoluble	n.a.	Flavone
Quercetin	Yellow powder	Methanol-microsoluble; Chloroform and ethyl acetate-insoluble	313–314	Flavone
Kaempferol	Yellow powder	Methanol-microsoluble; chloroform- and ethyl acetate-insoluble	276–278	Flavone
Genkwanin	Yellow powder	Methanol- and water-insoluble	293–296	Flavone
Rosmarinic acid	White powder	Water- and methanol-soluble; acetone-insoluble	171–175	Polyphenol
Methyl rosmarinate	Yellow syrup	Methanol- and ethyl acetate-soluble; water-insoluble	–	Polyphenol
Podophyllotoxin	White powder	Methanol-soluble; water-insoluble	183–184	Lignan
Picropodophyllotoxin	White powder	Acetone-soluble; water-insoluble	225–227	Lignan

TABLE II. UV and ESI-MS data for the isolated compounds

Compound	Max. absorption in UV, nm	ESI-MS	Exact mass	Molecular formula	Ref.
Quercetin 3- <i>O</i> - β -D-glucopyranoside	268, 364	487.0857[M+Na] ⁺ , 463.0887[M-H] [–]	465.391	C ₂₁ H ₂₀ O ₁₂	19
Apigenin	268, 336	271.0635[M+H] ⁺	270.241	C ₁₅ H ₁₀ O ₅	20
Sorbifolin	262, 325	323.0542[M+Na] ⁺ , 299.0536[M-H] [–]	300.268	C ₁₆ H ₁₂ O ₆	21
Quercetin	283, 336	303.0445[M+H] ⁺ , 301.0344[M-H] [–]	302.201	C ₁₅ H ₁₀ O ₇	22
Kaempferol	222, 267, 366	285.0378[M-H] [–]	286.241	C ₁₅ H ₁₀ O ₆	23
Genkwanin	–	283.0619[M-H] [–]	284.268	C ₁₆ H ₁₂ O ₅	24

TABLE II. Continued

Compound	Max. absorption in UV, nm	ESI-MS	Exact mass	Molecular formula	Ref.
Rosmarinic acid	199, 261, 325	361.0931[M+H] ⁺ , 383.0746[M+Na] ⁺ , 359.0785[M-H] ⁻	360.32	C ₁₈ H ₁₆ O ₈	25
Methyl rosmarinate	205, 264, 331	374.0961[M+H] ⁺ , 397.0901[M+Na] ⁺ , 373.0933[M-H] ⁻	374.347	C ₁₉ H ₁₈ O ₈	26
Podophyllotoxin	203, 288	432.0534[M+NH ₄] ⁺ , 415.0847[M+H] ⁺	414.412	C ₂₂ H ₂₂ O ₈	27
Picropodophyllotoxin	264, 322	851.0301[2M+Na] ⁺ , 438.0634[M+Na+1] ⁺ , 415.0688[M+1] ⁺	414.412	C ₂₂ H ₂₂ O ₈	28

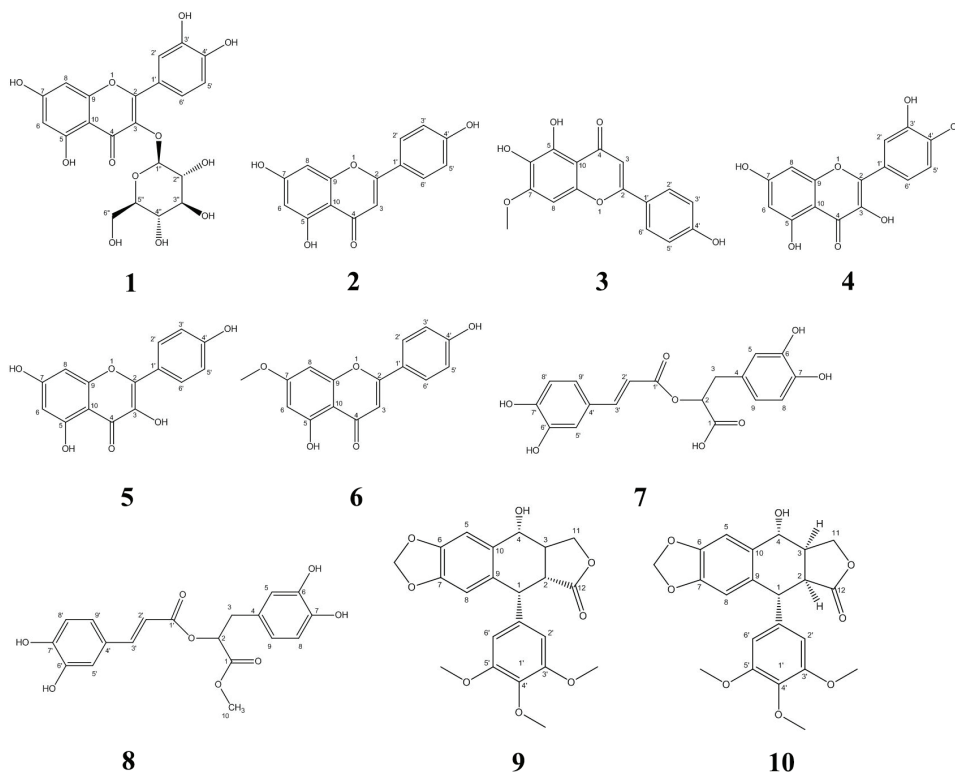


Fig 1. Chemical structures of 1–10. 1. Quercetin 3-*O*- β -D-glucopyranoside; 2. apigenin; 3. sorbifolin; 4. quercetin; 5. kaempferol; 6. genkwanin; 7. rosmarinic acid; 8. methyl rosmarinate; 9. podophyllotoxin; 10. picropodophyllotoxin

Most previous studies focused on the identification of polyphenols, sesquiterpenes, diterpenes, triterpenes and steroids extracted from *H. suaveolens*, but little attention was given to the flavonoids extracted from this plant. It was indi-

cated that flavonoids have antibacterial and antiviral, antitumor, antioxidant, anti-inflammatory, analgesic and other biological activities. The results showed that compounds obtained from the aerial parts of *H. suaveolens* were flavonoid constituents mostly. Generally, dissociative flavonoids were extracted by ethanol (90–95 %), flavonoid glycosides were extracted by ethanol (60 %), while both could be extracted by ethanol (70 %). In this study, the flavonoid constituents of the aerial parts of *H. suaveolens* were studied by the ultrasonic extraction method using ethanol (70 and 90 %), which could be used for the comprehensive extraction of flavonoids. However, in this experiment, tailing of peaks of preparation liquid chromatogram could easily occur when extracting flavonoids monomer. In this case, the hydrothermal re-crystallization purification method can be tried to remove water-soluble impurities and improve the purity of the product when the percentage of methanol in methanol-water is more than 30 %. The compounds podophyllotoxin and picropodophyllotoxin obtained in this research belong to the functional group of aryl lignans that are mainly derived from plants of the family Berberidaceae, and are isomers to each other. It was reported that podophyllotoxin had potent inhibitory effect on tumors and virus, which reveal the huge exploitation and utilization value of the roots of *H. suaveolens*.³¹

This research provides essential information for further studies on the medicinal values of *H. suaveolens*. However, *H. suaveolens* has not been recorded in the Chinese Pharmacopoeia. In order to develop the curative value of it, a series of further scientific research, such as its pharmacological research, clinical tests and formulation of the quality standard have to be completed.

CONCLUSIONS

The chemical compounds isolated from the aerial parts and roots of *Hyptis suaveolens* (L.) Poit. were identified by comparison of physico-chemical analysis and their spectral data. The components obtained from the aerial parts were identified as quercetin 3-*O*- β -D-glucopyranoside (**1**), apigenin (**2**), sorbifolin (**3**), quercetin (**4**), kaempferol (**5**), genkwanin (**6**), rosmarinic acid (**7**) and methyl rosmarinate (**8**), while the components obtained from the roots were identified as podophyllotoxin (**9**) and picropodophyllotoxin (**10**). In this regard, compounds **1**²⁹ and **7–9**^{30,31} have been reported to be found in *H. suaveolens*, while compounds **2–6** are found in *H. suaveolens* for the first time and compound **10** is isolated from the genus of *Hyptis* for the first time.

SUPPLEMENTARY MATERIAL

The ¹H-NMR and ¹³C-NMR data, as well as all recorded spectra and preparation liquid chromatograms, are available electronically at the pages of journal website: <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

ИЗВОД

ИСПИТИВАЊЕ ХЕМИЈСКОГ САСТАВА *Hyptis suaveolens* (L.) Poit.

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Циљ истраживања је био да се изврши идентификација хемијских једињења која су изолована из надземних делова и корена биљке *Hyptis suaveolens* (L.) Poit. Једињења су изолована и пречишћена комбиновањем различитих хроматографских и препаративних техника, које укључују хроматографију на колони силика-гела, хроматографију на реверсној фази модификованог силика-гела, хроматографију на колони Sephadex LH-20, семи-препаративну HPLC хроматографију и кристализацију. Хемијске структуре једињења анализиране су и утврђене на основу физичко-хемијских особина и спектроскопске анализе. Осам једињења је изоловано из надземних делова *H. suaveolens* и идентификована су као кверцетин-3-O- β -D-глукопиранозид (1), апигенин (2), сорбинолин (3), кверцетин (4), камферол (5), генкванин (6), рузмаринска киселина (7) и метил-розмаринат (8). Два једињења су изолована из корена *H. suaveolens* и идентификована су као подофилотоксин (9) и пикроподофилотоксин (10). Једињења 2–6 изолована су по први пут из *H. suaveolens* док је једињење 10 изоловано из рода *Hyptis* по први пут. Резултати истраживања пружају научну заснованост за резвијање примене *H. suaveolens* у медицини и имају важан теоретски значај за ширу употребу *H. suaveolens*.

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