ACTIVE CONSTITUENTS IN CHINESE, AYURVEDIC AND THAI HERBAL MEDICINES: APPLICABLE SEPARATION PROCEDURES

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Abstract

A simple and applicable column chromatographic and prep. HPLC methods with UV detection are described for the isolation of active constituents including new active compounds from the alcoholic extract of Chinese herbal medicine: Gastrodia rhizome (Orchidaceae) and Curcuma rhizome (Zingiberaceae), Ayurvedic medicine: Asparagus root (Liliaceae), and Thai herbal medicines: Derris stem (Leguminosae) and Ardisia fruit (Myrsinaceae), guided by the biological activity assay. These methods show good specificity with respect to commonly prescribed traditional medicines, and it could be successfully applied for phytochemical and pharmacological studies.

Key words: Gastrodia rhizome, Curcuma rhizome, Asparagus root, Derris stem, Ardisia fruit, Chinese herbal medicine, Ayurvedic medicine, Thai herbal medicine

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สารออกฤทธิ์ในยาจากสมุนไพรจีน สมุนไพรอายุรเวท และสมุนไพรไทย: การประยุกต์วิธีการแยกสาร

*ฟูมิโอะ ไอคีกามิ**

บทคัดย่อ

ประยุกต์วิธีการแยกสารออกฤทธิ์อย่างง่ายโดยใช้คอลัมน์โครมาโตกราฟี และเอชพีแอลซีชนิดใช้แยกสารด้วยหัววัดรังสีเหนือม่วง แยกสารออกฤทธิ์จากสารสกัดแอลกอฮอล์ที่ติดตามการตรวจกรองฤทธิ์ทางชีวภาพแล้ว ของยาจากสมุนไพรจีน คือ เหง้า *Gastrodia elata* (วงศ์ Orchidaceae) และ เหง้าขมิ้นชัน (วงศ์ Zingiberaceae) สมุนไพรอายุรเวท คือ รากสามสิบ (วงศ์ Liliaceae) และสมุนไพรไทย คือ ลำต้นเถาวัลย์เปรียง (วงศ์ Leguminosae) และ ผลพิลังกาสา (วงศ์ Myrsinaceae) วิธีการแยกที่ใช้ให้ความจำเพาะดีกับยาจากสมุนไพรที่นิยมใช้อยู่ และสามารถประยุกต์สู่การศึกษาทางพฤกษเคมีและเภสัชวิทยาได้

คำสำคัญ : เหง้า *Gastrodia elata*, เหง้าขมิ้นชัน, รากสามสิบ, ลำต้นเถาวัลย์เปรียง, ผลพิลังกาสา, สมุนไพรจีน, สมุนไพรอายุรเวท, สมุนไพรไทย

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Introduction

The unique role of traditional oriental medicines including Chinese, Ayurvedic and Thai herbal medicines is gradually attracting worldwide attention in modern medical treatment. Since these traditional medicines have been produced by combining multiple crude drugs and are effective in the treatment of certain disorders and disease states, active constituents of these crude drugs should be studied for evidence of the pharmacological effects of herbal medicines in medical treatment.

Gastrodia rhizome, the steamed and dried rhizomes of *Gastrodia elata* Blume (Orchidaceae) (Chinese name: Tienma), has been considered as one of the very important Chinese herbal medicines and used for the medical treatments of headaches, migraine, dizziness, epilepsy, rheumatism, neuralgia, paralysis and other neuralgic and nervous affections[1]. Curcuma rhizome, the dried rhizomes of *Curcuma longa* L. (Zingiberaceae) (Common name: turmeric), has been used for aromatic stomachics and cholagogues. In traditional Chinese medicine, this crude drug is used as a component of ointments for pustular dermatitis and for diseases that are associated with abdominal pains. In India and the neighbor countries, turmeric is widely used not only as a spice or pigment but also as a traditional medicine for biliary disorders, anorexia, hepatic disorders and rheumatism[2]. Asparagus root, the dried, decorticated roots of *Asparagus racemosus* Willd. (Liliaceae), has earlier been used in Ayurvedic medicine as a stimulant, restorative, anti-abortifacient and demulcent in India and Thailand[3]. Dried stems of *Derris scandens* Benth. (Leguminosae) has been used for anti-dysenteric, diuretic and for relief of muscular pain in Thailand[4]. *Ardisia colorata* Roxb. (Myrsinaceae) has been used as a folk medicine to treat liver disease, cough and diarrhea in Thailand[4].

Phytochemical studies of Gastrodia rhizome have revealed the presence of several phenolic compounds and much interest in pharmacologically active constituents from *G. elata* led to isolate a new bioactive compound, named gastrol, together with several phenolic compounds, guided by the smooth muscle relaxant activity in the guinea-pig ileum[5]. Analytical studies have so far revealed that Curcuma rhizome contains bioactive volatile oils including turmerones as well as curcuminoids such as curcumin, monodemethoxycurcumin and bisdemethoxycurcumin, guided by the anti-inflammatory activity in the carrageenin-induced paw edema in rats[6]. The pharmacological effect of Asparagus root in Ayurvedic and Thai herbal medicines cannot be explained by several steroidal and sugar constituents

previously isolated. Further investigation on the separation of a bioactive constituent led to isolate a novel cage-type alkaloid, named asparagamine A, showing an anti-oxytocin activity[7]. Chromatographic separation of EtOH extracts of the stems of *D. scandens* led to isolate six new diprenylisoflavones, named derrisisoflavones A-F, together with six known isoflavones, and their structures were elucidated by spectroscopic analyses as well as the anti-dermaptophyte activity against *Trichophyton mentagrophytes*[8]. For evidence of the traditional use of *A. colorata* in Thailand, the antioxidant principles were studied and new ardisiphenols A-C, demethoxybergenin and several known compounds were isolated from the fruits of this plant, guided by the scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals[9].

In this paper the isolation procedures of these active constituents of Gastrodia rhizome, Curcuma rhizome, Asparagus root, Derris stem and Ardisia fruit in Chinese, Ayurvedic and Thai herbal medicines have been described, respectively.

Materials and Methods

Chemicals

MeOH, EtOH, *n*-hexane, benzene, EtOAc, *n*-butanol, $CHCl_3$ were purchased from Wako Pure Chemical Industries (Tokyo, Japan) and used for study after distillation by using a glass apparatus. Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA). Kieselgel 60 (70-230 mesh, 230-400 mesh) and precoated TLC plates (silica gel $60F_{254}$, 20 x 20 cm, 0.25 mm; RP-$18F_{254}$, 20 x 20 cm, 0.25 mm) were purchased from Merck Japan Ltd. (Tokyo, Japan). Chromatorex NH-DM1020 (100-200 mesh) and precoated TLC plate (NH silica gel, 0.25 mm) were purchased from Fuji Silisia Ltd. (Tokyo, Japan). Cosmosil C_{18}-OPN (Nacalai Tesque, Japan) for column chromatography and TSK gel ODS120A column (4.6 mm i.d. x 150 mm) (TOSOH, Japan) for analytical HPLC were used. All other chemicals used were of the highest commercial grade available, purchased from Wako Pure Chemical Industries.

Plant materials

Rhizomes of *G. elata* Blume were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan) and were also purchased in Yunnan (China) by Dr Qing Lin, and verified by Dr Qing Lin, Yunnan Institute of Traditional Chinese Medicine and Materia Medica,

Kunming, Yunnan, China. A voucher specimen (JH015393) is deposited in the herbarium, Graduate School of Pharmaceutical Sciences, Chiba University, Japan.

Dried rhizomes of *C. longa* L. used were a product of China and were purchased from Shin-a-Tsuko Co., Ltd. (Tokyo, Japan) and deposited as the voucher specimen (SS200106) in Sanover Co., Ltd. (Saitama, Japan).

The roots of *A. racemosus* Willd., the stems of *D. scandens* Benth. at Nakorn Pathom Province and the fruits of *A. colorata* Roxb. at Rayong Province were respectively collected by Dr. Nijsiri Ruangrungsi, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. These voucher specimens are deposited in the herbarium, Graduate School of Pharm. Sci., Chiba Univ., Japan.

Apparatus

The prep. HPLC system consisted of Hitachi model 655 high-pressure pump and UV detector, and a Hitachi D-2500 Chromato-Integrator (Tokyo, Japan). LiChrosorb Si60 column (5 μm, 4.6 mm i.d. x 250 mm) (Kanto Chemical Co., Japan), Cosmosil ODS column (5 μm, 8 mm i.d. x 250 mm) (Nacalai Tesque, Japan) or Inertsil ODS column (20 mm i.d. x 250 mm) (Gaskurokogyo Co., Japan) was used. Gas chromatography with mass spectrometry (GC-MS) system consisted of a gas chromatograph (HP5890 Series II Gas Chromatograph, Hewlett Packard, USA) coupled with a mass spectrometer (HP5971A Mass Selective Detector, Hewlett Packard, USA) and a mass data system (HP G1030A MS Chemstation, Hewlett Packard, USA). Silica gel column chromatography was performed with several size glass columns prepared by manufacturer (Chemitec, Tokyo, Japan). UV detection system on the post-column chromatography consisted of Atto model AC-5000 UV monitor (254 nm) and SJ-1410SR minicollector (Atto Corp., Tokyo, Japan).

Extraction and isolation

Gastrodia rhizome: Dried and ground rhizomes of *G. elata* (3.0 kg) were extracted with 99% MeOH (5 L x 3) at 45°C. The MeOH extract (143 g/155 g), after removal of the solvent by evaporation, was suspended in water and partitioned with *n*-hexane, EtOAc and *n*-butanol, successively, to give EtOAc fraction (6.0 g)[5].

Curcuma rhizome: Dried and ground rhizomes of *C. longa* (500 g) were extracted with *n*-hexane (1 L x 3) at 45°C. The residue, after removal of the solvent, was

suspended in water and partitioned with benzene, EtOAc and *n*-butanol, successively, to give *n*-hexane (34.5 g), benzene (26.7 g), EtOAc (16.6 g), *n*-butanol (1.6 g) and water (2.0 g) fractions[6].

Asparagus root: Air-dried roots of *A. racemosus* (130 g) were ground and extracted by 75% EtOH (500 ml x 3). The combined extract was concentrated suspension and partitioned between water (400 ml) and EtOAc (400 ml x 3) to give EtOAc fraction (1.5 g)[7].

Derris stem: Dried stems of *D. scandens* (600 g) were mechanically pulverized and extracted with 75% EtOH (4 L x 3). A half of EtOH extract (41 g) was suspended in water and extracted with *n*-hexane, benzene, EtOAc, and *n*-butanol, successively, to give *n*-hexane (0.19 g), benzene (6.41 g), EtOAc (5.1 g) and *n*-butanol (7.16 g) fractions[8].

Ardisia fruit: Dried fruits of *A. colorata* (500 g) were extracted with 75% EtOH (1.5 L x 3) at room temperature. The solvent was evaporated under reduced pressure to give the extract (62 g). The extract (58 g) was partitioned with water-*n*-hexane, and the remaining water layer was successively extracted with EtOAc and *n*-butanol (each 600 ml x 3) to give *n*-hexane (12 g), EtOAc (5 g), *n*-butanol (7 g) and water (30 g) fractions[9].

Chromatographic conditions

Gastrodia rhizome: After evaluating the activity of the resulting fractions in Gastrodia rhizome, guided by the inhibitory activity on KCl (40 m*M*)-induced contraction in the guinea-pig ileum, a portion of the EtOAc-soluble fraction (5.9 g) was subjected to silica gel column chromatography (36 mm i.d. x 46 cm, 250 g) with solvent 1 ($CHCl_3$-MeOH) of increasing polarities (10:0 to 50:50, v/v, each of fractions collected 400 ml) to give 13 fractions. Fr. 3 (528 mg) was re-chromatographed over silica gel (22 mm i.d. x 27 cm, 50 g) with solvent 2 (benzene-EtOAc) of increasing polarities (10:0 to 80:20, v/v) to yield 4-hydroxybenzyl methyl ether (250 mg). Fr. 4 (555 mg) was subjected to silica gel column chromatography (22 mm i.d. x 27 cm, 50 g) with solvent 2 (10:0 to 80:20, v/v) to yield 4-(4'-hydroxybenzyloxy) benzyl alcohol (2.8 mg), 4-hydroxybenzaldehyde (45 mg) and 4-hydroxybenzyl methyl ether (212 mg). Fr. 7 (171 mg) was subjected to silica gel column chromatography (14.5 mm i.d. x 20 cm, 15 g) with solvent 2 (10:0 to 80:20, v/v) to yield 4,4'-methylenebisphenol (3.5 mg), trimer of 4-hydroxybenzyl alcohol (5.7 mg) and 2,4-bis (4-hydroxybenzyl) benzyl alcohol (10 mg). Fr. 8 (204 mg) and 9 (123 mg) were combined and repeatedly chromatographed over silica gel with solvents 1 and 2 in a similar manner to

yield 4-hydroxybenzyl alcohol (0.8 mg) and bis(4-hydroxybenzyl) ether (154 mg). Fr. 10-12 (total 349 mg) were combined and subjected to silica gel column chromatography (22 mm i.d. x 21 cm, 40 g) with solvent 2 (10:0 to 0:10, v/v) to yield 2,4-bis(4-hydroxybenzyl) phenol (3.5 mg), 4,4'-dihydroxybenzyl sulfoxide (7.6 mg) and a fraction (42 mg) rich in gastrol. Gastrol (15 mg, 0.0006%/g dried rhizomes) was finally purified from the gastrol-rich fraction by prep. HPLC (LiChrosorb Si60 column, 4.6 mm i.d. x 250 mm), using $CHCl_3$-MeOH (9:1, v/v) system.

Curcuma rhizome: In analytical studies on Curcuma rhizome, the *n*-hexane, benzene and EtOAc extracts were dissolved in acetone for GC-MS analysis. The conditions used were as follows: column, DB-1 fused silica capillary (0.22 mm x 30 m; J & W Scientific); column temperature, 100°C (kept for 3 min), 100-300°C (increased at a rate of 15°C/min) and 300°C (kept constant for 5 min); carrier gas, He 33.1 ml/min; ionizing mode, EI (70 eV). Extracts were dissolved in MeOH, and analyzed by HPLC which was carried out on L-column ODS (4.6 mm i.d. x 150 mm) at room temperature. The mobile phase was CH_3CN-50m*M* NaH_2PO_4 (62.5:37.5, v/v) or 100% MeOH at a flow rate of 0.5 ml/min. Absorbance of the effluent was detected at 380 or 200 nm.

Asparagus root: A portion (1.3 g) of the EtOAc fraction (1.5 g) was subjected to silica gel column chromatography (28 mm i.d. x 37 cm, 100 g) with elution of EtOAc-MeOH (99:1 to 98:2, v/v) to afford asparagamine A (167 mg, 0.15%/dry wt.), crude asparagamine B fraction (72 mg) and asparagamine C (20 mg, 0.017%/dry wt.), monitoring with Dragendorff's reagent. The resulting asparagamine B fraction was repeatedly chromatographed on silica gel column chromatography (14 mm i.d. x 11 cm, 7 g) with elution of $CHCl_3$-MeOH (95:5, v/v) and benzene-MeOH (98:2, v/v) to give asparagamine B (8 mg, 0.007%/dry wt.).

Derris stem: A portion of the most anti-fungal active benzene extract (5 g) was subjected to silica gel column chromatography (36 mm i.d. x 36 cm, 185 g) with elution of benzene-EtOAc (100:0 to 92:8, v/v) to afford ten fractions (fr. 1-10). Among them, two fraction, Fr. 3 and 5, elution at approx. 10% and 12% EtOAc, were purified as 5,7,4'-trihydroxy-6,8-diprenylisoflavone (49 mg) and lupalbigenin (326 mg), respectively. Fr. 7 (a complex mixture, 570 mg, elution at approx. 25% EtOAc) was chromatographed repeatedly on silica gel column chromatography (22 mm i.d. x 26 cm, 55 g) eluted with *n*-hexane-acetone (80:20, v/v) and on Chromatorex NH-DM1020 column chromatography (10 mm i.d. x 23 cm, 7.5 g) eluted with $CHCl_3$-MeOH (70:30 to 94:6, v/v) to afford derrisisoflavone A (14 mg),

derrisisoflavone B (7 mg) and scandinone (34 mg). Fr. 6 (390 mg) was chromatographed repeatedly on silica gel column chromatography (14 mm i.d. x 30 cm, 25 g) eluted with *n*-hexane-acetone (75:25, v/v), and submitted to prep. HPLC ($CHCl_3$-MeOH-conc.NH_4OH, 99:1:0.1, v/v/v) to afford erysenegalensein E (5 mg) and lupinisol A (6 mg). Fr. 8 (a complex mixture, 1100 mg, elution at approx. 30% EtOAc) was chromatographed repeatedly on silica gel column chromatography (26 mm i.d. x 26 cm, 55 g) eluted with *n*-hexane-acetone (90:10 to 78:22, v/v), $CHCl_3$-MeOH (99:1, v/v) systems, and submitted to prep. HPLC (LiChrosorb Si60, 5 μm, 4.6 mm i.d. x 250 mm) (*n*-hexane-EtOH, 93:7, v/v) to afford derrisisoflavone C (4 mg), and also submitted to prep. HPLC ($CHCl_3$-EtOH, 100:0 to 99:1, v/v) to afford derrisisoflavone D (4 mg), derrisisoflavone E (8 mg), derrisisoflavone F (11 mg), lupinisoflavone G (17 mg) together with scandinone (178 mg).

Ardisia fruit: A portion (5.2 g) of the *n*-hexane-soluble fraction (12 g) was subjected to silica gel column chromatography (25 mm i.d. x 37 cm) with $CHCl_3$-MeOH of increasing polarity to give 8 fractions. Fr. 4 was applied to silica gel column chromatography (18 mm i.d. x 23 cm) with *n*-hexane-EtOAc of increasing polarity to give 7 fractions. Subfr. 4 was subjected to ODS HPLC (MeOH-0.2% AcOH, 85:15, v/v) to provide ardisiphenol A (87 mg, 0.043%/g dried fruits), B (170 mg, 0.084%), and C (118 mg, 0.058%). Subfr. 2 was also subjected to ODS HPLC (MeOH-0.2% AcOH; 85:15, v/v) to provide alkylresorcinols (A, 45 mg, 0.022%; B, 107 mg, 0.053%; C, 75 mg, 0.037%). Subsequently, a portion (2.0 g) of the EtOAc-soluble fraction (5 g) was separated by silica gel column chromatography (22 mm i.d. x 22 cm) with $CHCl_3$-MeOH of increasing polarity to give 7 fractions. Fr. 1 was recrystallized to give embelin (598 mg, 0.32%) and Fr. 4 was subjected to ODS column chromatography (12 mm i.d. x 16 cm) with acetone-water to afford myricetin (3 mg, 0.0016%) and quercetin (8 mg, 0.0043%). A portion (4.5 g) of the *n*-butanol-soluble fraction (7 g) was separated by silica gel column chromatography (26 mm i.d. x 30 cm) with $CHCl_3$-MeOH-water of increasing polarity to give 10 fractions. Fr. 3 was purified by ODS HPLC (MeOH-water, 55:45, v/v) to give kaempferol (4 mg, 0.0013%), and Fr. 6 was separated by ODS HPLC (MeOH-water, 20:80, v/v) to give bergenin (13 mg, 0.0043%) and demethoxybergenin (11 mg, 0.0036%). Fr. 7 was subjected to ODS column chromatography (20 mm i.d. x 17 cm) with MeOH-water to afford gallic acid (16 mg, 0.0053%), norbergenin (51 mg, 0.017%) and quercetin 3-*O*-β-D-glucopyranoside (6 mg, 0.002%).

Results and Discussion

Chromatography

Gastrodia rhizome: The MeOH extract from Gastrodia rhizome was separated into *n*-hexane, EtOAc, *n*-butanol and water-soluble fractions. After evaluating the activity of the EtOAc fraction, several secondary fractions obtained through the separation process of the EtOAc-soluble fraction were subjected to a series of column chromatographic and prep. HPLC separations to yield 11 phenolic compounds including a new compound, named gastrol, as one of the major active components, guided by the inhibitory activity on KCl (40 m*M*)-induced contraction in the guinea-pig ileum. Although gastrodin has been thought to be the major active component responsible for the clinical effects of Gastrodia rhizome[1], it was not found in the EtOAc-soluble fraction following this method.

Curcuma rhizome: The GC-MS analysis of volatile oils contained in the *C. longa* extracts revealed eight constituents, *trans*-caryophyllene, α-curcumene, α-zingiberene, β-bisabolene, β-sesquiphellandrene, ar-turmerone, α-turmerone and β-turmerone in the *n*-hexane and benzene fractions. Curcuminoids such as curcumin, monodemethoxycurcumin and bisdemethoxycurcumin were identified in the benzene and EtOAc fractions, and their curcuminoid contents were 33.1 and 50.5%, respectively. The curcuminoid contents in the other fractions were very low; 0.4% in the *n*-hexane fraction, 2.2% in the *n*-butanol fraction and 0.4% in the water fraction.

Asparagus root: A new cage-type alkaloid, asparagamine A was isolated from the 75% EtOH extract of air-dried roots of *A. racemosus* as a major component (0.15%/dry wt.) together with by-products asparagamine B and C by chromatographic (silica gel) separation. They were positive to Dragendorff's and I_2-platinate reagents. The relative stereostructure was elucidated by spectroscopic, chemical and single crystal X-ray analyses as a novel polycyclic pyrrolizidine derivative. This is not only the first alkaloid to be isolated from the genous *Asparagus* but also the first pyrrolizidine derivative with carbon substituents at C-5 and C-8. Asparagamine A in dose form 10 to 100 μg/ml showed an anti-tumor activity on several tumor models *in vitro* in a dose dependent manner. In addition, asparagamine A showed an inhibitory effect on oxytocin-induced construction of rat uterus. The inhibition (%) values of asparagamine A were 67.4 and 38.8% at 10^{-5} and 10^{-6} mg/ml when the construction was induced by oxytocin at 10^{-3} IU/ml. Asparagamine A is a major component of *A. racemosus* and is thought to be responsible for a significant

portion of the anti-abortifacient activity of this Ayurvedic medicinal plant.

Derris stem: Chromatographic separation of EtOH extracts of the stems of *D. scandens* has yielded six new diprenylisoflavones, named derrisisoflavones A-F, together with six known isoflavones: lupalbigenin, scandinone, erysenegalensein E, lupinisol A, lupinisoflavone G and 5,7,4′-trihydroxy-6,8-diprenylisoflavone. The 75% EtOH extract of the stems was partitioned with *n*-hexane, benzene, EtOAc and *n*-butanol, successively. The benzene extract was concentrated and subjected to silica gel, Chromatorex NH column chromatographies and prep. HPLC to yield 12 compounds. Among them, 5,7,4′-trihydroxy-6,8-diprenylisoflavone, lupalbigenin and scandinone were estimated as major constituents of the benzene-soluble fraction from the yields and TLC profiles, while derrisisoflavones A-F, erysenegalensein E, lupinisol A and lupinisoflavone G were minor components. To the best of our knowledge, this is the first isolation of derrisisoflavones A-F, while more than a thousand isoflavone derivatives have been reported. Anti-dermaptophyte activity of the isolated isoflavones from *D. scandens* against *Trichophyton mentagrophytes* was examined by the microdilution method[10]. Derrisisoflavone C, 5,7,4′-trihydroxy-6,8-diprenylisoflavone and lupalbigenin, at least, may be an anti-dermaptophyte principle of *D. scandens* from the majority in the chromatograms and the magnitude of the activity.

Ardisia fruit: The 75% ethanol extract of the dried fruits of *A. colorata* was separated into *n*-hexane, EtOAc, *n*-butanol and water-soluble fractions. They were fractionated by repeated column chromatographies according to the scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals in the TLC autographic assay[11]. The *n*-hexane-soluble fraction yielded novel ardisiphenols A-C and 3 known alkylresorcinols. The EtOAc-soluble fraction afforded embelin as the major constituent, myricetin and quercetin. A new bergenin derivative, demethoxybergenin was isolated from the *n*-butanol-soluble fraction, together with bergenin, norbergenin, kaempferol, quercetin 3-*O*-β-D-glucopyranoside and gallic acid. Their structures were determined by NMR, MS(/MS) analyses and other spectroscopic methods. Demethoxybergenin isolated from the *n*-butanol fraction can be an artifact. Ardisiphenols showed a weak antioxidant activity but showed a strong cytotoxicity against the murine breast cancer cell line, FM3A cells.

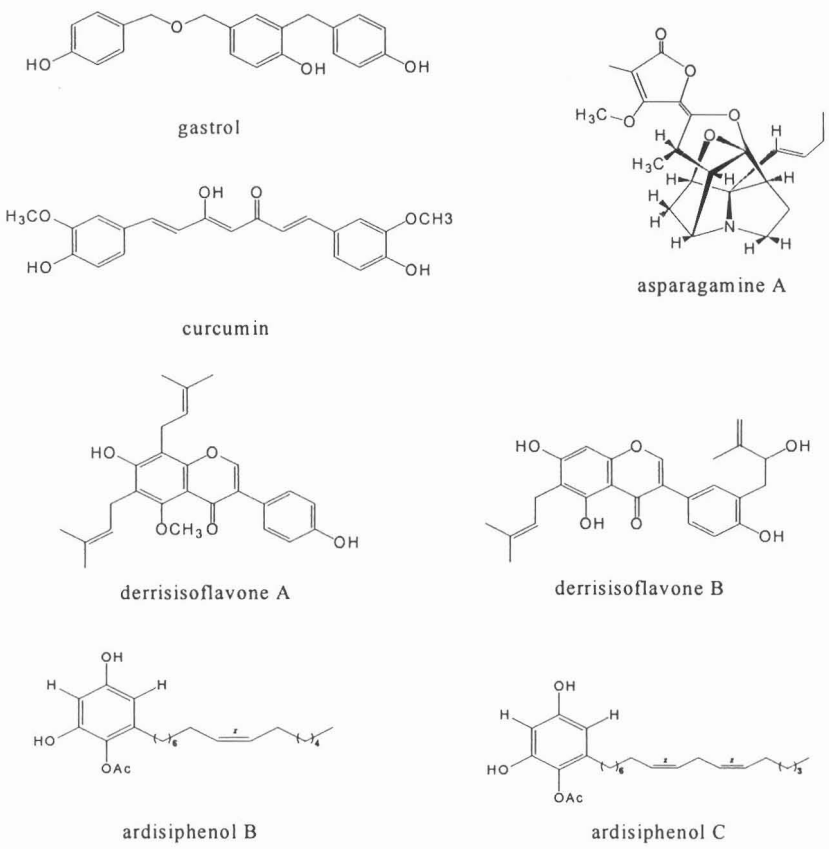

Fig. 1 Chemical structures of some active constituents isolated from traditional herbal medicines

Conclusion

The present results showed that our chromatographic methods with silica gel column chromatography and prep. HPLC, by monitoring the UV absorption at 254 nm, and also guided by the biological activity assay, are applicable for the separation of several bioactive constituents from Chinese herbal medicines, traditional oriental medicines and some medicinal resources, for evidence of the pharmacological effects in medical treatment.

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