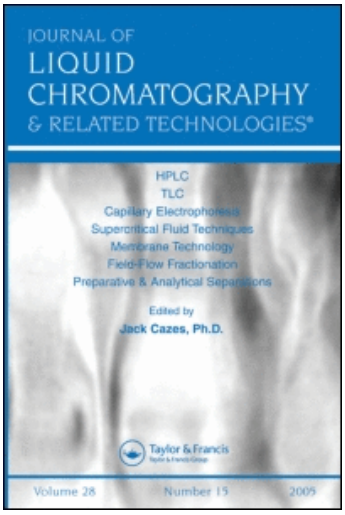

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CENTRIFUGAL PARTITION CHROMATOGRAPHY: APPLICATION TO NATURAL PRODUCTS IN 1994-2009

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CENTRIFUGAL PARTITION CHROMATOGRAPHY: APPLICATION TO NATURAL PRODUCTS IN 1994–2009

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□ *Centrifugal partition chromatography (CPC) is a type of counter current separation technique and was first invented by Sanki Eng. Ltd. in Kyoto. Since the year of 1982, CPC has been used to isolate or fractionate bioactive materials and has shown its efficiency in the purification process of natural products from plants and microorganisms. This review focuses on the CPC applications for the purification of over 250 compounds from 60 plant materials and microorganisms between 1994 to September 2009.*

Keywords centrifugal partition chromatography, CPC application, microorganism, natural product, plant

INTRODUCTION

In the year of 1982, a new centrifugal counter-current chromatograph (CCCC) instrument was invented by Sanki Eng. Ltd. in Kyoto. This instrument first employed two rotary seal joints and consisted of twelve cartridges that were arranged around the rotor of a centrifuge. The inner volume of each column cartridge was 15 mL and the single-axis joint rotation mechanism produced constant centrifugal field to retain a liquid stationary phase.[1,2] This instrument was announced to peoples at the 18th International Symposium on Advances in Chromatography in 1982, and the name "Centrifugal Partition Chromatography (CPC)" became the generic name for this apparatus later on.[3,4] Currently, CPC has been one of predominant counter-current separation (CS) tools along with counter-current chromatography

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(CCC) that applies two axis gyration mechanism developed by Ito et al.[5]

CPC (hydrostatic instrument) is composed of a number of partition channels which are linked in cascade by ducts. The single-axis centrifuge generates centrifugal force to retain stationary phase while the mobile phases are passing. Mixing and settling take place in individual partition cells that compose a group of partition disks,[2,6,7] while modern CCC (type-J hydrodynamic instrument) is composed of a continuous multi layer coil separation column, and mixing and settling occurred via type-J planetary motion.[8] Although the mechanical type and separation principle are different, it is certain that the separation is based on the difference in distribution of target molecules between two immiscible liquid phases.

In the past few decades, numerous papers have been published to deal with counter-current separations and their applications. Recently, two CS reviews have analyzed the published papers of counter-current separations into journals, authors, methodologies, and applications by statistical method.[9,10] This phenomenon reflects that the counter-current separations are not alternative methods to solid support based conventional column chromatography, but stand as an independent area of liquid-liquid chromatography.

CS has been increasingly applied to separate diverse constituents from natural sources, such that

1. Bioactive natural products are frequently found in very small amounts and may be lost due to irreversible adsorption or chemical reactions during the conventional column chromatography process that uses solid adsorbents; whereas, CS utilizes only liquid-liquid phases as the stationary and the mobile ones, where all the target materials can theoretically be recovered without loss.
2. The gram-scale of sample mass can be introduced to CS instruments and the sample scale-up process from laboratory scale to pilot is predictable,[11] which is considered a major advantage when it comes to the production of standard marker compounds from herbal medicines in the pharmaceutical area. Indeed, high-performance liquid chromatography (HPLC) or other purifying methods by conventional column chromatography have difficulties in loading large amounts of sample mass.
3. Conventional column chromatography methods require time- and labor-consuming processes to separate and purify target molecules; however, CS enables us to save separation time, solvent consumption, costs, and labors, once optimal separation conditions are selected for target compounds. Actually, a few steps have been required to isolate target compounds once CS methods were involved in separation processes,

and these facts imply that CS is economical and an environmentally-friendly technique.

Even though many articles concerning CS have been issued, the main focus has been on counter-current chromatography (CCC) methods such as high-speed counter chromatography (HSCCC). There are more manufacturers and users of HSCCC than those of CPC. However, there has been a recent article that deals with CPC application to natural products during the time period since 1994;[12] however, it is not easy for English-speaking peoples to access and refer to this review as it is written in Chinese.

The present article describes the exclusive CPC applications as a tool for the separation or fractionation of natural products. The mechanical theory and principles of CPC were not mentioned herein because several articles and reviews have fully covered it. The data referred to in this review were obtained with the aid of SciFinder® database. The key word "centrifugal partition chromatography" was input as a research topic. The data obtained were restricted by publication year (1994–2009) and language (English), and articles that were not related to natural products were excluded.

CPC APPLICATIONS TO NATURAL PRODUCT

Phenolic Constituents

Simple Phenolics

Two phenylethanoid glycosides, plantamajoside (45.6 mg **1**) and acetoside (**2**) or isoacteoside (**3**) of 293.8 mg, were isolated and purified from the aerial parts of *Plantago asiatica* (Plantaginaceae) by high performance CPC (HPCPC) using ethyl acetate-*n*-butanol-ethanol-water (5:5:1:10, v/v). However, the structure (**2** or **3**) of the isolated compound was not resolved.[13]

Sequential CPC led to the isolation of three phenylpropanoids, myristicin (**4**), elemicin (**5**), and *trans*-isoelemicin (**6**) from *Diplolophium buchanani* (Umbelliferae). A portion (1.7 g) of the CH_2Cl_2 extract was fractionated, by CPC using the upper phase of the solvent system (*n*-hexane-ethyl acetate-methanol-water = 10:5:5:1, v/v) as a mobile phase, into nine sub-fractions (I-IX). Further, CPC separation of sub-fraction II with a solvent system (*n*-hexane-methyl *t*-butyl ether-acetonitrile, 5:1:5, ascending mode), and sub-fraction IV with a solvent system (*n*-heptane-acetonitrile-methanol, 6:3:1, v/v, ascending mode) afforded **4** (69 mg) and a mixture of **5** and **6** (75 mg), respectively.[14]

Rosmarinic acid (**7**) was successfully purified by ion-exchange CPC using benzalkonium chloride as a strong exchanger and iodide as a displacer from callus culture of *Lavandula vera* (Lamiaceae). The separation was

achieved by using a two-phase solvent system of chloroform-*n*-butanol-water (9:2:9, v/v, ascending mode).[15]

A CPC hyphenated with an evaporative scattering light detector facilitated preparative separation of oraposide (450 mg **8**) and verbascoside (1200 mg **9**) from the crude extract (6 g) of *Orobanche rapum* (Orobanchaceae). This separation was conducted by using a solvent system (ethyl acetate-acetone-water, 8.7:3.9:87.4, v/v, descending mode).[16]

CPC run with a solvent system of *n*-hexane-ethyl acetate-ethanol-water (4:5:3:3, v/v) and sequential HPLC purification purified *trans-p*-ethylcoumarate (**10**), caffeic acid (**11**), and vanillic acid (**12**) from a blended red wine extract. The residue in the stationary phase of the first CPC run was applied to the second CPC run with a solvent system composed of *n*-hexane-ethyl acetate-ethanol-water (4:5:3:3, v/v, descending mode) and, followed by HPLC purification on its fractions, syringic acid (**13**), tyrosol (**14**), and *trans-p*-coumaroyl 6″-glucoside (**15**) were isolated.[17]

Salting-out gradient CPC with either LiCl or $(NH_4)_2SO_4/KNO_3$ was applied to separate three chlorogenic acids, 5-caffeoylquinic acid (**16**), 5-feruloylquinic acid (**17**), and 3,4-dicaffeoylquinic acid (**18**) from green coffee bean (*Coffea arabica*). Using a LiCl salting-out gradient solvent system [ethyl acetate-*n*-hexane-0.01 M pH 2.5 phosphate buffer/LiCl (68:32:100, v/v, descending mode), 5.0 M, 2.5 M, and 0.1 M of LiCl], 5-caffeoylquinic acid (**16**), 5-feruloylquinic acid (**17**), and 3,4-dicaffeoylquinic acid (**18**) were well separated, with 88%, 85%, and 70% of recovery rate, respectively. In $(NH_4)_2SO_4/KNO_3$ salting-out gradient CPC run [ethyl acetate-*n*-hexane-pH 2.5 $(NH_4)_2SO_4$ (70:30:100; v/v), descending mode, 3.0 M and 1.5 M of $(NH_4)_2SO_4$, and 1.5 M of KNO_3], **16** (81% recovery), 3.6 mg of **17** (60% recovery), and 0.8 mg of **18** (80% recovery) were obtained (Figure 1). Additionally, two biphasic systems, chloroform-*n*-butanol-0.01 M pH 2.5 phosphate buffer (84:16:100; v/v) system and chloroform-*n*-butanol-0.01 M pH 2.5 phosphate buffer/5.0 M LiCl (82:18:100; v/v), were proposed as CPC run for both geometric isomers of feruloylquinic acid and dicaffeoylquinic acid, and geometric isomers of caffeoylquinic acid, respectively.[18]

Xanthohumol (40 mg **19**) and (*E*)-2″-(2‴-hydroxy-isopropyl)-dihydrofurano[2″,3″:4′,3″]-2′, 4-dihydroxy-6′-methoxychalcone (2 mg **20**), were purified from the extract of *Humulus lupulus* by normal-phase CPC using *n*-heptane-toluene-acetone-water system (24.8:2.8:50:22.4, v/v, ascending mode).[19] Chemical structures of aforementioned compounds and CPC experimental conditions were described in Figure 2 and Table 1, respectively.

Lignans

An enriched lignan extract (150 mg) of *Forsythia koreana* (Oleaceae) was subjected to, and eluted with the lower phase of the two-phase solvent

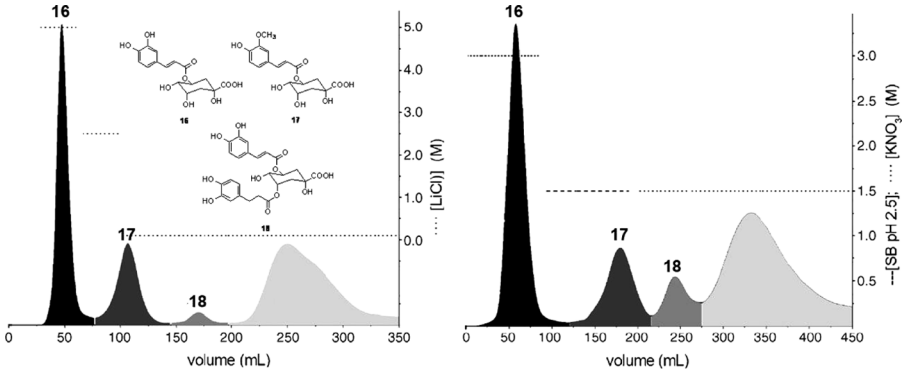


FIGURE 1 A CPC separation of the major chlorogenic acids present in green coffee beans with a LiCl salting-out gradient. Experimental conditions: rotation speed: 800 rpm; solvent system: *n*-hexane-ethyl acetate-0.01 M phosphate buffer/LiCl (pH 2.5) (32:68:100; v/v); salting-out gradient: 5.0 M (60 mL), 2.5 M (40 mL), and 0.1 M (250 mL) LiCl; mobile phase: lower aqueous phase; flow rate: 2.4 mL/min; retention of stationary phase: 76%; injection volume: 5 mL; back pressure: 50 bar (5.0 M LiCl), 40 bar (2.5 M LiCl), and 30 bar (0.1 M LiCl); sample: 50 mg chlorogenic acids-enriched green coffee bean extract dissolved in 2.5 mL of mobile phase and 2.5 mL of stationary phase. 5-CQA (5-caffeoylquinic acid, 16), 5-FQA (5-Feruloylquinic acid, 17), 3,4-diCQA (3,4-Dicaffeoylquinic acid, 18); B CPC separation of the major chlorogenic acids present in green coffee beans with a $(NH_4)_2SO_4/KNO_3$ salting-out gradient. Experimental conditions: rotation speed: 600 rpm; solvent system: *n*-hexane-ethyl acetate-$(NH_4)_2SO_4$ (pH 2.5) (30:70:100; v/v); salting-out gradient: 3.0 M (85 mL) and 1.5 M (105 mL) $(NH_4)_2SO_4$, and 1.5 M (260 mL) KNO_3; mobile phase: lower aqueous phase; flow rate: 2.0 mL/min; retention of stationary phase: 80%; injection volume: 5 mL; back pressure: 22 bar (3.0 M $(NH_4)_2SO_4$), 18 bar (1.5 M $(NH_4)_2SO_4$) and 16 bar (1.5 M KNO_3); sample: 50 mg chlorogenic acids-enriched green coffee bean extract dissolved in 2.5 mL of mobile phase and 2.5 mL of stationary phase. 5-CQA (5-caffeoylquinic acid, 16), 5-FQA (5-Feruloylquinic acid, 17), 3,4-diCQA (3,4-Dicaffeoylquinic acid, 18). (Adapted from J. Chromatogr. A 2009, *1216*, 4245–4251.)

system composed of *n*-hexane-ethyl acetate-methanol-water (5:5:5:5, v/v). By this chromatographic work, two dibenzylbutyrolactone lignans, matairesinol (20.2 mg **21**), and arctigenin (18.5 mg **22**), were obtained (Figure 3 and Table 1).[20]

Coumarins

The dichloromethane soluble fraction (1.7 g) of *Diplolophium buchanani* (Umbelliferae) was fractionated by CPC using the solvent system of *n*-hexane-ethyl acetate-methanol-water (10:5:5:1, v/v) into nine sub-fractions. Recrystallization in sub-fraction 8 furnished oxypeucedanin (25 mg **23**) while the second CPC run with a solvent system of chloroform-ethyl acetate-methanol-water (5:3:6:4, v/v, ascending mode) on the sixth sub-fraction, followed by an additional gel-filtration on Sephadex LH-20, gave oxypeucedanin hydrate (19 mg **24**) (Figure 4 and Table 1).[14]

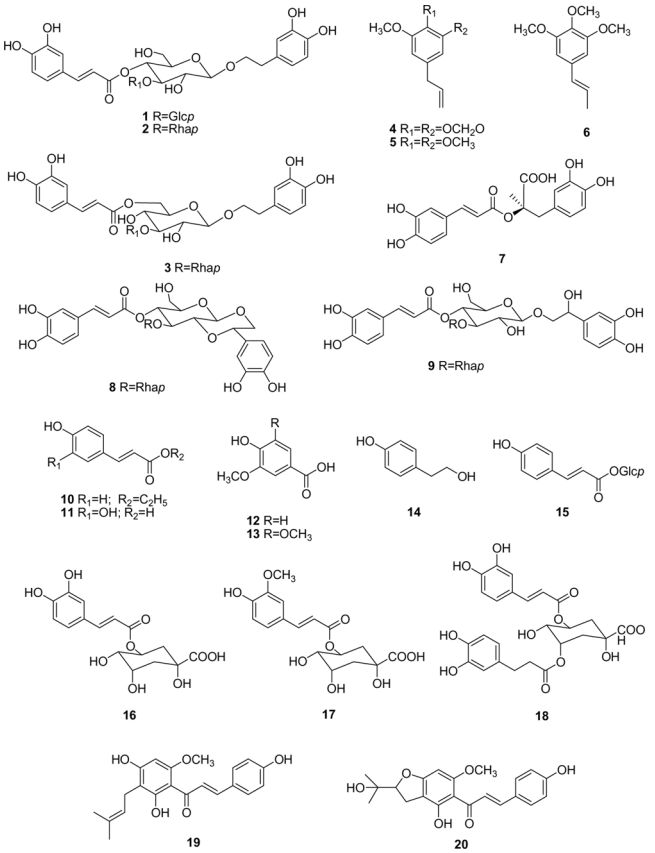


FIGURE 2 Chemical structures of simple phenolics separated or fractionated by CPC.

Anthocyanins

CPC application to the separation of anthocyanins was initially performed using a gradient elution with an organic phase of ethyl acetate-*n*-butanol-water (77:15:8, v/v) and then, an organic phase of ethyl acetate-*n*-butanol-water (40:46:14, v/v). The stationary phase was the aqueous phase of ethyl acetate-*n*-butanol-water (5:5:90, v/v) acidified with TFA (pH 1–3). With this CPC method, seven anthocyanins [malvidin 3-glucoside (**25**), peonidin 3-glucoside (**26**), petunidin 3-glucoside (**27**), cyanidin 3-glucoside (**28**), cyannidin 3-rutinoside (**29**), delphinidin 3-glucoside (**30**), delphinidin 3-rutinoside (**31**)] were purified from Champagne vintage by-product (*Vitis vinifera*) and blackcurrant (*Ribes nigrum*).[21] Chemical structures and CPC experimental conditions were summarized in Figure 5, Figure 6, and Table 1, respectively.

TABLE 1 Phenolic Constituents Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Simple phenolic constituents						
Plantamajoside (1) Acetoside (2) Isoacteoside (3)	*Plantago asiatica* (Plantaginaceae)	EtOAc-BuOH-EtOH-H_2O (5:5:1:10)	Descending	Isocratic	S	[13]
Myristicin (4)	*Diplolophium buchanani* (Umbelliferae)	(i) Hex-EtOAc-MeOH-H_2O (10:5:5:1) (ii) Hex-M*t*BE-MeCN (5:1:5)	Ascending Ascending	Isocratic	S	[14]
Elemicin (5) Trans-isoelemicin (6)	*Diplolophium buchanani* (Umbelliferae)	Hex-EtOAc-MeOH-H_2O (10:5:5:1)	Ascending	Isocratic	F	[14]
Rosemarinic acid (7)	*Lavandula vera* (Lamiaceae)	$CHCl_3$-BuOH-H_2O (9:2:9)	Ascending	Ion-exchage	S	[15]
Oraposide (8) Verbascoside (9)	*Orobanche rapum* (Orobanchaceae)	(i) EtOAc-acetone-H_2O (8.7:3.9:87.4) (ii) Hep-MeCN-MeOH (6:3:1)	Descending Ascending	Isocratic	S	[16]
Trans-p-ethylcoumarate (10)	Red wine	Hex-EtOAc-MeOH-H_2O (4:5:3:3)	Ascending	Isocratic	F	[17]
Caffeic acid (11) Vanilic acid (12) Syringic acid (13), Tyrosol (14) Trans-p-coumaroyl 6″-glucoside (15)	Red wine	(i) Hex-EtOAc-MeOH-H_2O (4:5:3:3) (ii) Hex-EtOAc-MeOH-H_2O (1:8:2:7)	Descending Ascending	Isocratic	F	[17]
5-Caffeoylquinic acid (16) 5-Feruloylquinic acid (17) 3,4-Dicaffeoylquinic acid (18)	*Coffea arabica* (Rubiaceae)	Hex-EtOAc-0.01 M phosphate-buffer/LiCl (32:68:100) Hex-EtOAc-pH 2.5 $(NH_4)_2SO_4$ (30:70:100; v/v)	Descending	Salting-out gradient	S	[18]
Xanthohumol (19) (*E*)-2″-(2‴-hydroxy-isopropyl)-dihydrofurano[2″,3″:4′,3′]-2′,4-dihydroxy-6′-methoxychalcone (20)	*Humulus lupulus* (Cannabaceae)	Hep-Tol-Me_2CO-water (24.8:2.8:50:22.4)	Ascending	Isocratic	S	[19]
Lignans						
Arctigenin (21) Matairesinol (22)	*Forsythia koreana* (Oleaceae)	Hex-EtOAc-MeOH-H_2O (5:5:5:5)	Descending	Isocratic	S	[20]

Coumarins						
Oxypeucedanin (23) Oxypeucedanin hydrate (24)	*Diplolophium buchanani* (Umbelliferae)	Hex-EtOAc-MeOH-H_2O (10:5:5:1)	Ascending	Isocratic	S	[14]
Anthocyanins						
Malvidin 3-glucoside (25) Peonidin 3-glucoside (26) Petunidin 3-glucoside (27) Cyanidin 3-glucoside (28) Cyannidin 3-rutinoside (29) Delphinidin 3-glucoside (30) Delphinidin 3-rutinoside (31)	*Vitis vinifera* (Vitaceae) *Ribes nigrum* (Grossulariaceae)	EtOAc-BuOH-H_2O (acidified by TFA (pH 1–3) (5:5:90 LP as a SP) (77:15:8 to 40:46:14 UP as a MP)	Ascending	Isocratic & Gradient	S	[21]
Flavonoids						
Hispidulin (32)	*Clerodendrum petasites* (Lamiaceae)	$CHCl_3$-MeOH-IPA-H_2O (4.5:6:1:4)	Descending	Isocratic	S	[22]
5,2′,4′-Trihydroxy-7-methoxy-3′-methyl isoflavone (33) 5,4′-Dihydroxy-7,2′-dimethoxy-3′-methyl isoflavone (34) 5,4′-Dihydroxy-7,2′-dimethoxyisoflavone (35) 5,7-4′-Trihydroxy-8-(2-hydroxy-3-methyl butenyl)flavanone (36) Tricin (37) Cajanin (38) Parvisoflavone-A and B (39–40)	*Eriophorum scheuchzeri* (Cyperaceae)	Hep-EtOAc-MeOH-H_2O (6:5:6:5)	Descending	Isocratic	S	[23]
Quercetin (41) Myricetin (42) Taxifoliol (43)	Red wine	Hex-EtOAc-MeOH-H_2O (4:5:3:3)	Ascending	Isocratic	F	[17]
Astilbin (44) Dihydromyricetin-3-rhamnoside (45) Isorhamnetin (46) Catechin (47) Epicatechin (48) Isoquercitrin (49)	Red wine	(i) Hex-EtOAc-MeOH-H_2O (4:5:3:3) (ii) Hex-EtOAc-MeOH-H_2O (1:8:2:7)	Descending Ascending	Isocratic Isocratic	F	[17]

(*Continued*)

TABLE 1 Continued

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Myricetin 3-glucoside (50)						
Naringin (51)	*Poncirus trifoliate* (Rutaceae)	EtOAc-MeCN-H_2O (3:2:5)	Descending	Isocratic	S	[24]
Neoponcirin (52)						
Poncirin (53)						
6,8-Diprenylnaringenin (54)	*Humulus lupulus* (Cannabaceae)	Hep-Toluene-acetone-water (24.8:2.8:50:22.4)	Ascending	Isocratic	S	[19]
Isoxanthohumol (55)						
Isoquercitrin (49)	Golden Apple	EtOAc-EtOH-H2O (4.5:1:4.5)	Descending	Isocratic	S	[25]
peltatoside (56)						
Rutin (57)						
Nicotiflorin (58)						
Hyperoside (59)						
Guajaverin (60)						
Quercitrin (61)						
Avicularin (62)						
Asgragalin (63)						
Stilbenes						
(+)-Ampelopsin A (64)	*Vitis vinifera* (Vitaceae)	Hep-EtOAc-MeOH-H_2O (1:2:1:2)	Ascending	Isocratic	S	[26]
E-Piceatannol (65)						
(+)-e-*E*-viniferin (66)	*Vitis vinifera* (Vitaceae)	(i) Hep-EtOAc-MeOH-H_2O (1:2:1:2)	Ascending Descending	Isocratic	F&S	[26]
E-vitisin C (67)		(ii) Hep-EtOAc-MeOH-H_2O (5:6:5:6)				
Cis-Resveratrol (68)	*Vitis vinifera* (Vitaceae)	(i) Hep-EtOAc-MeOH-H_2O (1:2:1:2)	Ascending	Isocratic	F	[17,26]
		(ii) Hep-EtOAc-MeOH-H_2O (5:6:5:6)				
Trans-resveratrol (69)	Red wine	Hex-EtOAc-EtOH-H_2O (4:5:3:3)	Ascending	Isocratic	F	[17]
Pallidol (70)	Red wine	(i) Hex-EtOAc-MeOH-H_2O (4:5:3:3)	Descending Ascending	Isocratic	F	[17]
Parthenocissin (71)						

Cis-piceid (72)		(ii) Hex-EtOAc-MeOH-H_2O(1:8:2:7)				
Trans-Piceid (73)						
(+)-Ampelopsin A (64)	*Vitis thunbergii* (Vitaceae)	$CHCl_3$-MeOH-H_2O (6:7:4)	Descening	Isocratic	F	[27]
(+)-Vitisin A (74)						
(−)-Vitisin B (75)						
Flavonolignans						
Silycristin (76)	*Silybum marianum* (Asteraceae)	Hep-EtOAc-MeOH-H_2O (1:4:3:4)	Descending	Isocratic	F&S	[28]
Silydianin (77)						
Mixture of silibinin and isosilybinin (78)						
Xanthones						
2-Hydroxy-1-methoxyxanthone (79)	*Kielmeyera coriacea* (Clusiaceae)	pet-ether-EtOAc-MeOH -H_2O (10:5:5:1)	Ascending	Isocratic	F	[29]
3-Hydroxy-2,4-dimethoxyxanthone (80)						
4-Hydroxy-2,3-dimethoxyxanthone (81)						
1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (82)						
1,3,7-Trihydroxy-2-(3-hydroxy-3-methylbutyl)-xanthone (83)						
1,3,5-Trihydroxy-2-(3-methylbut-2-enyl)-xanthone (84)						
Kielcorin (85)						
Osajaxanthone (86)						
Magniferin (87)	*Anemarrhena sphodeloides* (Liliaceae)	EtOAc-IPA- H_2O (3:2:5)	Descending	Isocratic	S	[30]
Corymbiferin 1-*O*-*β*-D-glucopyranoside (88)	*Gentianella amarelle* (Gentianaceae)	$CHCl_3$-MeOH-H_2O (45:30:25)	Descending	Isocratic	S	[31]
Triptexanthoside C (89)	*Gentianella amarelle* (Gentianaceae)	(i) $CHCl_3$-MeOH-H_2O (45:30:25) (ii) $CHCl_3$-MeOH-H_2O (45:22:33)	Descending	Isocratic	F&S	[31]
Veratriloside (90)						
Corymbiferin 3-*O*-*β*-D-glucopyranoside (91)	*Gentianella amarelle* (Gentianaceae)	(i) $CHCl_3$-MeOH-H_2O (45:30:25) (ii) CyHex-EtOAc-MeOH- H_2O (1:5:1:5, 1:3:1:3, 5:5:3:3)	Descending Ascending	Isocratic	F&S	[31]
Norswertianolin (92)	*Gentianella amarelle* (Gentianaceae)	$CHCl_3$-MeOH-H_2O (45:30:25)	Descending	Isocratic	F	[31]
Swertianolin (93)	*Gentianella amarelle* (Gentianaceae)	(i) $CHCl_3$-MeOH-H_2O (45:30:25) (ii) CyHex-EtOAc-MeOH-H_2O (1:5:1:5)	Descending Ascending	Isocratic	F&S	[31]

(*Continued*)

TABLE 1 Continued

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Swertiabisxanthone-I 8′-*O*-b-D-glucopyranoside (94)	*Gentianella amarelle* (Gentianaceae)	$CHCl_3$-MeOH-H_2O (45:30:25)	Descending	Isocratic	F	[31]
Swertiabisxanthone-I (95) Bellidin (96) Bellidifolin (97)	*Gentianella amarelle* (Gentianaceae)	(i) $CHCl_3$-MeOH-H_2O (45:30:25) (ii) CyHex-EtOAc-MeOH-H_2O (5:5:3:3)	Descending	Isocratic	F	[31]
3-Isomangostin (98) Gartanine (99) α-Mangostin (100) Desoxygartanine (101) β-Mangostin (102) 9-Hydroxycalabaxanthone (103)	*Garcina mangostana* (Clusiaceae)	Hep-EtOAc-MeOH-H_2O (2:1:2:1)	Descending	Isocratic	F&S	[32]

[a]BuOH: *n*-butanol; $CHCl_3$: chloroform; CyHex: cyclohexane; EtOAc: ethyl acetate; H_2O: water; Hep: *n*-heptane; Hex: *n*-hexane; IPA: isopropyl alcohol; Me_2CO: acetone; MeCN: acetonitrile; MeOH: methanol; M*t*BE: methyl *tert*-butyl ether; pet-ether: petroleum ether; TFA: trifluoroacetic acid; Tol: toluene; LP: lower phase; UP: upper phase; SP: stationary phase; MP: mobile phase.

[b]CPC was used for separation (S) or fractionation (F).

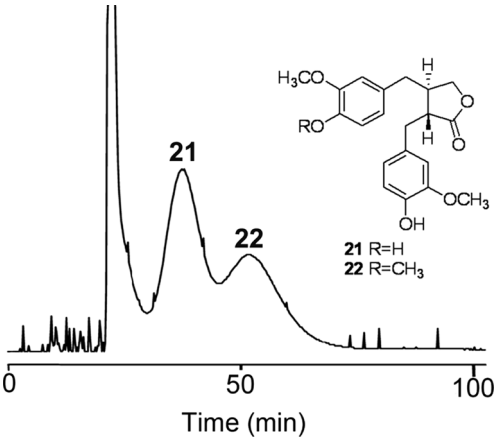
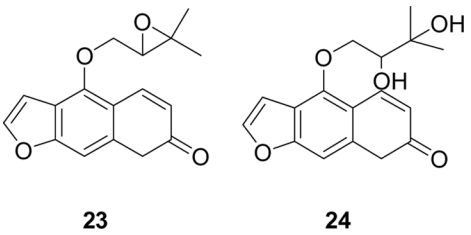


FIGURE 3 Preparative CPC separation of enriched lignan fraction from *Forsythia koreana*. CPC solvent system: *n*-hexane-ethyl acetate-methanol-water (1:1:1:1, v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow rate: 2 mL/min; rotation speed: 800 rpm; backpressure: 31.4 bar; sample: 150 mg dissolved in 5 mL stationary phase of the solvent system. Peaks 21: matairesinol, 22: arctigenin. (Adapted from J. Sep. Sci. 2006, *29*, 656–659.)



23 **24**

FIGURE 4 Chemical structures of coumarins separated or fractionated by CPC.

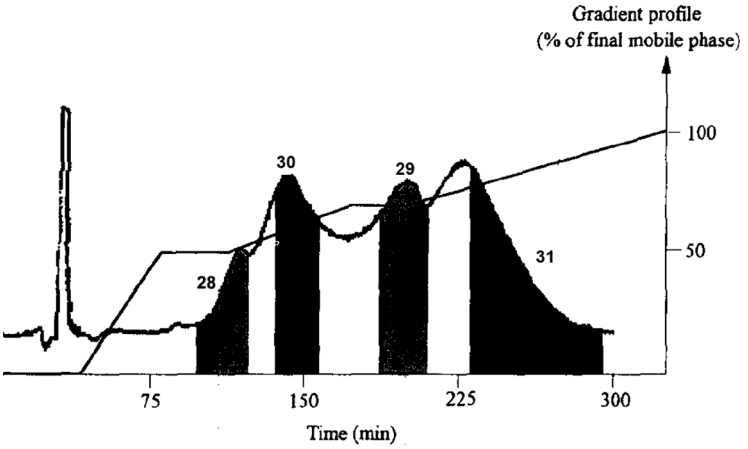
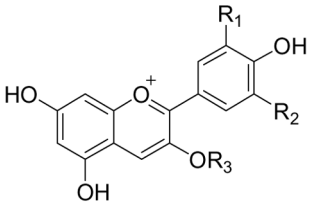


FIGURE 5 CPC separation of four anthocyanins from blackcurrant in a laboratory-scale gradient run. The flow-rate was 3 mL/min at 1400 rpm. (Adapted from *J. Chromatogr. A* 1997, *763*, 345–352.)



25 Malvidin 3-glucoside: R_1=OCH_3; R_2=OCH_3 R_3=Glc*p*
26 Peonidin 3-glucoside: R_1=OCH_3; R_2=H; R_3=Glc*p*
27 Petunidin 3-glucoside: R_1=OCH_3; R_2=OH; R_3=Glc*p*
28 Cyanidin 3-glucoside: R_1=OH; R_2=H; R_3=Glc*p*
29 Cyanidin 3-rutinoside: R_1=OH; R_2=H; R_3=Rutinose
30 Delphinidin 3-glucoside: R_1=OH; R_2=OH; R_3=Glc*p*
31 Delphinidin 3-rutinoside: R_1=OH; R_2=OH; R_3=Rutinose

FIGURE 6 Chemical structures of anthocyanins separated or fractionated by CPC.

Flavonoids

CPC was employed to isolate a bronchodilator flavonoid from *Clerodendrum petasites* (Lamiaceae). Partition with ethyl acetate-methanol-water (43:22:35, v/v) of the crude ethanol extract of *C. petasites* and consecutive redissolvation of the organic residue in 10% EtOH gave an ethanol-insoluble extract. This extract was separated by a CPC utilizing two-phase solvent system composed of chloroform-methanol-*n*-propanol-water (45:60:10:40, v/v, descending mode) and gave hispidulin (**32**).[22]

Activity-guided isolation of antifungal, antibacterial, and antioxidant compounds from the dichloromethane extract of the aerial parts of *Eriophorum scheuchzeri* led to the isolation of eight flavonoids (**33–40**) including three new isoflavones [5,2′,4′-trihydroxy-7-methoxy-3′-methylisoflavone (**33**), 5,4′-dihydroxy-7,2′-dimethoxy-3′-methylisoflavone (**34**), 5,4′-dihydroxy-7, 2′-dimethoxyisoflavone (**35**)], and a new flavanone [5,7-4′-trihydroxy-8-(2-hydroxy-3-methylbutenyl)flavanone (**36**)]. The fractionation or isolation of active materials was performed by CPC using a two-phase system composed of *n*-heptane-ethyl acetate-methanol-water (6:5:6:5, v/v, descending mode), and further purification step was carried out by HPLC to obtain these compounds.[23]

CPC was applied to separate flavonoids from the red wine extract. A two-phase solvent of *n*-hexane-ethyl acetate-ethanol-water (4:5:3:3, v/v, ascending mode) was used to give four fractions and semi-preparative HPLC of fourth fraction afforded three flavonoids, quercetin (**41**), myricetin (**42**) and taxifoliol (**43**). A fraction obtained by CPC using *n*-hexane-ethyl acetate-ethanol-water (4:5:3:3, v/v, descending mode) was submitted to a second run of CPC with solvents in the ratio of 7:2:1:8 (v/v, ascending mode) to give six fractions and these fractions were further purified by semi-preparative HPLC to give two new compounds, astilbin (**44**), dihydromyricetin-3-rhamnoside (**45**), and five known compounds,

isorhamnetin (**46**), catechin (**47**), epicatechin (**48**), quercetin 3-glucoside (**49**), and myricetin 3-glucoside (**50**).[17]

Three flavanone glycosides, naringin (50.0 mg **51**), neoponcirin (16.8 mg **52**), and poncirin (71.9 mg **53**), were separated from the crude extract (524 mg) of *Poncirus trifoliata* (Rutaceae) using a preparative CPC run with a two-phase solvent system [ethyl acetate-acetonitrile-water (3:2:5, v/v, descending mode)] (Figure 7).[24]

6,8-Diprenylnaringenin (1 mg **54**) and isoxanthohumol (50 mg **55**) was purified from the extract (1 g) of *Humulus lupulus* by normal-phase FCPC. A two-phase solvent system composed of *n*-heptane-toluene-acetone-water system (24.8:2.8:50:22.4, v/v, ascending mode) was used for the optimal separation.[19]

The CPC separation of nine flavonoids from the extract of golden apple, isoquercitrin (**49**), peltatoside (**56**), rutin (**57**), nicotiflorin (**58**), hyperoside (**59**), guajaverin (**60**), quercitrin (**61**), avicularin (**62**), and asgragalin (**63**), was fulfilled. This procedure was performed on CPC-ESIMS instrument equipped with an active flow-splitter device.[25] An ethyl acetate-ethanol-water system (4.5:1:4.5, v/v, descending mode) was used for the optimal CPC experiment. Chemical structures and CPC experimental conditions were mentioned in Figure 8 and Table 1, respectively.

Stilbenes

Two step CPC separation methyl *tert*-butyl ether (M*t*BE) extract of the stem of *Vitis vinifera* afforded five stilbenes, (+)-ampelopsin A (**64**), *E*-piceatannol (**65**), (+) *E*-(ε)-viniferin (**66**), vitisin C (**67**), and *cis*-resveratrol (**68**). In the first step, reversed-phase CPC mode using a solvent system of

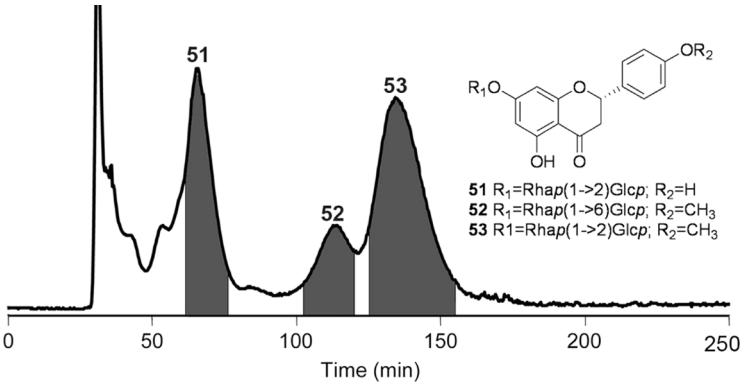


FIGURE 7 Preparative CPC separation of the crude extract from *Poncirus trifoliata*. CPC solvent system: ethyl acetate-acetonitrile-water (3:2:5, v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow rate: 2 mL/min; rotation speed: 1200 rpm; backpressure: 42 bar; sample: 524 mg dissolved in 5 mL of 1:1 miture of lower and upper phase. Peaks 51: naringin ; 52: neoponcirin; 53: poncirin. (Adapted from *J. Sep. Sci.* 2007, *30*, 2693–2697.)

n-heptane-ethyl acetate-methanol-water (1:2:1:2, v/v) was used to fractionate the M*t*BE extract into 132 fractions. From fractions 22–29, compound **65** was obtained and from fractions 89–104, (+)-ampelopsin A (**64**). The second CPC separation on combined fractions 12–15, using *n*-heptane-ethyl acetate-methanol-water (5:6:5:6, v/v), afforded **66** and **67** in the descending mode while *cis*-resveratrol (**68**) in the ascending mode (Figure 9).[26]

Two new resveratrol dimers (**70** and **71**) and four stilbenes (**68, 69, 72** and **73**) were isolated from red wine extract with the aid of CPC and semi-preparative HPLC. A normal-phase CPC (Sanki, model LLB-M) using *n*-hexane-ethyl acetate-ethanol-water (4:5:3:3, v/v) system gave a mixture of *cis*-resveratrol (**68**) and *trans*-resveratrol (**69**), and this mixture was purified by semi-preparative HPLC. Two-step CPC run was used to isolate compounds **70–73**. In the first run of CPC, *n*-hexane-ethyl acetate-ethanol-water system (4:5:3:3, v/v, descending mode) gave a fraction containing **70–73**,

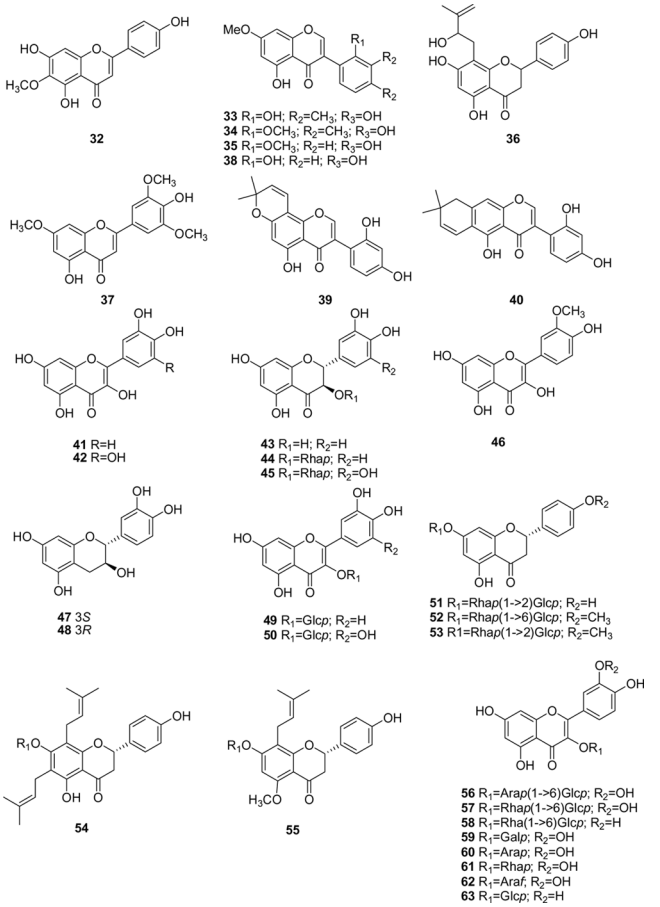


FIGURE 8 Chemical structures of flavonoids separated or fractionated by CPC.

and the second run of normal-phase CPC was done using the same solvents with the ratio of 1:8:2:7 (v/v) to give six major fraction. Then, these fractions were chromatographed on semi-preparative HPLC to give pallidol (**70**), parthenocissin (**71**), *cis*-piceid (**72**), *trans*-piceid (**73**).[17]

The crude methanol extract (1 g) from *Vitis thunbergii* var. *taiwaniana* (Vitaceae) was subjected to CPC to give oligostilbenes fractions and then, subsequent Sephadex LH-20 gel filtration for each CPC peak fraction yielded three oligostilbenes, (+)-ampelopsin A (**64**), (+)-vitisin A (**74**), and (-)-vitisin A (**75**). A two-phase solvent system composed of chloroform-methanol-water (6:7:4, v/v) was used and the lower phase of it was eluted as a mobile phase.[27] Chemical structures and CPC experimental conditions were mentioned in Figure 10 and Table 1, respectively.

Flavonolignan

The separation of flavonolignans, known as silymarin, from milk thistle (*Silybum marianum*) was conducted on fast centrifugal partition chromatography (FCPC) with a two-phase solvent system consisting of *n*-heptane-ethyl acetate-methanol-water (1:4:3:4, v/v, descending mode). This separation work gave impure silychristin (**76**) with a purity of 70.2%, and silydianin (**77**) of 93.7%, and a mixture of silybinin and isosilybinin (**78**). The mixture of silybinin and isosilybinin was not separated in this study (Figure 11 and Table 1).[28]

Xanthones

Eight xanthones (**79–86**) were obtained by CPC using petroleum ether-ethyl acetate-methanol water system (10:5:5:1, v/v, ascending mode),

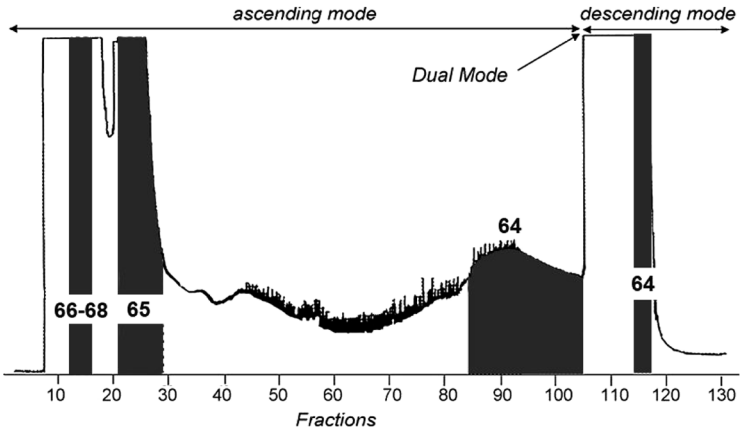


FIGURE 9 CPC chromatogram of M*t*BE extract from *Vitis vinifera*. CPC conditions: rotation speed, 1000 rpm; solvent system, *n*-heptane-ethyl acetate-methanol-water (1:2:1:2; v/v). Peaks 64: (+)-ampelopsin A; 65: *E*-piceatannol: 66: (+)-*E*-(ε)-viniferin; 67: *E*-vitisin C: 68: *E*-resveratrol. (Adapted from *J. Chromatogr. B* 2009, *877*, 1000–1004.)

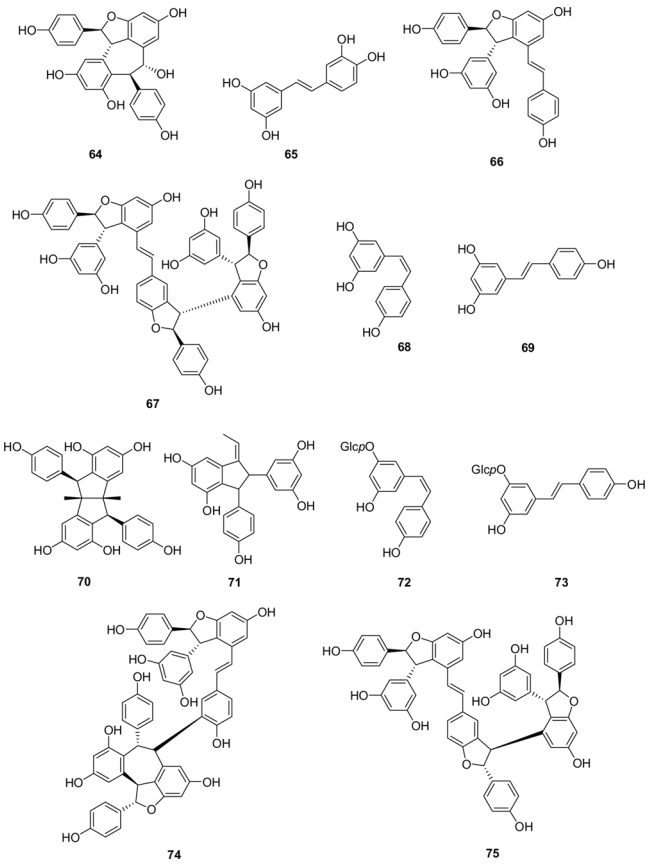


FIGURE 10 Chemical structures of stilbenes separated or fractionated by CPC.

and further purification of Sephadex LH-20 gel filtration and recrystallization. From the dichloromethane-soluble extract (500 mg) of *Kielmeyera coriacea* (Clusiaceae), 2-hydroxy-1-methoxyxanthone (**79**), 3-hydroxy-2,4-dimethoxyxanthone (**80**), 4-hydroxy-2,3-dimethoxyxanthone (**81**), 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**82**), 1,3,7-trihydroxy-2-(3-hydroxy-3-methylbutyl)-xanthone (**83**), 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone (**84**), kielcorin (**85**), and osajaxanthone (**86**) were successfully isolated.[29]

Magniferin (22.5 mg, **87**), a xanthone *C*-glycoside, was purified by reversed-phase CPC run using a solvent system of ethyl acetate-isopropyl alcohol-water (3:2:5, v/v) from the crude methanol extract (957 mg) of *Anemarrhena asphodeloides* (Liliaceae).[30]

Preparative CPC, semi-preparative CPC and Sephadex LH-20 gel filtration were used to isolate 10 xanthone derivatives (**88-97**) from *Gentianella amarelle* (Gentianaceae). The *n*-butanol soluble extract (3 g) of *G. amarelle* was chromatographed on normal-phase CPC (9 fractions) and reversed-phase

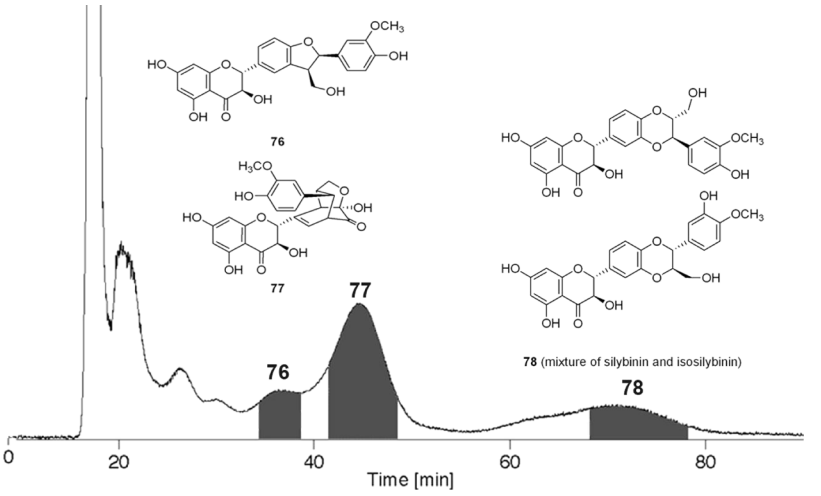


FIGURE 11 FCPC trace of pressurized hot water milk thistle seed extract. CPC conditions: rotational speed, 1300 rpm; solvent system, *n*-heptane-ethyl acetate-methanol-water (1:4:3:4, v/v, descending mode); flow rate: 4 mL/min. Peaks 76: sliycristin ; 77: silydianin; 78: mixture of silibinin and isosilybinin. (Adapted from J. Liq. Chromatogr. Relat. Technol. 2008, *31*, 3001–3011.)

CPC (3 fractions) to give totally 12 fractions using chloroform-methanol-water system (45:30:25, v/v). Crystallization in the seventh fraction gave corymbiferin 1-*O*-glucoside (**88**). Fractions 4 and 5 were further separated by semi-preparative CPC utilizing chloroform-methanol-water system (45:22:33, v/v, descending mode) to yield triptexanthoside C (**89**) and veratriloside (**90**). A new xanthone glycoside, corymbiferin 3-*O*-*β*-D-glucopyranoside (**91**) was obtained by CPC using cyclohexane-ethyl acetate-methanol-water systems (1:5:1:5, 1:3:1:3, 5:5:3:3, v/v) from the other fractions. Norswertianolin (**92**) and a new compound, swertiabisxanthone-I 8′-*O*-*β*-D-glucopyranoside (**94**), were purified by further Sephadex LH-20 gel filtration on fraction 10. CPC separation for fraction 8, using cyclohexane-ethyl acetate-methanol-water system (1:5:1:5, v/v, ascending), yielded swertianolin (**93**). The diethyl ether-soluble extract (1 g) was loaded on CPC using cyclohexane-ethyl acetate-methanol-water (5:5:3:3, v/v, descending) to give four factions, and these four fractions were purified successively by Sephadex LH-20 gel filtration and crystallization method to provide swertiabisxanthone-I (**95**), bellidin (**96**), and bellidifolin (**97**).[31]

Simultaneous separation and identification of six xanthones, 3-isomangostin (**98**), gartanin (**99**), α-mangostin (**100**), deoxygartanin (**101**), *β*-mangostin (**102**), 9-hydroxycalabaxanthone (**103**) from mangosteen (*Garcinia mangostana*, Clusiaceae) was fulfilled by aid of CPC-ESIMS instrument using *n*-heptane-ethyl acetate-methanol-water system (2:1:2:1, v/v, descending mode). A variable flow splitter and an additional stream

of ethanol/1 M ammonium acetate (95:5, v/v) were adopted to facilitate ionization of all analytes and dual mode CPC reduced analysis time and solvent consumption (Figure 12).[32] Chemical structures and CPC experimental conditions were mentioned in Figure 13 and Table 1, respectively.

Monoterpenes

An iridoid glycoside, geniposide (**104**), was purified by CPC using ethyl acetate-isopropyl alcohol-water (3:2:5, v/v, descending mode) from *Gardenia jasminoides* (Rubiaceae). From five hundred milligrams of 80% methanolic extract, 56.2 mg of geniposide was obtained with over 95% purity (Figure 14 and Table 2).[33]

Sesquiterpenes

Three sesquiterpene lactones (**105–107**) were obtained by one step-CPC strategy. The 22.5 g of chloroform soluble extract from *Xanthium macrocarpum* (Asteraceae) was chromatographed on Kromaton FCPC column that was constituted by two rotors with 40 circular partition disks each (total volume 5160 mL). The upper phase of *n*-hexanes-ethyl acetate-methanol-water system (1:1:1:1, v/v) was used as mobile phase; thus, the flow mode of CPC was descending with the rotational speed at 800 rpm and flow rate at 60 mL/min. As a result, xanthathin (1664 mg

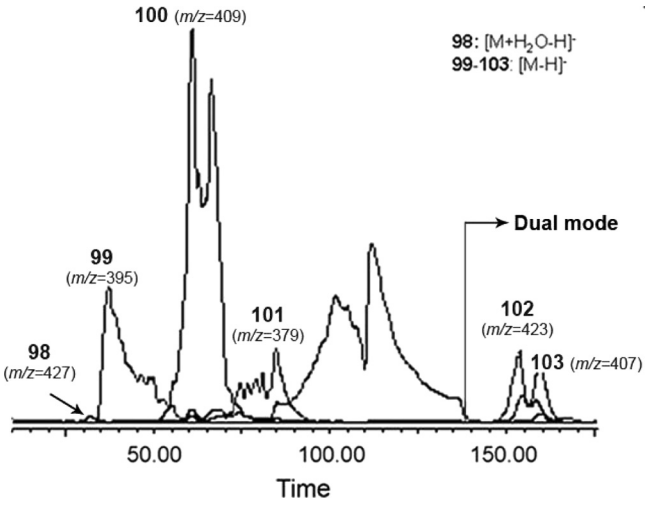


FIGURE 12 CPC-ESI-MS with a variable flow splitter smoothed chromatograms of the crude extract of *mangosteen*, SIM $m/z = 379$, 395, 407, 409, 423, 427 (CPC: column 50 mL, 2000 rpm, 1 mL/min, 10 mg injected). Peaks 98: 3-isomangostin; 99: gartanine; 100: α-mangostin; 101: desoxygartanine; 102: β-mangostin; 103: 9-hydroxycalabaxanthone. (Adapted from *J. Chromatogr. A* 2009, *1216*, 1390–1394.)

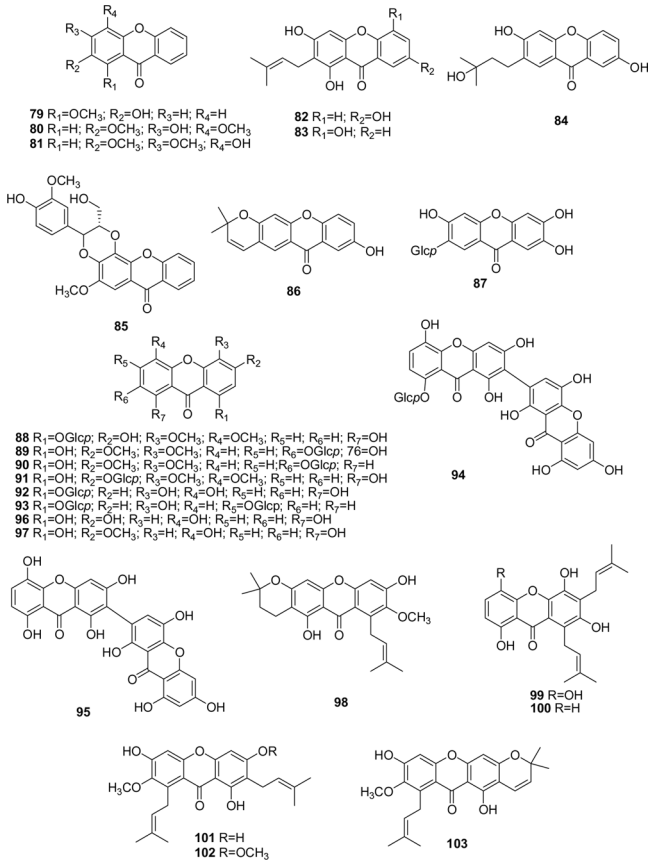


FIGURE 13 Chemical structures of xanthones separated or fractionated by CPC.

105), 4-*epi*-xanthanol (522 mg **106**), and 4-*epi*-isoxanthanol (713 mg **107**) could be obtained and these results were superior to those of silica gel column chromatography method (Figure 15 and Table 3).[34]

Diterpenes

The *n*-butanol soluble extract of *Juniperus communis* (Cupressaceae) was submitted to CPC instrument using a chloroform-methanol-isopropyl alcohol-water system (5:6:1:4, dual mode) to afford twelve fractions. Each fraction was monitored by HPLC-UV and further purified by MPLC and HPLC. By this method, a new labdane diterpene, 15,16-epoxy-12-hydroxy-8(17),13(16),14-labdatrien-19-oic acid (**108**) and four known compounds, imbricatolic acid (**109**), isocupressic acid (**110**), sandaracopimaric acid (**111**), and isopimaric acid (**112**) were obtained.[35]

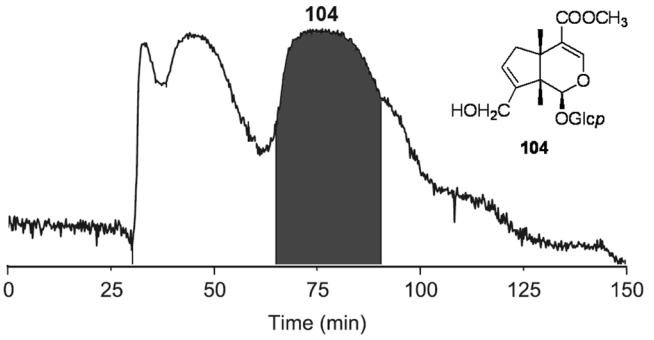


FIGURE 14 CPC chromatogram of 80% methanolic extract of *Gardenia* fruits. CPC condition: rotational speed, 1200 rpm; solvent system, ethyl acetate-isopropanol-water (3:2:5, v/v, descending mode); flow rate: 2 mL/min. Peak 104: geniposide. (Adapted from *Phytochem. Anal.* 2007, *18*, 115–117.)

TABLE 2 Monoterpene Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Geniposide **(104)**	*Gardenia jasminoides* (Rubiaceae)	EtOAc-IPA-H_2O (3:2:5)	Descending	Isocratic	S	[33]

[a]EtOAc: ethyl acetate; H_2O: water; IPA: Isopropyl alcohol.
[b]CPC was used for separation (S) or fractionation (F).

CPC application on the separation of salvinorin A (**113**), a potent naturally occurring non-nitrogenous *k*-opioid selective agonist, was carried out by using a two-phase solvent system composed of *n*-hexane–dichloromethane–methanol–water (8:8:9:2, v/v, descending mode), further silica gel column chromatography (*n*-hexane-ethyl acetate, 1:1, v/v), and recrystallization method. The MeOH extract (2.5 g) of *Salvia divinorum* (Lamiaceae) gave 100 mg of salvinorin A (Figure 16).[36] Chemical structures and CPC experimental conditions were mentioned in Figure 17 and Table 4, respectively.

Triterpenes

Activity-guided CPC were employed to isolate trichomonas inhibitory compound from *Cussonia holstii* (Araliaceae). An active fraction (222 mg), obtained by silica gel column chromatography of dichloromethane extract (727 mg) from *C. holstii*, was subjected to CPC using *n*-hexane-ethyl acetate-methanol-water system (7:8:5:3, v/v, descending mode). After screening of anti-trichomonas activities of CPC peak fractions, the most active fraction (71.8 mg) was chromatographed on CPC using *n*-hexane-ethyl

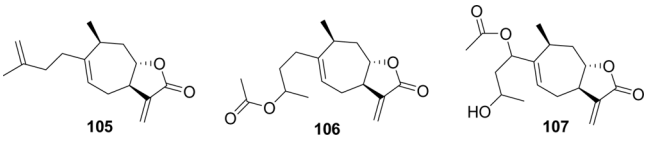


FIGURE 15 Chemical structures of sesquiterpenes separated or fractionated by CPC.

TABLE 3 Sesquiterpenes Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Xanthathin (105) 4-*Epi*-xanthanol (106) 4-*Epi*-isoxanthanol (107)	*Xanthium macrocarpum* (Asteraceae)	Hex-EtOAc-MeOH-H_2O(1:1:1:1)	Descending	Isocratic	S	[34]

[a]EtOAc: ethylacetate; Hex: *n*-hexane; H_2O: water; MeOH: methanol.
[b]CPC was used for separation (S) or fractionation (F).

acetate-methanol-water system (8:7:5:3, v/v, descending mode) to afford hederagenin (37.6 mg **114**).[37]

Two quinonemethide triterpenes, pristimerin (**115**), and netzahuaolcoyene (**116**), were purified from *Maytenus ilicifolia* (Celastraceae) by FCPC run. Conventional column chromatography methods such as flash chromatography or preparative TLC were not feasible to isolate these two compounds. A mixture (200–300 mg) of these compounds was chromatographed on CPC using *n*-heptane-ethyl acetate-methanol-water system

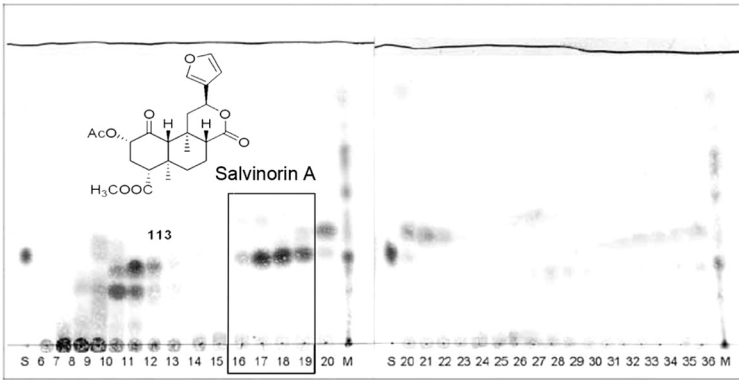
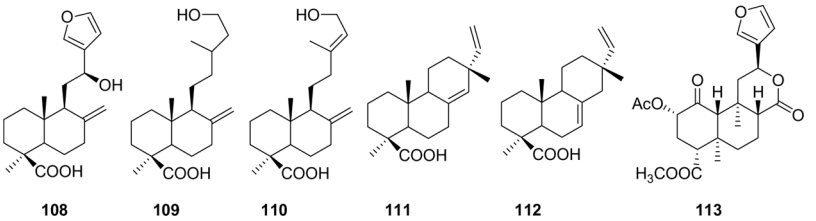


FIGURE 16 TLC monitoring of fractions from CPC separation of the methanol extract of *Salvia divinorum* leaves. Spots were detected by heating after spraying with vanillin-phosphoric acid reagent. (Adapted from *J. Liq. Chromatogr. Relat. Technol.* 2007, *30*, 1105–1114.)



108 109 110 111 112 113

FIGURE 17 Chemical structures of diterpenes separated or fractionated by CPC.

(8:1:6:1, v/v, ascending mode), and two or three repeated CPC runs afforded pristimerin (**115**) and netzahuaolcoyene (**116**).[38]

Prostane-type triterpenes, alisol B (**117**) and alisol B 23-actate (**118**), were separated from *Alisma orientale* by CPC-ELSD technique. Alisol B (37.5 mg **117**) and alisol B 23-actate (205.3 mg **118**) were obtained from the chloroform-soluble extract (870 mg) using *n*-hexane-ethyl acetate-methanol-water system (10:2:10:7, v/v, ascending mode) (Figure 18).[39] Chemical structures and CPC experimental conditions were mentioned in Figure 19 and Table 5, respectively.

Saponins

Nine triterpenoid saponins (**119–127**) From *Zizyphus lotus* (Rhamnaceae), jujuboside A (**119**) and three new compounds, jujuboside C (**120**), lotoside I (**121**), and lotoside II (**122**) were separated by one-step CPC fractionation using an ethyl acetate-*n*-butanol-water system. The lower phase of ethyl acetate-*n*-butanol-water system (1:1:18, v/v) was used as a stationary phase, and the upper phase of ethyl acetate-*n*-butanol-water system (95:1:4 to 20:23:7, v/v) used as a gradient mobile phase. Furthermore, four new

TABLE 4 Diterpenes Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
15,16-Epoxy-12-hydroxy-8(17), 13(16),14-labdatrien-19-oic acid (108)	*Juniperus communis* (Cupressaceae)	$CHCl_3$-MeOH-IPA-H_2O (5:6:1:4)	Dual mode	Isocratic	F	[35]
Imbricatolic acid (109)						
Isocupressic acid (110)						
Sandaracopimaric acid (111)						
Isopimaric acid (112)						
Salvinorin A (113)	*Salvia divinorum* (Lamiaceae)	Hex-CH_2Cl_2-MeOH-H_2O (8:8:9:2)	Descending	Isocratic	F	[36]

[a]BuOH: *n*-butanol; $CHCl_3$: chloroform; CH_2Cl_2: methylene chloride; H_2O: water; Hex: *n*-hexane; IPA: Isopropyl alcohol; MeOH: methanol.

[b]CPC was used for separation (S) or fractionation (F).

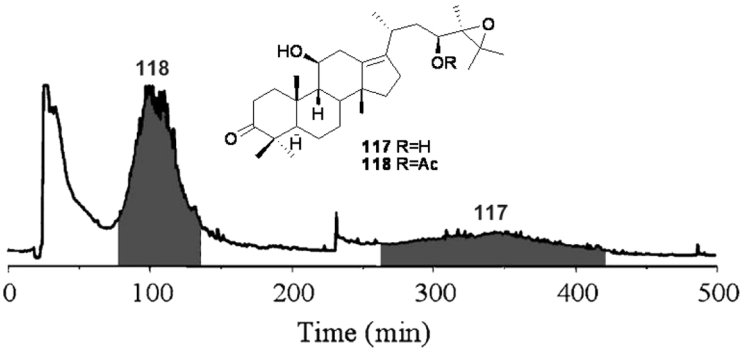


FIGURE 18 A CPC separation of chloroform soluble extract of *Alisma orientale*. CPC conditions: rotational speed, 800 rpm; solvent system, *n*-hexane-ethyl acetate-methanol-water system (10:2:10:7,v/v, ascending mode); flow rate: 2 m:/min. Peaks 117: alisol B; Peak 118: alisol B 23-acetate. (Adapted from *Chromatographia* 2009, *69*, 791–793.)

saponins, 3-*O*-α-L-rhamnopyranosyl-(1->6)-*β*-D-glucopyranosyljujubogenin-20-*O*-(2,3,4-*O*-triacetyl)-α-L-rhamnopyranoside (**123**), 3-*O*-α-L-rhamnopyranosyl-(1->6)-*β*-D-glucopyranosyljuju- bogenin-20-*O*-α-L-rhamnopyranoside (**124**), 3-*O*-α-L-rhamnopyranosyl-(1->2)-[(4-sul-fo)-*β*-D-glucopyranosyl-(1->3)]-α-L-arabinopyranosyl jujubogenin (**125**), and 3-*O*-α-L-rhamnopyranosyl-(1->2)-[(4-sulfo)-*β*-D-glucopyranosyl-(1->3)]-*β*-D-galactopyranosyl-(20*R*,22*R*)-16*β*,22:16α,30-diepoxy-dammar-24-ene-3*β*,20-diol (**126**) along with a known compound, jujuboside B (**127**) were isolated by CPC method using a gradient of ethyl acetate-*n*-butanol-water system (95:1:4 to 20:23:7, v/v, ascending mode).[40,41]

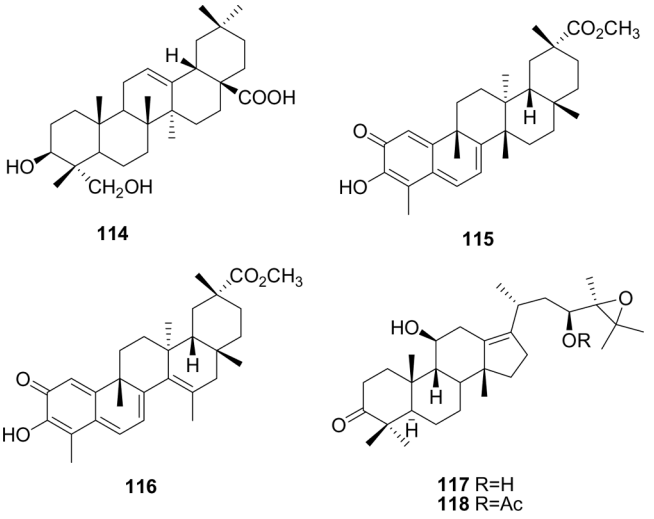


FIGURE 19 Chemical structures of triterpenes separated or fractionated by CPC.

TABLE 5 Triterpenes Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Hederagenin (114)	*Cussonia holstii* (Araliaceae)	(i) Hex-EtOAc-MeOH-H_2O (7:8:5:3) (ii) Hex-EtOAc-MeOH-H_2O (8:7:5:3)	Descending Descending	Isocratic Isocratic	F	[37]
Pristimerin (115) Netzahuaolcoyene (116)	*Maytenus ilicifolia* (Celastraceae)	Hep-EtOAc-MeOH-H_2O (8:1:6:1)	Ascending	Isocratic	S	[38]
Alisol B (117) Alisol B 23-acetate (118)	*Alisma orientale* (Alismataceae)	Hex-EtOAc-MeOH-H_2O (10:2:10:7)	Ascending	Isocratic	S	[39]

[a]EtOAc: ethyl acetate; H_2O: water; Hep: *n*-heptane; Hex: *n*-hexane; MeOH: methanol.
[b]CPC was used for separation (S) or fractionation (F).

Diosgenin glycosides are the representative secondary metabolites found in *Dioscorea* species. A two-phase solvent system composed of chloroform-isopropyl alcohol-methanol-water (7:1:6:4, descending mode) was used for CPC separation. The enriched-saponin extract (300 mg) from the roots of *D. villosa* was chromatographed on CPC and yielded prosapogenin A of dioscin (11.1 mg **128**), dioscin (8.9 mg **129**), deltonin (29.2 mg **130**), and zingiberensis saponin I (6.2 mg **131**) with high purity (Figure 20).[42]

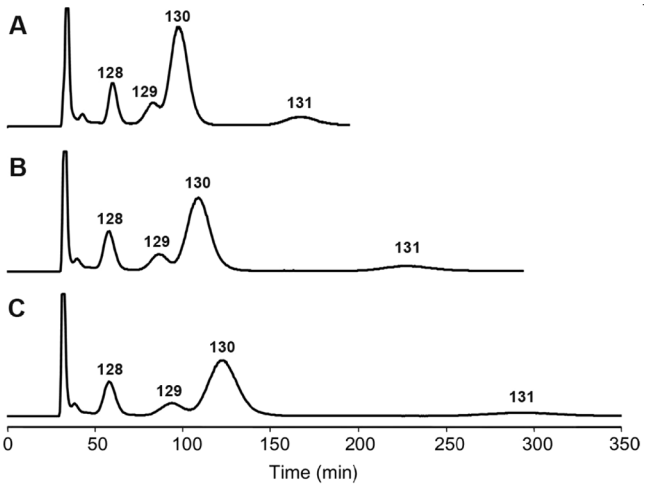


FIGURE 20 CPC separation of saponin-rich extract from *D. villosa*. CPC donditions: rotational speed, 800 rpm; solvent systems, chloroform-methanol-isopropyl alcohol-water (v/v) systems as 5:6:1:4 (A), 7:6:1:4 (B) and 9:6:1:4 (C); stationary phase: upper phase; mobile phase: lower phase; flow rate: 2.5 mL/min; ELSD tube temperature: 508C; nebulizer N_2 gas pressure: 3.0 bar; gain level: 2. Peaks 128: prosapogenin A of dioscin; 129: dioscin; 130: deltonin; 131: zingiberensis saponin I. (Adapted from *J. Sep. Sci.* 2008, *31*, 2486–2491.)

Lancemaside A (**132**) originated from *Codonopsis* species was known to prevent or ameliorates symptoms associated with male climacteric disorder. A CPC separation method was developed to purify lancemaside A (**132**) along with two saponins, foetidissimoside A (**133**), and astersaponin Hb (**134**) from *Codonopsis lanceolate* (Campanulaceae), using a *n*-hexane-*n*-butanol-methanol-0.1% formic acid (3:4:1:6, v/v, ascending mode). From the saponin-enriched fraction (0.5 g) of *C. lanceolate*, repeated CPC run (6 times), followed by simple preparative HPLC, provided **132** (85.5 ± 2.6 mg), **133** (12.8 ± 1.2 mg), and **134** (5.7 ± 0.7 mg).[43]

Saikosaponins-a (**135**) and -c (**136**) are the main constituents of *Bupleurum falcatum* (Umbelliferae), and simple separation of **135** (36.1 mg) and **136** (28.7 mg) was accomplished by CPC-ELSD method with an ethyl acetate-*n*-butanol-water system (15:1:3:15, v/v, ascending mode) from the saponin-rich extract (370 mg).[44] Chemical structures and CPC experimental conditions were shown in Figure 21 and Table 6, respectively.

Alkaloids

Naphthyltetrahydroisoquinoline alkaloids with inhibitory activity against human immunodeficiency virus (HIV), michellamines A (**137**) and B (**138**), were separated from *Ancistrocladus karupensis* (Ancistrocladaceae) by CPC runs and sequential preparative-HPLC. An optimal two-phase solvent system, chloroform-methanol-0.5% HBr (5:5:3, v/v, descending mode), was used for this separation.[45]

Eighteen polar phenolic alkaloids (**139-156**) were separated from three lauraceous plants (*Litsea cubeba, Phoebe formosana,* and *Neolitsea konishii*). This separation work was conducted using CPC runs with a two-phase solvent systems composed of chloroform-methanol-water (containing 0–l% acetic acid) (2:2:1 or 5:5:3, v/v, ascending mode) and, in part, conventional column chromatographic purification.[46]

A mixture of three structurally similar diterpene alkaloids, lappaconitine (**157**), *N*-deacetyllapaconitine (**158**), and oxolapaconitine (**159**), and "Merck Potent Aconitine", a mixture of aconitine (**160**) and 3-deoxyaconitine (**161**), were successfully separated by HPCPC run with a biphasic solvent system of *n*-hexane-dichloromethane-methanol-water system (15:15:24:8, v/v, descending mode). Using aforementioned CPC condition, nine alkaloids (**161, 165–172**) were isolated from *Aconitum falconeri*. Reversed-phase CPC application using C_6H_6-$CHCl_3$-MeOH-H_2O (5:5:7:2) resulted in the isolation of talatizamine (**162**), isotalatizidine **(163),** and cammaconitine (**164**) from a mixture of these compounds. The same CPC separation condition as for compounds **162–164** was employed for the separation of alkaloid-rich

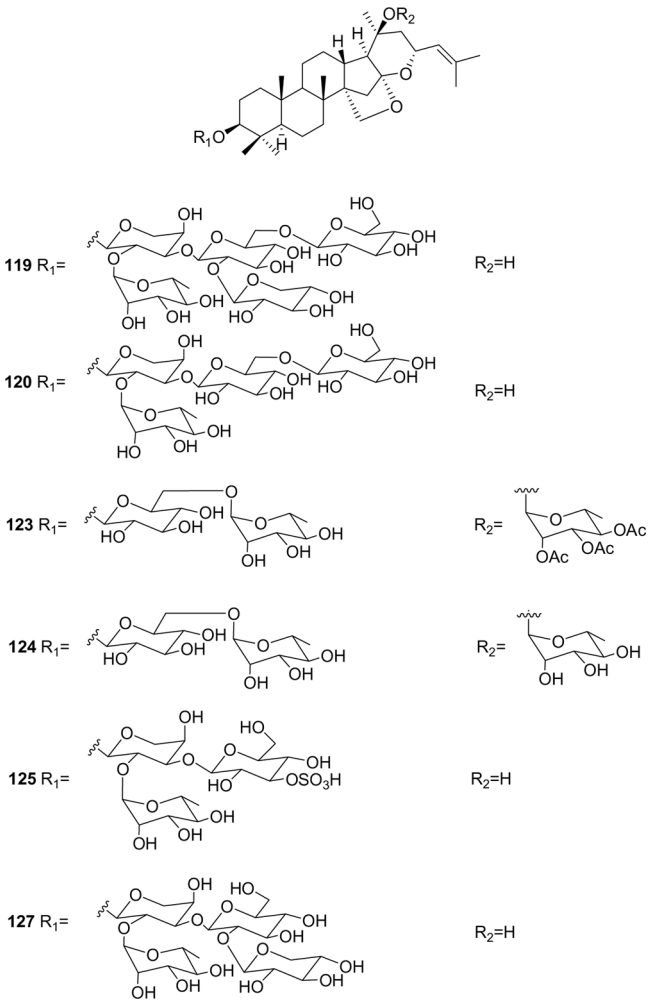


FIGURE 21 Chemical structures of saponins separated or fractionated by CPC.

extract from *Consolida ambigua* (Ranunculacea) and gave a new compound, 13-acetylvakhmatine (**173**), and three known alkaloids (**174–176**).[47]

An acetylcholinesterase (AchE) inhibitor, ungimonorine (**177**) present in *Narcissus* 'Sir Winston Churchill' extract, was isolated by combining CPC fractionation and on-line HPLC-UV/MS biochemical detection. In this study, the first CPC run was conducted using a solvent system composed of *n*-heptane-ethyl acetate-methanol-water (6:1:6:1, v/v), and the second run on the active fraction was performed with a solvent system composed of ethyl acetate-methanol-water (43:22:35, v/v), followed by HPLC purification. The elution mode of CPC runs was not stated in this study.[48]

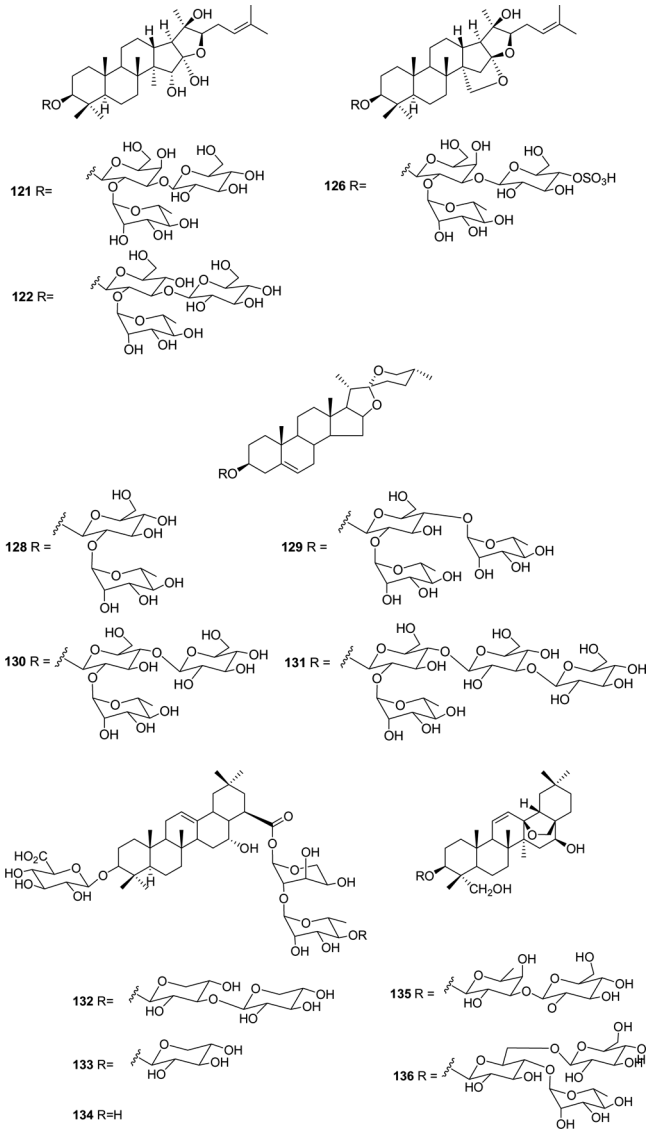


FIGURE 21 Continued.

Nine new zizyphine A-type cyclopeptide alkaloids, paliurines A-F (**178–183),** and paliurines G-I (**185–187**), were isolated from the alkaloid-enriched extract of *Paliurus ramossisimus* (Rhamnaceae) by CPC and conventional column chromatography, along with three known compounds (**184, 188, and 189**). A two-phase solvent system composed of chloroform-methanol-6% acetic acid (5:5:3, v/v, ascending mode) facilitated this CPC separation.[49,50]

TABLE 6 Saponins Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F	Ref
Jujuboside A (119) Jujuboside C (120) Lotosides I–II (121, 122)	*Zizyphus lotus* (Rhamnaceae)	EtOAc-BuOH-H_2O (1:1:18 LP as a SP) (95:1:4 to 20:23:7 UP as a MP)	Ascending	Gradient	S	[40]
3-*O*-α-L-rhamnopyranosyl-(1->6)-β-D-glucopyranosyljujubogenin-20-*O*-(2,3,4-*O*-triacetyl)-α-L-rhamnopyranoside (123) 3-*O*-α-L-rhamnopyranosyl-(1->6)-β-D-glucopyranosyljujubogenin-20-*O*-α-L-rhamnopyranoside (124) 3-*O*-α-L-rhamnopyranosyl-(1->2)-[(4-sulfo)-β-D-glucopyranosyl-(1->3)]-α-L-arabinopyranosyljujubogenin (125) 3-*O*-α-L-rhamnopyranosyl-(1->2)-[(4-sulfo)-β-D-glucopyranosyl-(1->3)]-β-D-galactopyranosyl-(20*R*,22*R*)-16β,22:16α,30-diepoxy dammar-24-ene-3β,20-diol (126) Jujuboside B (127)	*Zizyphus lotus* (Rhamnaceae)	EtOAc-BuOH-H_2O (95:1:4 to 46:40:14 UP as a MP)	Ascending	Gradient	S	[41]
Prosapogenin A of dioscin (128) Dioscin (129) Deltonin (130) Zingiberensis saponin I (131)	*Dioscorea villosa* (Dioscoreaceae)	$CHCl_3$-IPA-MeOH-H_2O (7:1:6:4)	Descending	Isocratic	S	[42]
Lancemaside A (132) Roetidissimoside A (133) Astersaponin Hb (134)	*Codonopsis lanceolata* (Campanulaceae)	Hex-BuOH-MeOH-0.1% HCOOH (3:4:1:6)	Ascending	Isocratic	F	[43]
Saikosaponins-a, c (135, 136)	*Bupleurum falcatum* (Umbelliferae)	EtOAc-BuOH-H_2O (15:1:3:15)	Ascending	Isocratic	S	[44]

[a]BuOH: *n*-butanol; $CHCl_3$: chloroform; EtOAc: ethyl acetate; H_2O: water; Hep: *n*-heptane; Hex: *n*-hexane; MeOH: methanol; HCOOH: formic acid; IPA: isopropyl alcohol; LP: lower phase; UP: upper phase; SP: stationary phase; MP: mobile phase.

[b]CPC was used for separation (S) or fractionation (F).

Methanol extract (1.47 g) of *Enantia chlorantha* (Annonaceae) was subjected to CPC using dichloromethane-methanol-water (potassium percholate) (48:16:36, v/v, descending mode) to give **190** (600 mg) and alkaloidal mixture (146 mg) containing **191–193**. A portion of the mixture (70 mg) was run on CPC with dichloromethane-methanol-water (5.7×10^{-3} M NaOH, pH 11.8) (48:16:36, v/v, descending mode) to afford **191** (16 mg) and **192** (13 mg). Finally, compound **193** (16 mg) was obtained by reversing the flow mode (dual mode).[51]

Sinomenine (44.3 mg **194**) was isolated from the crude methanol extract (400 mg) of *Sinomenium acutum* (Menispermaceae) in one-step CPC. The optimal two-phase solvent system was proposed as *n*-hexane-ethyl acetate-methanol-0.1% triethylamine (1:6:2:8, v/v, ascending mode).[52]

pH-Zone refining CPC using a two-phase solvent system consisting of methyl *tert*-butyl ether-acetonitrile-water (4:1:5, v/v, ascending mode) was applied to separate and purify several alkaloids from plant sources. Acidic and basic modifiers were added to the aqueous stationary phase and organic mobile phase, respectively, and vice versa. A mixture of cyclopeptides, lotusines A-G (**195–201**), present in *Zizyphus lotus* (Rhamnaceae) was resolved by CPC run with the acidified stationary phase (10 mM methanesulfonic acid) and basified mobile phase (5 mM of triethylamine).[53] In case of the separation of lysergol (210 mg **202**) and chanoclavine (182 mg **203**) from the crude extract (4 g) of *Ipomoea muricata*, the aqueous and organic phases were acidified and basified with trifluoroacetic acid (10 mM) and triethylamine (10 mM), respectively.[54] The same biphasic system with modifiers [triethylamine (1.5 mM) in the aqueous phase and methanesulfonic acid (2 mM) in the organic stationary phase] was applied to resolve oubatchensine (**204**), and (-)-13aα-antofine (**205**) from *Cryptocarya oubatchensis* (Lauraceae).[55] Renault et al. utilized pH-zone refining CPC to separate vindoline (**206**), vindolinin (**207**), catharanthine (**208**) and vinblastine (**209**) from the crude mixture of *Catharanthus roseus* alkaloids. The experimental conditions were selected after numerical simulation on synthetic mixture of vindoline, catharanthamine and vinblastine. Four samples with different amounts (800 mg, 1.6 g, 2.4 g, and 7.0 g) were subjected to CPC using methyl *tert*-butyl ether-acetonitrile-water (4:1:5, v/v, ascending mode). The aqueous stationary phase was acidified with 10 mM HCl and the organic mobile phase was basified with 8 mM triethylamine. The yields and purities of **206–209** were dependent on loading sample mass (Figure 22).[56]

Huperazines A (360 mg **210**) and B (300 mg **211**), acetylcholine esterase inhibitors, were successfully isolated from the crude extract (4.9 g) of *Huperzia serrata* (Lycopodiaceae) using pH-zone refining CPC. The separation was fulfilled using a *n*-heptane-ethyl acetate-*n*-propanol-water system (10:30:15:45, v/v, ascending mode) with the addition of

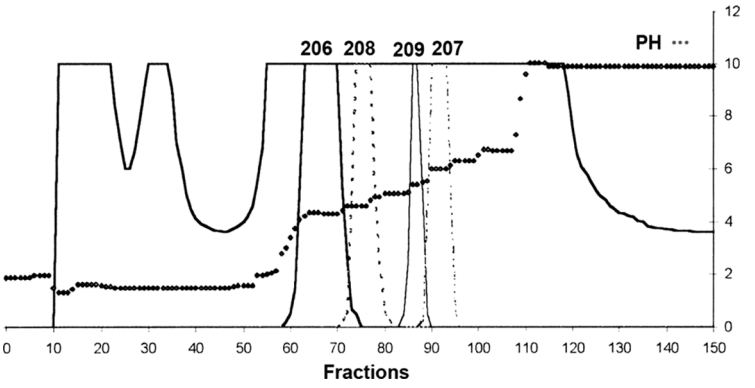


FIGURE 22 UV chromatogram and pH profile for the separation with 2.4 g of tartrate alkaloids from *Catharanthus roseus*. CPC conditions: rotational speed: 800 rpm; solvent system, methyl *tert*-butyl ether-acetonitrile-water (4:1:5, v/v, ascending mode); The aqueous stationary phase was acidified with 10 mM of HCl as a retainer and basified organic mobile phase (8 mM of triethylamine) was utilized as a displacer; flow rate, 3 mL/min. Peaks 206: vindoline; 207: vindolinin; 208: catharanthine; 209: vinblastine. (Adapted from *J. Chromatogr. A* 1999, *849*, 421–431.)

triethylamine (8 mM) and methane sulfonic acid (6 mM) as a displacer and a retainer, respectively. (Figure 23).[57]

pH-Zone refining CPC using an ethyl acetate-*n*-butanol-water system (1:4:5, ascending mode) and sequential MPLC led to the isolation of solamargine (15 mg **212**) and solasonine (30 mg **213**) from the extract (1 g) of *Solanum xanthocarpum* (Solanaceae). Triethylamine (5 mM) was added to the organic mobile phase, and trifloroacetic acid (10 mM) to the aqueous stationary phase.[58] Chemical structures and CPC experimental conditions were shown in Figure 24 and Table 7, respectively.

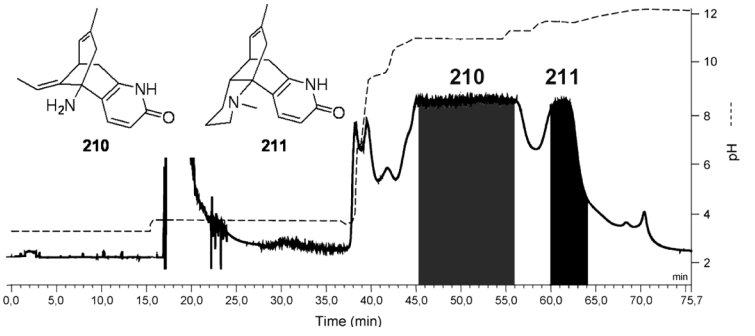


FIGURE 23 pH-zone refining UV chromatogram, pH profile and HPLC control for the separation of 1.4 g of alkaloid extract from *Huperzia serrata*. CPC conditions: rotational speed, 1400 rpm; solvent system: *n*-heptane-ethyl acetate-propanol-water (10:30:15:45, v/v, ascending mode); stationary phase: lower aqueous phase with methanesulfonic acid (6 mM) as a retainer; mobile phase: upper organic phase with triethylamine (8 mM) as a displacer; flow rate: 6 mL/min; retention of stationary phase: 70%; back pressure: 42–44 bar. Peaks 210: huperazine A; 211 huperazine B. (Adapted from *J. Chromatogr. A* 2007, *1140*, 101–106.)

Antibiotics

A Japanese group involved CPC instrument using a two-phase solvent system composed of chloroform-methanol-water (5:6:4, v/v, descending mode) to separate efficiently several antibiotics from *Streptomyces* sp.

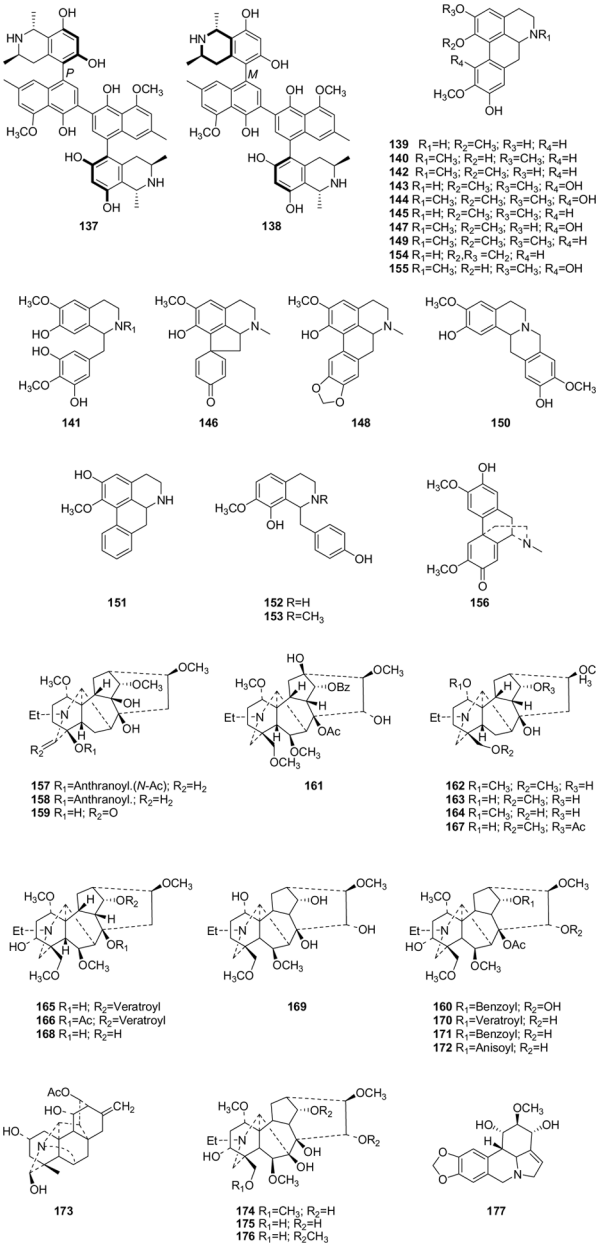


FIGURE 24 Chemical structures of alkaloids separated or fractionated by CPC.

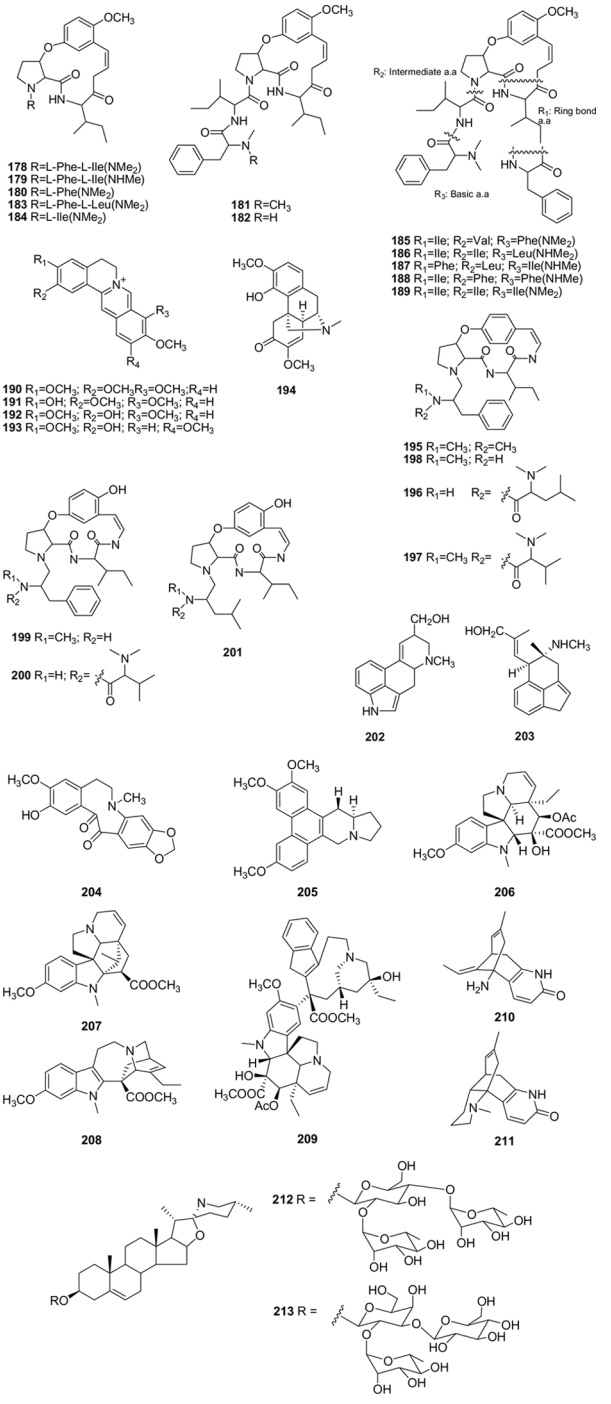


FIGURE 24 Continued.

TABLE 7 Alkaloids Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Michellamine A (137) Michellamine B (138)	*Ancistrocladus korupensis* (Ancistrocladaceae) *Litsea cubeba* (Lauraceae)	$CHCl_3$-MeOH-0.5% HBr (5:5:3)	Descending	Isocratic	F	[45]
Laurolistine (139)	*Phoebe formosana* (Lauraceae) *Neolitsea konishii* (Lauraceae)	$CHCl_3$-MeOH-0.5% AcOH (2:2:1) $CHCl_3$-MeOH-1% AcOH (5:5:3)	Ascending	Isocratic	S	[46]
Isoboldine (140) Reticuline (141)	*Neolitsea konishii* (Lauraceae)	$CHCl_3$-MeOH-1% AcOH (5:5:3)	Ascending	Isocratic	S	[46]
Boldine (142) Norisocorydine (143) Isocorydine (144)	*Litsea cubeba* (Lauraceae)	$CHCl_3$-MeOH-0.5% AcOH (2:2:1)	Ascending	Isocratic	S	[46]
Laurotetanine (145) Isoboldine (140) Glaziovine (146) N-methyllindcarpine (147) Isodemesticine (148) N-Methyllaurotetanine (149)	*Litsea cubeba* (Lauraceae)	$CHCl_3$-MeOH-0.5% AcOH (2:2:1)	Ascending	Isocratic	F	[46]
Coreximine (150) Asimilobine (151) Norjuziphine (152) Juziphine (153)	*Phoebe formosana* (Lauraceae)	$CHCl_3$-MeOH-1% AcOH (5:5:3)	Ascending	Isocratic	F	[46]
N-Methyllaurotetanine (149) Actinodaphnine (154) Corytuberine (155) Pallidine (156)	*Neolitsea konishii* (Lauraceae)	$CHCl_3$-MeOH-1% AcOH (5:5:3)	Ascending	Isocratic	F	[46]
Lappaconitine (157) N-Deacetyllapaconitine (158) Oxolapaconitine (159)	Alkaloid mixture	Hex-CH_2Cl_2-MeOH-H_2O (15:15:24:8)	Descending	Isocratic	S	[47]
Aconitine (160) 3-Deoxyaconitine (161)	Merk Potent Aconitine	Hex-$CHCl_3$-MeOH-H_2O (15:15:24:8)	Descending	Isocratic	F	[47]
Talatizamine (162)	Alkaloid mixture	C_6H_6-$CHCl_3$-MeOH-H_2O (5:5:7:2)	Descending	Isocratic	F	[47]

(*Continued*)

TABLE 7 Continued

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Isotalatizidine (163) Cammaconitine (164) Falconerine (165) 8-Acetylfalconerine (166)	*Aconitum falconeri* (Ranunculaceae)	Hex-CH_2Cl_2-MeOH-H_2O (15:15:24:8)	Descending	Isocratic	S	[47]
Isotalzatidine (163) Falconerine (165) Condelphine (167) Ezochasmanine (168) Neoline (169) Pesudaconitine (170) Indaconitine (171) Yunaconitine (172)	*Aconitum falconeri* (Ranunculaceae)	Hex-CH_2Cl_2-MeOH-H_2O (15:15:24:8)	Descending	Isocratic	F	[47]
13-Acetylvakhmatine (173)	*Consolida ambigua* (Ranunculaceae)	C_6H_6-$CHCl_3$-MeOH-H_2O (5:5:7:2)	Descending	Isocratic	S	[47]
Delcosine (174) Takaosamine (175) Gigactonine (176)	*Consolida ambigua* (Ranunculaceae)	C_6H_6-$CHCl_3$-MeOH-H_2O (5:5:7:2)	Descending	Isocratic	S	[47]
Ungimonorine (177)	*Narcissus 'Sir Winston Churchill'*	(i) Hep-EtOAc-MeOH-H_2O (6:1:6:1) (ii) EtOAc-MeOH-H_2O (43:22:35)	Ascending Dual mode	Isocratic	F	[48]
Paliurines A-F (178–183) Sativnine G (184)	*Paliurus ramosisimus* (Rhamnaceae)	$CHCl_3$-MeOH-6% AcOH (5:5:3)	Ascending	Isocratic	F	[49]
Paliurines G-I (185–187) Nummularine H (188) Daechuine-S3 (189)	*Paliurus ramosisimus* (Rhamnaceae)	$CHCl_3$-MeOH-6% AcOH (5:5:3)	Ascending	Isocratic	F	[50]
Palmatine (190)	*Enantia chlorantha* (Annonaceae)	CH_2Cl_2-MeOH-H_2O (potassium percholate) (48:16:36)	Descending	Isocratic	S	[51]
Jatrorrhizine (191) Columbamine (192) Pseudocolumbamine (193)	*Enantia chlorantha* (Annonaceae)	(i)CH_2Cl_2-MeOH-H_2O (potassium percholate) (48:16:36) (ii) CH_2Cl_2-MeOH- NaOH (5.7×10^{-3} M pH 11.8) (48:16:36)	Descending	Isocratic	S	[51]
Sinomenine (194)	*Sinomenium acutum* (Menispermaceae)	Hex-EtOAc-MeOH-0.1% TEA (1:6:2:8)	Descending	Isocratic	S	[52]

Lotusines A-C (195–197)	*Zizyphus lotus* (Rhamnaceae)	M*t*BE-MeCN-H_2O (4:1:5)	Ascending	pH zone-refining	F	[53]
Lotusines D-G (198–201)	*Zizyphus lotus* (Rhamnaceae)	M*t*BE-MeCN-H_2O (4:1:5)	Ascending	pH zone-refining	S	[53]
Lysergol (202)	*Ipomoea muricata* (Convolvulaceae)	M*t*BE-MeCN-H_2O (4:1:5)	Ascending	pH-zone refining	S	[54]
Chanoclavine (203)						
Oubatchensine (204)	*Cryptocarya ubatchensis* (Lauraceae)	M*t*BE-MeCN-H_2O (4:1:5)	Ascending	pH-zone refining	S	[55]
(−)-13aα-Antofine (205)						
Vindoline (206)	*Catharanthus roseus* (Apocynaceae)	M*t*BE-MeCN-H_2O (4:1:5)	Ascending	pH-zone refining	S	[56]
Vindolinin (207)						
Catharanthine (208)						
Vinblastine (209)						
Huperazines A-B (210–211)	*Huperzia serrata* (Lycopodiaceae)	Hep-EtOAc-PrOH-H_2O (10:30:15:45)	Ascending	pH zone-refining	S	[57]
Solamargine (212)	*Solanum xanthocarpum*	EtOAc-BuOH-H_2O (1:4:5)	Ascending	pH-zone refining	S	[58]
Solasonine (213)	(Solanaceae)					

[a]AcOH: acetic acid; BuOH: *n*-butanol; $CHCl_3$: chloroform; CH_2Cl_2: methylene chloride; C_6H_6: benzene; EtOAc: ethyl acetate; H_2O: water; HBr: hydrogen bromide; Hep: *n*-heptane; Hex: *n*-hexane; MeCN: acetonitrile; MeOH: methanol; M*t*BE: methyl *tert*-butyl ether; PrOH: propanol; TEA: triethylamine;.

[b]CPC was used for separation (S) or fractionation (F).

A new antibiotic, aldecalmycin (**214**), was isolated from *Streptomyces* sp.[59] The crude extract (1.0 g) from a slant culture of the strain MJ147–72F6 was fractionated by RP-HPLC and the following CPC purification to give **214** (290 mg). Helliquinomycin (**215**), an inhibitor of DNA helicase, was isolated from *Streptomyces* sp. MJ-929SF2, using column chromatography methods including CPC in a fractionation step.[60] Epostatin (**216**, 9.7 mg) was separated from the *n*-butanol soluble extract (81.9 mg) of *Streptomyces* sp. MJ995-OF5 and CPC experiment was used at the final purification step.[61] In addition, CPC was involved in final purification procedure for the separation of 612 mg of pure polyketomycin (**217**) from the 2 g of extract from *Streptomyces* sp. MK277-AF1.[62] Another two-phase solvent system, ethyl acetate-K_2HPO_4 (10 mM) (1:1, v/v, descending mode) was applied in Sanki LLB-M instrument to purify 16-membered lactone named tubelactomicin A (37. 9 mg **218**) from the 236 mg of active extract of *Nocardia* sp. MK703–102F1. Tubelactomicin A showed potent antibacterial activities against drug-resistant strains.[63] A novel antifungal agent, formamicin (**219**), was isolated from *Saccharothrix* sp. MK27–91F2 by CPC procedure as a purification step using an *n*-hexane-ethanol-water system (100:85:15, v/v, dual mode), and this compound exhibited strong antibicrobial activity against phytopathogenic fungi.[64]

The Sanki-LLN instrument (1000 rpm, 3 mL/min) was involved in the separation of three new macrolide antibiotics. The 1 g of crude extract from *Streptomyces violaceusniger* (strain RS-22) was initially resolved by CPC using an *n*-butanol-ethanol-water system (10:2.5:10, v/v, ascending mode), and 725 mg of active CPC peak fractions was re-chromatographed on a preparative HPLC to yield RS-22A (14.5 mg **220**), RS-22B (27 mg **221**) and RS-22C (28.7 mg **222**). These compounds showed antimicrobial activities against fungi and Gram-negative bacteria.[65]

Two diastereomeric antibiotics namely sparoxomycins A1 (**223**) and A2 (**224**) were fractionated by CPC. A two-phase solvent system composed of *n*-butanol-ethanol-water (10:2.5:10, v/v, ascending mode) was utilized and consecutive conventional column chromatography were applied to give 1.55 mg of **223** and 2.7 mg of **224** from the culture broth of *Streptomyces sparsogenes* SN-2325.[66]

YM-47522 (**225**), produced by *Bacillus* sp. (strain YL-03709B), was isolated by a purification process which involved reversed-phase CPC using a chloroform-methanol-water system (2:2:1, v/v). YM-47522 showed potent *in vitro* antifungal activity against *Rhodotrorula acuta* and *Pichia angusta* (MIC 0.05 and 0.75 μg/mL) and weak antifungal activities against *Candida albicans* (MIC 25 μg/mL) and *Cryptococcus neofromans* (MIC 6.25 μg/mL).[67]

CPC was used as the first step in the separation of nine new antibiotics (**226–234**) along with seven known compounds (**235–241**) from *Penicillium* sp. FK1–2140. A two-phase solvent system, *n*-hexane-chloroform-acetonitrile

(5:1:5, v/v, descending mode) was applied to fractionate the 1.75 g of acetonitrile soluble extract. Further purification step was performed by preparative HPLC to give new yaequinolones A1 (**226**), A2 (**227**), B-F (**228–232**), J1 (**233**) and J2 (**234**), and seven known compounds, quinolinones A (**235**) and B (**236**), peniprequinolone (**237**), peniprequinolones A (**238**), peniprequinolone B (**239**), 4′-methoxycyclopeptin (**240**) and *trans*-dehydro-4′-methoxycyclopeptin (**241**). Among the constituents, yaequinolone F (**232**) exhibited the most potent growth inhibitory activity against brine shrimp (*Artemia salina*) with MIC value of 0.19 μg/mL.[68] Chemical structures and CPC experimental conditions were shown in Figure 25 and Table 8, respectively.

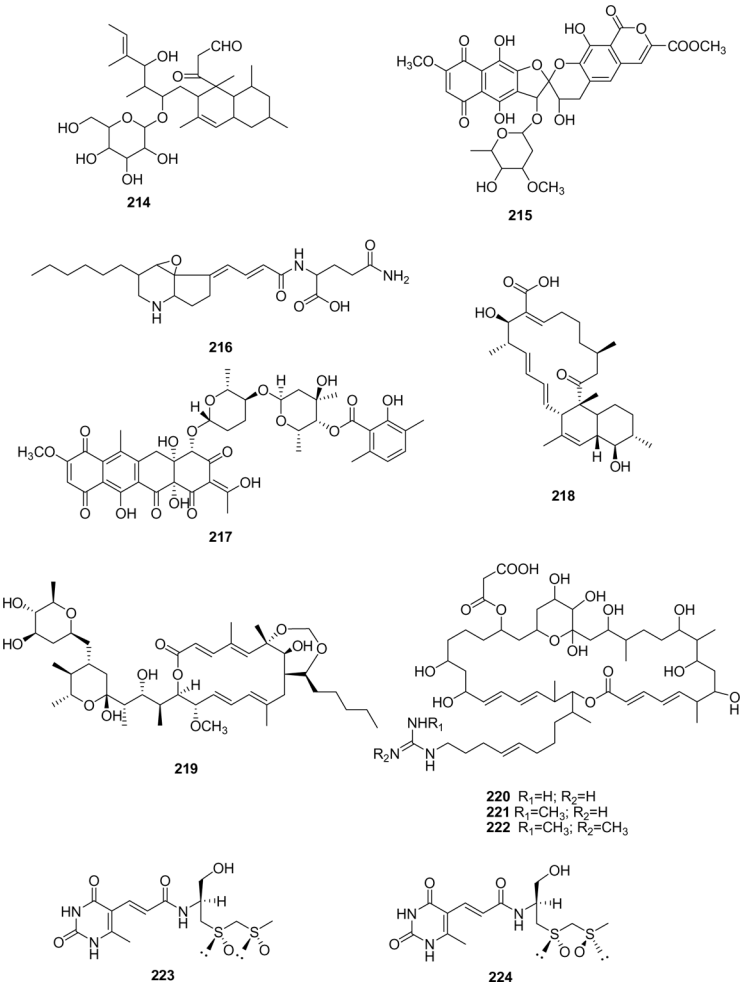


FIGURE 25 Chemical structures of antibiotics separated or fractionated by CPC.

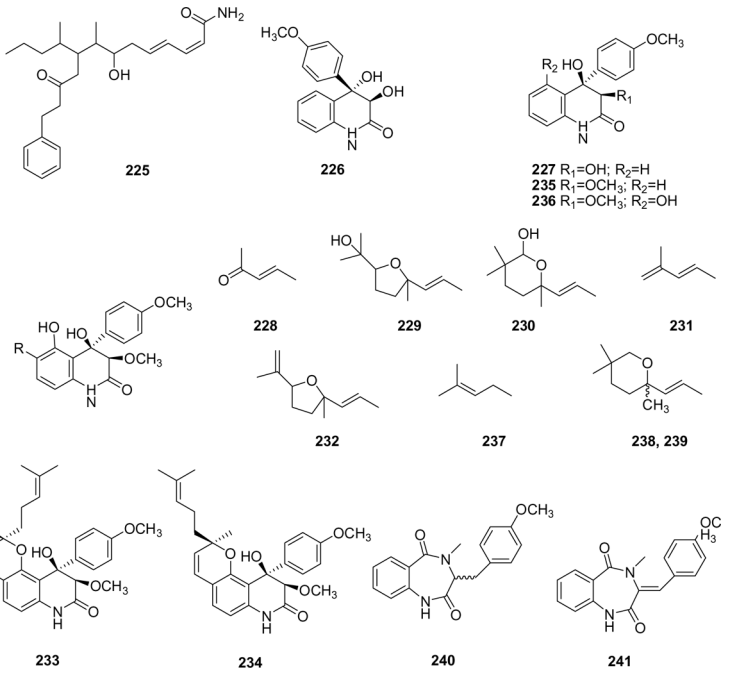


FIGURE 25 Continued.

Miscellaneous

CPC was used to separate α- acids (**242–244**) and *β*-bitter acids (**245–247**) from the crude supercritical carbon dioxide extract of *Humulus lupulus* (Cannabaceae). Aqueous phase of toluene-0.1 M triethanolamine-HCl (pH 8.4) in water was used as a mobile phase to separate cohumulone (**242**), humulone (**243**), and adhumulone (**244**). After separation of α-bitter acids, the mobile phase was changed to the aqueous phase of 0.2 M diethanolamine-H_3PO_4 (pH 9.75) in water-methanol (4:1, v/v) for elution of colupulone (**245**), lupulone (**246**), and adlupulone (**247**).[69]

Kava lactones from *Piper methysticum* (Piperaceae) have been known to be the major responsible constituents that induce hepatotoxicity in human. Thus, the necessity of separation of kava lactones has been needed to investigate their live toxicity. In order to obtain this goal, a one-step CPC method was developed to separates six kava lactones from supercritical carbon dioxide extract of *P. methysticum*. A two-phase solvent system composed of *n*-hexane-acetone-methanol-water (4:1:3:1, v/v) was used to resolve the 1 g of supercritical carbon dioxide extract, and the upper and lower phase of this solvent mixture were applied as a mobile and a stationary phase, respectively. This method could afford desmethoxyyangonin (53.9 mg **248**), yangonin (29.5 mg

TABLE 8 Antibiotics Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Aldecalmycin (214)	*Streptomyces* sp. (MJ147072F6)	$CHCl_3$-MeOH-H_2O (5:6:4)	Descending	Isocratic	S	[59]
Helliquimomycin (215)	*Streptomyces* sp. (MJ-929SF2)	$CHCl_3$-MeOH-H_2O (5:6:4)	Descending	Isocratic	F	[60]
Epostatin (216)	*Streptomyces* sp. (MJ995-OF5)	$CHCl_3$-MeOH-H_2O (5:6:4)	Descending	Isocratic	S	[61]
Polyketomycin (217)	*Streptomyces* sp. (MK277-AF1)	$CHCl_3$-MeOH-H_2O (5:6:4)	Descending	Isocratic	S	[62]
Tubelactomicin A (218)	*Nocardia* sp. (MK703-102F1)	EtOAc-K_2HPO_4 (10 mM) (1:1)	Descending	Isocratic	S	[63]
Formamicin (219)	*Saccharothix sp.* (MK27-91F2)	Hex-EtOH-H_2O (100:85:15)	Dual mode	Isocratic	F	[64]
RS-22A (220)	*Streptomyces violaceusniger* (RS-22)	BuOH-EtOH- H_2O (10:2.5:10)	Ascending	Isocratic	F	[65]
RS-22B (221)						
RS-22C (222)						
Sparoxomycins A1 and A2 (223–224)	*Streptomyces sparsogenes* (SN-2325)	BuOH-EtOH- H_2O (10:2.5:10)	Ascending	Isocratic	F	[66]
YM-47522 (225)	*Bacillus* sp. (YL-037909B)	$CHCl_3$-MeOH-H_2O (2:2:1)	Ascending	Isocratic	F	[67]
Yaequinolones A1 and A2 (226, 227)	*Penicillium* sp. (FK1-2140)	Hex-$CHCl_3$-MeCN (5:1:5)	Descending	Isocratic	F	[68]
Yaequinolones B-F (228–232)						
Yaequinolones J1 and J2 (233–234)						
Quinolinones A-B (235, 236)						
Peniprequinolone (237)						
Peniprequinolones A and B (238–239)						
4′-Methoxycyclopeptin (240)						
Trans-dehydro-4′-methoxycyclopeptin (241)						

[a]BuOH: *n*-butanol; $CHCl_3$: chloroform; EtOH: ethanol; H_2O: water; Hex: *n*-hexane; MeCN: acetonitrile; MeOH: methanol; K_2HPO_4: dipotassium phosphate.
[b]CPC was used for separation (S) or fractionation (F).

249), dihydrokavain (168.2 mg **250**), dihydromethysticin (71 mg **251**), kavain (138 mg **252**), and methysticin (50.7 mg **253**) with high purities.[70]

CPC was applied to separate neutral and acidic cannabinoids from *Cannabis sativa* (Cannabaceae). The neutral cannabinoids, Δ^9-tetrahydrocannabinol (**254**), cannabidiol (**255**), cannabinol (**256**), and cannabigerol (**257**) were purified by Sanki LLB-M instrument (600 rpm, 5 mL/min) using a two-phase system composed of *n*-hexane-acetone-acetonitrile (5:2:3, v/v). The *n*-hexane-rich upper phase was used as a mobile phase, and the acetonitrile-rich lower phase was used as a stationary phase; whereas, the use of *n*-hexane-methanol-water (5:3:2, v/v) resulted in the isolation of three acidic cannabinoids, Δ^9-tetrahydrocannabinolic acid-A (**258**), cannabidiolic acid (**259**), and cannabigerolic acid (**260**) by reversed-phase CPC (500 rpm, 4 mL/min). The purities of seven cannabinoids were determined to be over 90%.[71]

Sinalbin (*p*-hydroxybenzylglucosinolate) was obtained on a multigram scale from a crude aqueous extract of *Sinapis alba* (Brassicaceae) by scaling up a strong ion-exchange CPC (SIXCPC). A two-phase solvent system, ethyl acetate-*n*-butanol-water (3:2:5 v/v, ascending mode) was used for the SIXCPC and 80 mM trioctylmethyl ammonium chloride was added to the organic stationary phase as an exchanger, whereas 80 mM potassium iodide added to the mobile phase was used as a displacer. By scaling-up of this method, 70.3 g of sinalbin (**261**) were obtained from 341 g of crude extract in a one-step process (Figure 26).[72]

A multiple dual mode CPC method was applied to separate two mycosporines from *Lichina pygmaea* (Lichinaceae). The separation was achieved by a two-phase solvent system composed of *n*-butanol-acetic acid-water (4:1:5, v/v) in the isocratic mode. In the initial separation process, the upper organic phase was eluted as a mobile phase; after then, successive changes of flow direction were performed to give L-glutamic acid derivative (**262**) and mycosporin-serinol (**263**) rich fractions. Further purification was achieved by C-18 columns.[73]

Pea seed proteins from *Pisum sativum* (Leguminosae) are polypeptides that show toxicity against stored product insects. Thus, a possibility has been increased that this polypeptide can be used as an environmentally-friendly and biodegradable insecticide. CPC was applied to purify pea albumin such as PA1b. The *n*-butanol-20 mM TFA (pH 2.2) system (1:1, v/v) gave a good partitioning of PA1b with *K* value of 1.7, and this solvent system could separate PA1b from other pea proteins. The total pea albumins were subjected to Kromaton CPC instrument with dual mode elution, and gave PA1b with above 95% purity. This CPC method gave better yield than that of anion-exchange and reversed-phase column chromatography method.[74] Chemical structures and CPC experimental conditions were listed in Figure 27 and Table 9, respectively.

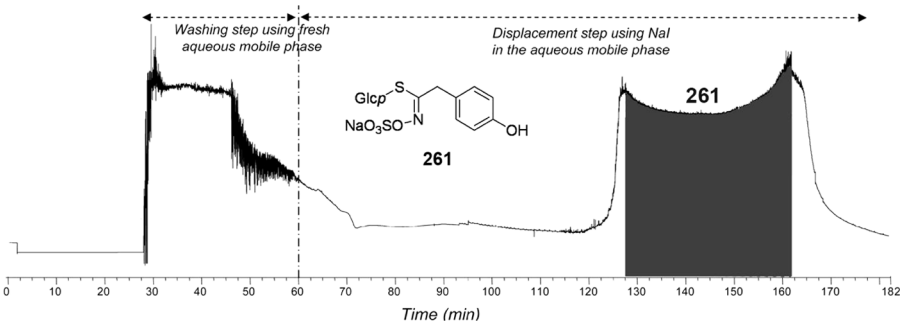


FIGURE 26 SIXCPC chromatogram of the laboratory-scale purification of sinalbin (261) from a white mustard seed extract. CPC condition: rotational speed, 1200 rpm; solvent system: ethyl acetate-*n*-butanol-water (3:2:5, v/v, descending mode); stationary phase: upper organic phase with Aliquat® 336 (80 mM) as ion-exchanger; mobile phase: lower aqueous phase with NaI (80 mM) as displacer; flow rate: 2 mL/min; retention of stationary phase: 71%. (Adapted from *J. Sep. Sci.* 2009, *32*, 1801–1807.)

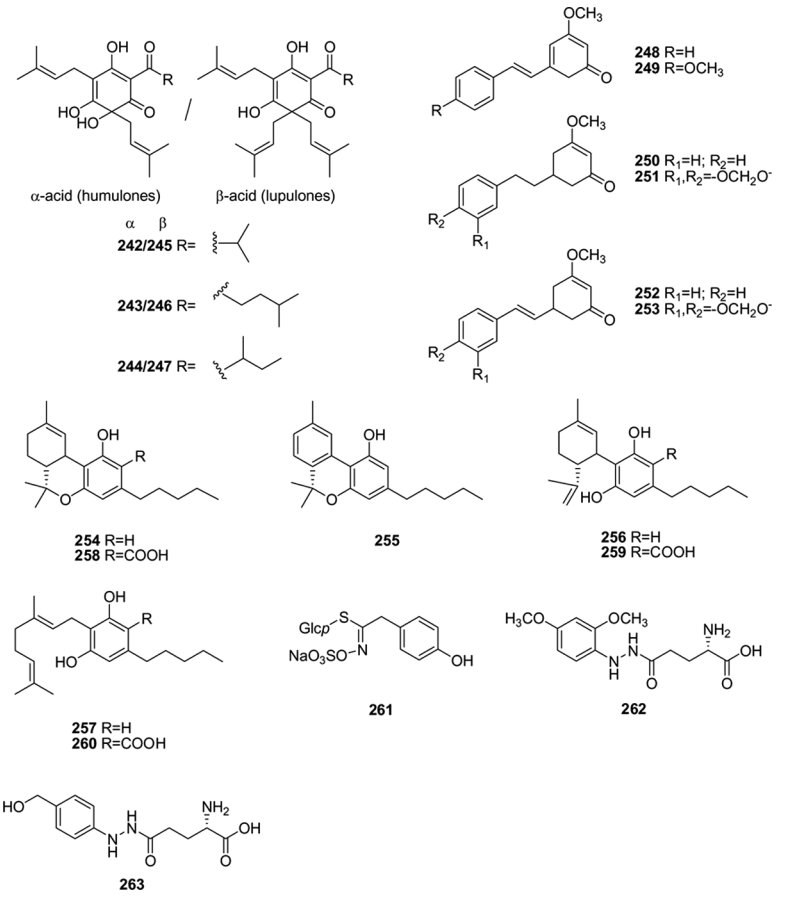


FIGURE 27 Chemical structures of miscellaneous compounds separated or fractionated by CPC.

TABLE 9 Miscellaneous Compounds Separated or Fractionated by Centrifugal Partition Chromatography

Compound	Source	Solvent System (Volume Ratio)[a]	Flow Mode	Elution Mode	S/F[b]	Ref
Cohumulone (242) Humulone (243) Adhumulone (244) Colupulone (245) Lupulone (246) Adlupulone (247)	*Humulus zupulus* (Cannabaceae)	(i) Tol-0.1 M TEA-HCl (pH 8.4) (ii) Toluene-0.2 M DEA-H_3PO_4 (pH 9.75)-MeOH (4:1, v/v)	Descending	Gradient	S	[69]
Desmethoxyyangonin (248) Yangonin (249) Dihydrokavain (250) Dihydromethysticin (251) Kavain (252) Methysticin (253)	*Piper methysticum* (Piperaceae)	Hex-EtOAc-MeOH-H_2O (4:1:3:1)	Ascending	Isocratic	S	[70]
Δ^9-Tetrahydrocannabinol (254) Cannabidiol (255) Cannabinol (256) Cannabigerol (257)	*Cannavis sativa* (Cannabaceae)	Hex-Me_2CO-MeCN (5:2:3)	Ascending	Isocratic	S	[71]
Δ^9-tetrahydrocannabinolic acid-A (258) Cannabidiolic acid (259) Cannabigerolic acid (260)	*Cannavis sativa* (Cannabaceae)	Hex-MeOH-H_2O (5:3:2)	Descending	Isocratic	S	[71]
Sinalbin (261)	*Sinapis alba* (Brassicaceae)	EtOAc-BuOH-H_2O (3:2:5)	Ascending	pH-zone refining	S	[72]
L-Glutamic acid derivative (262) Mycosporin-serinol (263)	*Lichina pygmaea* (Lichinaceae)	BuOH-AcOH-H_2O (4:1:5)	Multiple dual mode	Isocratic	S	[73]

[a]BuOH: *n*-butanol; DEA: diethanoamine; EtOAc: ethyl acetate; H_2O: water; Hex: *n*-hexane; HCl: hydrogen chloride; H_3PO_4: phosphoric acid; Me_2CO: acetone; MeCN: acetonitrile; MeOH: methanol; TEA: triethanolamine; Tol: toluene.

[b]CPC was used for separation (S) or fractionation (F).

CONCLUSIONS

Since 1982, CPC has been the favorite apparatus for preparative purposes. Especially, CPC has been applied for the separation of biologically active constituents and standard marker compounds from natural sources, which indicates that CPC techniques are superior to conventional column chromatography methods in terms of sample recovery, consumption of time, and costs. In this review, over 250 compounds from 60 plants and microorganisms have been described for the period of 1994 to September 2009. Currently, CPC manufacturers and user groups in the world have been undergoing trials and errors in order to enhance separation efficiency and to develop large-scale process. There is no doubt that countercurrent separation techniques play important roles in providing bioactive natural products in diverse scientific and industrial area.

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