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Review article

Capillary electrophoresis of phytochemical substances

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Abstract

Applications of capillary electrophoresis (CE) for analysis of phytochemical substances (e.g. flavonoids, alkaloids, terpenoids, phenolic acid, quinones and coumarins) are reviewed. For example, CE analysis of sixteen tea ingredients were achieved within 10 min with the good precision (RSDs% $< 1\%$ for intra-day and 2% for inter-day) and linearity ($R^2 > 0.990$). Quantitation of sanguinarine and chelerythrine, alkaloids from *Sanguinaria canadensis* L. or *Macleaya cordata* (Wild) Br. R. by CE showed excellent linearity ($R^2 > 0.998$), precision (RSD% = 1.8%) and detection limit (2.4–3.0 μM). Determination of antraquinone-1-sulphonate was also obtained by this technique with good linearity ($R^2 > 0.9999$), precision (RSD% = 2%) and detection limit (0.7 μg/ml). Results of CE analysis from several studies are comparable to those of high performance liquid chromatography (HPLC), but the former is more useful for complex mixture samples where the analysis demands higher resolving power. Advantages of CE are high efficiency, low cost, short analysis time and simplicity, whereas disadvantages include low sensitivity comparing to HPLC and limitation of the preparative scale.

Keywords: Capillary electrophoresis; Flavonoids; Alkaloids; Terpenoids; Phenolic acids; Quinones; Coumarins

1. Introduction

Currently, production of drugs from medicinal plants is an issue of interest worldwide since they possess bioactivity, which are useful for various symptoms (Table 1) [1–37]. Examples of phytochemical substances that are recently isolated and characterized are listed in Table 2 [38–62]. Analysis and quality assessments of these substances are essential in order to provide the highest efficacy, efficiency and safety for consumers. Thin-layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC) are valuable tools for analysis of phytochemical substances. TLC is the simplest and inexpensive method for qualitative analysis of these compounds. Detection in TLC can be performed either by direct visualization or viewing under ultraviolet radiation. GC is useful for volatile compounds, otherwise analytes have to be derivatized prior to GC analysis. Among several chromatographic methods, HPLC is the most widely used technique for both qualitative and quantitative analysis of phytochemical substances. Capillary electrophoresis (CE) is a micro-analyti-

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Table 1
Recent examples of medicinal plants with bioactivity

Activity	Source	References
Anti-inflammatory		
Alkaloids	*Cryptolepis sanguinolenta*	[1]
Polyphenol	*Cameillia sinensis*	[2]
Triterpenoids	*Polyepis racemosa* (quenual)	[3]
	Trichocereus pachanoi	[4]
	Nelumbo nucifera	[4]
	Diospyros leucomelas	[5]
Sesquiterpene	*Ferula linkii*	[6]
Flavonoids	*Haematoxylon campechianum*	[7]
	Tanacetum parthenium, *T. vulgare*	[8]
	Scutellaria baicalensis	[9]
Antipyretic		
Alkaloids	*C. sanguinolenta*	[10]
Analgesic		
Flavonoid	*Rheedia gardneriana*	[11]
	Dioclea grandiflora	[12]
Triterpenoids	*Sebastiania schottiana*	[13]
Sesquiterpene	*F. linkii*	[6]
	Lactuca virosa	[14]
Antitussive		
Sesquiterpene	*Lactuca virosa*	[14]
Antiulcer		
Flavonoid	*Citrus grandis*	[15]
Antipsoriatic		
Alkaloids	*Mahonia aquifolium*	[16]
Wound healing		
Phenolic compounds	*Croton lechleri*	[17]
Intestinal antiseptic		
Alkaloids	*Corydalis pallida* var. *tenuis*	[18]
Diarrhoea		
Flavonoids	*Psidium guajava*	[19]
Anhelmintic		
Alkaloid	*Evodia rutaecarpa*	[20]
Antiplatelet aggregation		
Alkaloids	*Melicope confusa*	[21]
	Zanthoxylum schinifolium	[22]
	Clausena excavata	[23]
Sesquiterpene	*Manglietiastrum sinicum*	[24]
	Tsoongiodendron odorum	[24]
Antimalarial		
Alkaloids	*C. sanguinolenta*	[10]
	Peschiera fushsiaefolia	[25]
	Corynanthe pachyceras	[26]
	Psychotria camponutans	[27]
Antimicrobial		
Flavonoid	*Soroseris hookeriana* (subsp.erysimoides)	[28]
Monoterpene		
Polyphenol	*Ca. sinensis*	[2]
Antibacterial		
Triterpenoids	*Syncarpia glomulifera*	[29]
Diterpene	*Salvia viridis*	[30]
Antiprotozoa		
Alkaloid	*Stephania dinklagai*	[31]
Flavonoid	*Citrus grandis*	[32]
Antiviral		
Flavonoids	*Alkanna orientalis*	[33]
Antifungal		
Flavonoids	*Hebe cupressoides*	[34]
Anti-tumor		
Triterpenoid	*Abies mariesii*	[35]
	Po. racemosa	[36]
	Myodocarpus gracilis	[37]
Antimutagenisis		
Polyphenol	*Ca. sinensis*	[2]
Anticancer		
Polyphenol	*Ca. sinensis*	[2]
Antioxidant		
Flavonoids	*Citrus grandis*	[21]
Polyphenol	*Ca. sinensis*	[10]
Anti-hyperglycemic		
Alkaloids	*C. sanguinolenta*	[1]

cal method, which is applicable for analyses of a wide range of compounds including natural products [63–65] and plant metabolites [66]. CE provides advantages in terms of speed, high efficiency, low cost and simplicity. Therefore, the method can be employed as an alternative for analysis of phytochemical substances.

The aim of this review is to describe the potential of CE and its application in phytochemical analysis. Two reviews on the CE of natural products [64,65] and one review on the CE of plant secondary metabolites [66] are currently available. Unlike previous reviews, the present work gives

information on the quantitative aspect and method validation of CE for analysis of phytochemical substances (e.g. flavonoids, alkaloids, terpenoids, phenolic acid, quinones and coumarins). The first part of this review focuses on the background of CE, which includes principle, instrument, and modes of CE. The second part describes the methods and applications of CE for phytochemical analysis cover the period from 1990 to 2000.

Table 2
Recent examples of isolated phytochemical substances

Compound	Source	References
Flavonoids		
Baicalin, baicalein	*S. radix*	[38]
Rutin	*Fagopyrum esculentum* Monech (Buckwheat)	[39]
Baicalin, baicalein, wagonin 7-*O*-glucuronide, wagonin, oroxylin 7-*O*-glucuronide, oroxylin A	*S. radix*	[40]
Icarin, epimedin B, epimedin C, querecetrin, querceti, luteolin	*E. brevicornum*, *E. humanense*, *E. coactum*, *E. truncatum*	[41,42]
Rutin, orienten, vitexin, quercetin, isovitexin, isoorientin	*Fa. esculentum*	[43]
Alkaloids		
Atropine, scopolamine	*Solanaceous plant*	[44]
	Datura candids, *D. aurea*	[45]
	Hyoscyamus alleus	[46,47]
Berberine, palmatine, jatrorrhizine	*R. coptidis*, *Mahonia genus*	[48,49]
Sophoridine, sophocarpine, oxymatrine, oxysophocarpine	*S. flavescens*	[50]
Isoquinoline alkaloids	*Eschscholzia californica*, *Hydrastis canadensis*, *Berberis vulgaris*, *Jateorhiza palmata*, *Chelidonium majus*	[51]
Psilocybin, baeocystris	*Ps. semilanceata*	[52]
Terpenoids triterpenoids		
Obtusol, zamanic acid	*Plumeria obtusa*	[53]
Ursolic acid, betulinic acid, β-amyrin, 27-E-4-hydroxycinnamoyl oxybetulinic acid	*Melilotus messanensis*, *M. messanensis*	[54]
6α-Hydroxy-3-epi-oleanolic acid, 3α, 27-dihydroxy-olean-12-ene	*Plumeria rubra*	[55]
Glomeric acid	*Pfaffia glomerata*	[56]
Taraxerone	*Neolitsea villosa*	[57]
Hookeroside A, B, C and D	*Pterocephalus hookeri*	[58]
Macrophyllicin	*Primula macrophylla*	[59]
Sesquiterpene		
Linderane, linderalactone, pseudoneolinderane, (+)-linderadine, villosine, isolinderalactone, pseudoneoliacine	*Neolitsea villosa*	[57]
Quinones		
Thymoquinone, dithymoquinone, thymohydroquinone, thymol	*Nigella sativa*	[60]
Coumarin		
Herniarin, coumarin, umbelliferone, aesculetin, dihychocoumarin, coumarinic acid, 4-hydroxycoumarin	*C. segetum*	[61]
Sagecoumarin	*Salvia offinalis*	[62]

2. Capillary electrophoresis

2.1. Principle

The unique characteristics of CE are that the separation is obtained by differential migration of solutes in an electric field and electrophoresis is performed in narrow-bore capillaries filled with electrolyte [67]. Migrations of analytes in CE are driven by two forces, the electrophoretic migration and the electro-osmotic flow (EOF). The EOF or 'bulk flow' is resulted from the charged of the inner wall of the capillaries during application of an electric field. The fused silica capillaries contain silanol groups (pI = 1.5), which are easily ionized under most conditions, resulting in the negatively charged of the inner wall [68]. Positively-charged ions from the electrolyte are attracted to the negatively-charged wall and an electrical double layer is formed. Upon applying a voltage, cations carrying water migrate toward the cathode. Therefore, a net flow of the electrolyte toward the cathode is obtained. In a typical CE instrument, analytes are introduced at the anode and are detected at the cathode, cations migrate first with the highest velocities toward the cathode by the combination effects of electrophoretic flow and EOF. Anions migrate last by the force difference of the EOF toward the cathode and the electrophoretic flow from the anode in the opposite direction. Neutral compounds migrate out from the capillary by the effect of EOF and are not well separated [69]. The EOF flow causes the flat profile during a separation, thus the driving force of flow is uniformly distributed, without the pressure drop from pump.

2.2. Instrument

The instrumentation of CE is simple, which consists of a capillary tube, electrolyte reservoirs, electrodes, a detector and a voltage power supply. Most capillary tubes are made of silica since it is inert and inexpensive. A typical tube is 25–75 cm long with an outer diameter of 300–400 μm and inner diameter of 25–75 μm. Detection of phytochemical substances in CE can be directly performed on-column using either direct [70] or indirect method [71,72]. The most widely used detector for monitoring of plant metabolites is an ultra-violet (UV) spectrophotometer [66]. Factors affecting phytochemical analysis by CE include types, concentrations and pHs of electrolytes, voltage, temperature, capillary dimension and sample loading methods [42,73–80]. Sample injection in CE can be performed by hydrodynamic, electrokinetic injection and on-capillary sample concentration. Hydrodynamic injection bases on differences of pressure at the inlet and the outlet, which can be done by applying pressure at the inlet, applying vacuum at the outlet or by elevating the inlet (siphon effect). In electrokinetic injection, a low voltage of 5–10 kV is applied during injection. The injection voltage is typically 3–5 times lower than the separating voltage [69]. On-capillary sample concentration is an injection technique, which samples are concentrated prior the separation. Isotachophoresis is one of CE mode that can be used for on-capillary sample concentration.

2.3. Modes

Several modes of CE are available for separation of various types of analytes: (1) capillary zone electrophoresis (CZE), (2) micellar electrokinetic chromatography (MEKC), (3) capillary gel electrophoresis (CGE), (4) capillary isoelectric focusing (CIEF), (5) capillary isotachophoresis (CITP), (6) capillary electrochromatography (CEC) and (7) non-aqueous CE [68]. Among these modes, CZE and MEKC are the most widely used methods that have been applied for phytochemical analysis [66]. CZE is the simplest and most versatile CE mode, which the separation is based on differences in the charge-to-mass ratio and analytes migrate into discrete zones at different velocities [69]. Anions and cations are separated by CZE due to electrophoretic migration and the EOF, while neutral species co-elute with the EOF. MEKC, a hybrid technique between electrophoresis and chromatography, is a CE mode in which surfactants above the critical micelle concentration (CMC) are added into the electrolyte to form micelles. Surfactants are molecules that contain

Table 3
Applications of CE to the analysis of flavonoids

	Compounds	References
Flavonoids	Tea polyphenol	[82]
	Medicarpin, vestitone	[83]
	Baicalin, baicalein (*S. radix*, *A. radix*)	[38]
	Flavonoid aglycone (*Fa. esculentum*)	[39]
	Icariin	[84]
	Baicalin, Baicalein, wogonin 7-*O*-glucoronide, wogonin, oroxylin A 7-*O*-glucuronide, oroxylin A (*S. radix*)	[40]
	Flavonoid glycoside, aglycone (*Eucommia ulmoides*)	[85]
	Icariin, epimedin B and C (*Epimedium* sp.)	[41]
	Flavonoid glycosides (*Epimedium* sp.)	[42]
	Kaempferol-3-rutinoside, rutin, aviclrin, quercitrin, isoquercitrin, isorhamnetin, kaempferol, quercetin	[76]
	Flavonoids glycosides (hesperidin, neohesperidin, narirutin)	[86]
	Flavonoid aglycones (Honey)	[87]
	Flavonoid aglycones (Honey)	[88]
	Flavonoid glycosides	[78]
	Flavonoid glycosides	[89]
	Flavonoid glycosides	[90]
	Flavonoid glycosides (*Tilia* sp.)	[91]
	Flavonoid C-glycosides (*Sambucus* sp.)	[92]
	Flavonoid glycosides	[73]
	Flavonoid aglycones and glycosides	[79]

both polar head groups (e.g. cationic, anion, neutral or zwitter ionic) and non-polar hydrocarbon tails. The hydrophilic polar head groups point outward, whereas the hydrophobic non-polar tails point toward the center of the micelles. During MEKC separation, non-polar portions of neutral solutes are incorporated into the micelles and migrate at the same velocity of the micelles, while the polar portions are free and migrate at the EOF velocity. The distribution coefficient between the micellar and non-micellar phase greatly influences the migration velocity of the analytes [81].

3. Application of capillary electrophoresis on phytochemical substances

Applications of CE on analyses of phytochemical substances are extensive (Tables 3–5), for example flavonoids [38–42,73,76,78,79,82–89], alkaloids [45–48,50,52,75,77,80,93–116], terpenoids [117–123] and other phenolic compounds such as phenolic acid, quinones (e.g. hydroquinone (HQ) and antraquinone) and coumarins [61,73,78,118,124–128]. Selected examples of recent analyses of these compounds by CE are discussed in this review.

3.1. Flavonoids

Flavonoids are major metabolites of numerous plants and are also known as natural pigments in several fruits and vegetables. Basic structure of these compounds contains phenolic rings with 2-phenylbenzopyrone (flavone) backbone, which are two phenolic rings connected with three carbon atoms (Fig. 1). Flavonoid derivatives differ in their substituents, the number and position of hydroxyl, methoxy groups and the number of sugar in the molecules. Flavonoids in medicinal plants are complex and usually appear as mixtures. In addition, the plant from individual source contains varied amount and quality of the flavonoids. CE particularly MEKC, are used for analysis of various flavonoids such as tea polyphenol, flavonoid aglycones, and flavonoid glycosides (Table 3).

Consumption of tea is gaining popularity since tea leaf contains polyphenols, which show anticarcinogenic effects in human. In green tea, the major polyphenols are (−)-epigallocatechin-3-gallate (EGCG) and other catechins, whereas in black tea they are theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin-3,3′-gallate. Lee and Ong [82] demonstrated the comparative analysis of tea cathechins, theaflavins in green and black teas and six other tea ingredients by CE and reversed-phase HPLC (RP-HPLC). The catechins are (+)-catechin (C), catechin gallate (CG), (−)-

Table 4
Applications of CE to the analysis of alkaloids

	Compounds	References
Tropane alkaloids	Hyoscyamine, littorine (*Datura* sp.)	[46]
	Atropine, homatropine, ipratropium, scopolamine, butyscopolamine	[93]
	Littorine, hyoscyamine (*Datura* sp.)	[45]
	Atropine, scopolamine, hyoscyamine, littorine (*Datura* sp.)	[47]
	Littorine, hyoscyamine	[94]
Quizolizidine alkaloids	Matrine, sophocarpine, oxymatrine, oxysophocarpine, sophoridine, cytisine, sophoramine, aloperine, lehmannine, dauricine (*Sophora* sp.)	[50]
Apoporphine alkaloids	Lindcarpine, laurolitsine, *N*-methyllindcarpine, boldine, norpredicentrine, isocorydine, laurotetanine, *N*-methyllaurotetanine, isoboldine	[80]
Purine alkaloids	Caffeine, theobromine, and theophylline	[95]
Quaternary alkaloids	Berberine, palmatine, jatrorrhizine (*Mahonia* sp.)	[96]
	Sanguinarine, chelerythrine	[97]
	Berberine (*R. coptidis*)	[48]
	Berberine, isoguanosine	[98]
	Berberine, palmatine	[99]
	Coptisine, berberine and palmitine (*Coptidis* sp.)	[100]
	Coptisine, berberine and palmitine (*Phellodendron* sp.)	[101]
	Coptisine, berberine and palmitine (*Phellodendron* sp.)	[102]
Indole alkaloids	Psilocybin, baeocystin (*Ps. semilanceata*)	[52]
Oxindole alkaloids	Pteropodine related alkaloids (*Uncaria tomentosa*)	[103]
Opium alkaloids	Morphine, 6-monoacetylmorphine, heroine	[104]
	Morphine	[105]
	Morphine, codeine, thebaine, noscapine, papaverine	[106]
	Morphine and cocaine	[107]
Imidazole alkaloids	Pilocapine and isopilocarpine (*Pilocarpus jaborandi)*	[108]
Protoalkaloids	Ephedrine and pseudoephedrine (*Ephedra*)	[109]
	Ephedrine alkaloids	[110]
Capsacinoids	Capsaicin, capsantin, etc. (*Capsicum* sp.)	[75]
Glucosinolates	Glucosinolates and desulphoglucosinolates (*Brassica* sp.)	[77]
	Glucosinolates and desulphoglucosinolates	[78]
Tobacco alkaloids	Nicotine, nornicotine, anabasine, anatabine	[111,112]
	Nicotine	[113]
	Nicotine, nornicotine, myosmine, anatabine, anabasine	[114]
Beta-carboline alkaloids	Harmine, harmol, harmaline	[115]
Various class of alkaloids	Indole alkaloids, protoberberines, benzophenanthridines, beta-caboline alkaloids, isoquinolines	[116]

Table 5
Applications of CE to the analysis of terpenoids and other phenolic compounds

	Compounds	References
Terpenoids		
Monoterpenes	Alpha-pinene, beta-pinene, camphene, limonine	[117]
	Monoterpene glycosides (Paeoniflorin, oxypaeoniflorin, etc.) (*Paeonia* sp.)	[118]
Diterpenes	Diterpene glycosides (Stevia rebaudiana)	[119]
	Gibberellins	[120]
Triterpenes	Cardiac glycosides (*Digitalis lanata*)	[121]
	Phytoecdysteroids (*Silene onites*)	[122]
	Ginseng saponins (*Panax ginseng*)	[123]
Phenolic acids	Gallic acid and derivatives (*Paeonia* sp. root)	[118]
	Arbutin, resorcinol, hydroquinone and gallic acid (*Arctostphylos uva-ursi*)	[124]
	Phenolic acid derivatives (apple juice)	[125]
	Phenolic acids (*Brassica* sp.)	[73]
	Phenolic acids	[78]
Quinones	Pyrroloquinoline quinone	[126]
	Hydroquinone and derivatives	[127]
	Anthraquinones	[128]
Coumarins	Coumarins	[78]
Closely related Courmarins	Herniarin, courmarin, umbelliferone, aesculetin, dihydrocoumarin, coumarinic acid, 4-hydroxycoumarin (*C. segetum*)	[61]

epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG), the theaflavins (TFs) are theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate and theaflavin-3,3′-gallate and other ingredients include caffeine, adenine, theophylline, quercetin (Q), gallic acid and caffeic acid. A gradient HPLC elution was performed on a C_{18} column (110×4.6 mm^2 I.D.) using two mobile phases containing different composition of acetonitrile and trifluoroacetic. Under these conditions, all analytes were separated within 27 min with the detection limits of 0.2 μg/ml for caffeine, 0.5 μg/ml for TFs, 0.1 μg/ml for EGC, EGCG and Q and 0.05 μg/ml for the rest of the analytes. The CE conditions were electrolyte consisting of acetonitrile–boric acid (pH 7.2; 200 mM), potassium dihydrogenphosphate (pH 4.5; 100 mM) containing β-cyclodextrin (20 mM) (72.5:27.5, v/v); capillary 40 cm × 50 μ I.D.; applied voltage 25 kV; temperature 30 °C; UV absorbance at 205 nm; injection pressure 2.5 kPa 3 s. CE provided baseline separation of sixteen tea ingredients within 10 min, but the sensitivity was about five times lower than HPLC method. In HPLC, the percentage of relative standard deviations (RSDs) of the retention times for intra-day and inter-day were < 3 and $< 5\%$, respectively, whereas in CE they were < 1 and 2%, respectively. Linearity of C, CG, EC and ECG (over a range of 0.05–50 μg/ml), EGC and EGCG (over a range of 0.05–100 μg/ml) and TFs (over a range of 0.5–100 μg/ml) was good with the correlation coefficient of > 0.9998 by HPLC and > 0.990 by CE. Both methods were used for quantitation of polyphenols in different teas including Japanese green tea, Long-jin, Jasmin, Chrysanthemum, Pu-erh, Iron Buddha, Oolong tea and Ceylong tea. TFs value from both methods were comparable with the correlation coefficient of > 0.97 although the values by CE analysis were generally higher than by HPLC. This was due to the instability of TFs in a longer analysis time in HPLC column. Analysis of green and black tea samples showed that the intra-day precision was $> 90\%$ and the inter-day was $> 75\%$ by both methods. The results indicated that HPLC and CE were capable for analysis of all known polyphenols in green and black tea. The former method was more sensitive, while the latter was more rapid and consumed less samples and solvent.

Larger et al. [129] analyzed cathechins from other tea including instant green, Darjeeling and black Assam tea by CE. In this study, different solvents (e.g. ethyl acetate, methanol and water) were used for extraction of various tea components. Results showed that the addition of organic modifier greatly affect CE separation of these tea components. A sharp caffeine peak was obtained in the presence of 10% acetonitrile in the running buffer, while a broad peak was obtained in the absence of acetonitrile. In addition, other factors such as micelle charge type, micelle concentration, surfactant type, buffer pH, buffer modifier (e.g. cyclodextrin) can affect CE separation of cathechins [130].

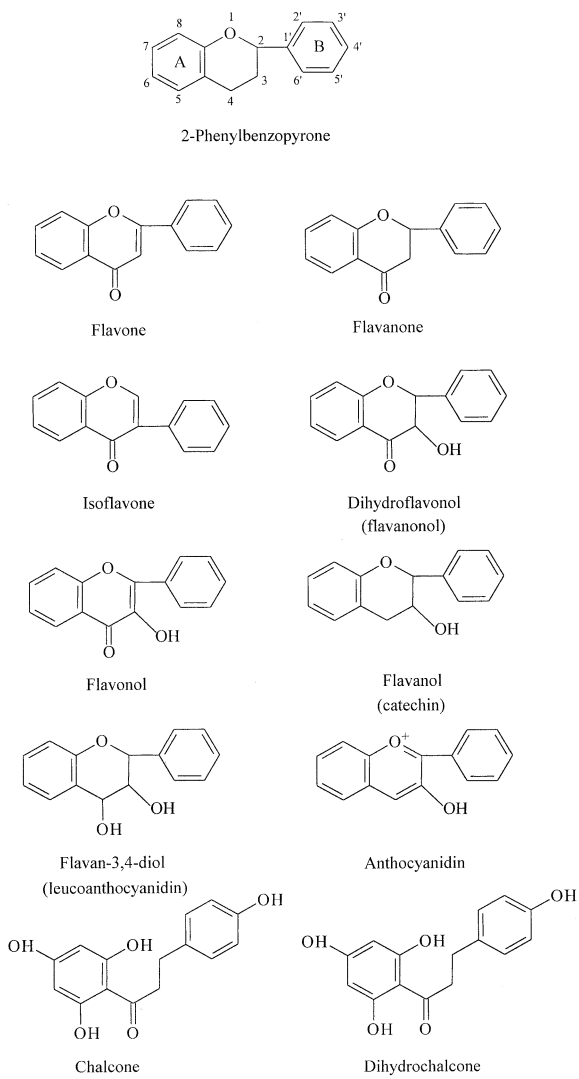

Fig. 1. Structures of various flvonoids.

Enantiomeric separation can be achieved by MEKC using cyclodextrins, chirally functional co-micelles, enantioselective metal chelation or bile salts as chiral selectors. This technique has been applied for analyses of several enantiomeric flavonoids. For example, MEKC separation of enantiomeric flavonoids of medicarpin and vestitone, from transgenic plant extracts were feasible using the optimized electrolyte consisting of methanol–sodium borate (pH 10.0; 25 mM) containing hydorxypropyl-β-cyclodextrin (2 mM) and hydroxylpropyl-γ-cyclodextrin (20 mM) (10:90, v/v) [83]. The resolution obtained was 1.47 for medicarpin enantiomers and 1.80 for vestitone enantiomers, respectively, and the total migration time was 12 min.

The use of markers can enhance the repeatability and reproducibility of CE of flavonoids. Liang et al. [41] determined flavonoids from extract of *Epimedium brevicornum*, *Epimedium humanense*, *Epimedium coactum*, and *Epimedium trancatum* such as icarin, epimedin B, epimedin C and eight other compounds. The relative migration times of flavonoids in the extract varied from 0.7 to 6.4%. Validation of the method showed that the repeatability of migration time calculated with two markers was $< 0.5\%$. In another study, MEKC with the two-marker (xanthene-9-carboxylic acid and meso-2,3-diphenylsuccinic acid) technique was successfully used to separate sixteen pharmacologically active flavonoids and one phenylethanoid glucoside isolated from *Epimedium* species [42]. Factors affecting the analysis were optimized, which included types of buffers, SDS concentrations, organic modifiers, voltage and effective capillary length. Baseline separation of fourteen flavonoids and one phenylethanoid glucoside was obtained within 20 min in the buffer system of sodium borate (pH 8.5; 20 mM) containing SDS (48 mM) and 1,3-diaminopropane (1 mM) and was monitored at wavelength 254 nm. Results showed that the two-marker technique enhanced the repeatability of analysis, the repeatability of identification of two

successive peaks and the reliability of identification.

Baicalin and baicalein are major flavonoids in *Scutellariar radix*, but are absent in *Astragali radix*. Thus, determination of baicalin and baicalein content can be used to differentiate *S. radix* from *A. radix*. Chen et al. [38] employed CE with and electrochemical detection to determine these two compounds. The optimum conditions were a 40 cm capillary tube with a separation voltage of 12 kV, the carbon disc electrode working at a potential of 0.90 V (vs. SCE) and the electrolyte consisting of sodium borate (pH 9.0; 100 mM). The conditions were stable and reproduced with the RSD% of $< 5\%$ for both migration time and peak current. Qi et al. [40] demonstrated the analysis of six flavonoids in *S. radix* by MEKC using an UV detector. The flavonoids including baicalin, baicalein, wogonin 7-*O*-glucuronide, wogonin, oroxylin A 7-*O*-glucuronide and oroxylin A could be analyzed within 15 min. The running electrolyte composed of sodium dihydrogen phosphate–sodium borate (pH 7.24; 20:25 mM) and SDS (20 mM) and the detection wavelength was 254 nm.

3.2. Alkaloids

Alkaloid (Fig. 2) is one of the most important and largest groups of plant secondary metabolites. It shows various clinically usefulness ranking from analgesic to antiarrhythmic. RP-HPLC and GC are the widely used method for analysis of alkaloids. Coupling of both techniques to mass spectrometry (MS) provides analytical and structural information of alkaloids. Analysis of complex alkaloid mixtures and certain alkaloids, however, are limited by these methods. Recently, CE has been employed for solving of various analytical problems including of analysis of plant secondary metabolites and natural products. Alkaloids are the second most frequent application of CE in phytochemical analysis (Table 4). These compounds are readily separated by CE using low pH electrolyte since they can be easily protonated giving the positive charges acidic solution.

Separation of quaternary alkaloids such as berberine, palmatine and jatrorrhizine was

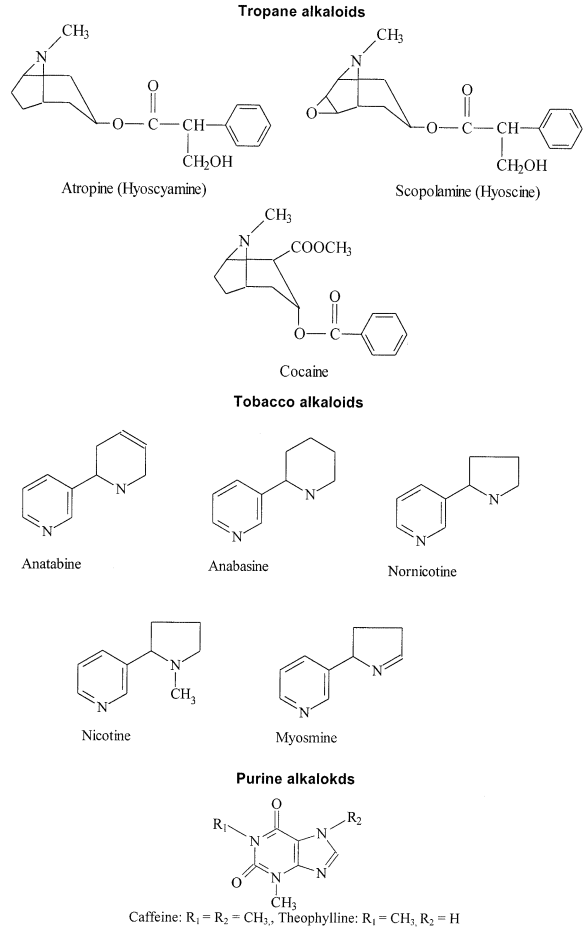

Fig. 2. Structures of various alkaloids.

demonstrated in methanol–sodium phosphate (pH 7.0; 100 mM) (33:67, v/v) using brucine as an internal standard [96]. The alkaloids were baseline separated within 5.0 min. The method showed good linearity in a range of 0.004986–0.4986 mg/ml ($r = 0.9990$, $n = 5$) for berberine, 0.005049–0.5049 mg/ml ($r = 0.9996$, $n = 5$) for palmatine and 0.005058–0.5058 mg/ml ($r = 0.9984$, $n = 5$) for jatrorrhizine. The precision calculated from RSD% were 1.56, 1.02 and 1.60% ($n = 6$) for berberine, palmatine and jatrorrhizine, respectively. The accuracy determined from recoveries were 96.00–101.66% for berberine, 100.15–102.97% for palmatine and 96.68–102.44% for jatrorrhizine, for all the alkaloids.

Sevcik et al. [97] compared the CE and HPLC analysis of sanguinarine and chelerythrine in plant extracts and pharmaceutical preparations. Both compounds are benzo[*c*]phenanthridinium alkaloids isolated from *Sanguinaria canadensis* L. or *Macleaya cordata* (Wild) Br. R. The CE conditions were electrolyte consisting of acetonitrile–phosphate Tris-buffer (pH 2.5; 50 mM) (50:50, v/v); capillary 75 cm (45 cm to UV detector) × 75 μ I.D.; applied voltage 30 kV; temperature 25 °C; UV absorbance at 260 nm; injection vacuum 0.3 s. The HPLC conditions were the solvent system containing methanol–water–triethylamine (78:22:0.1, v/v/v) adjusted to pH 6.15 with phosphoric acid; column: Nucleosil 120-5 C_{18}; flow rate 1 ml/min; temperature 40 °C; UV absorbance at 270 nm; injection volume 20 μl. In CE, the linear correlation over a range of 20–500 μM was $y = 2.373x + 0.0065$, $R^2 = 0.9988$ for sanguinarine and $y = 2.983x + 0.0019$, $R^2 = 0.9991$ for chelerythrine. The detection limits for CE analysis were 3 and 2.4 μM for sanguinarine and chelerythrine, respectively. The RSD% for the migration time was 1.8% for both alkaloids and for concentration were 4.9% sanguinarine for and 4.4% for chelerythrine. In HPLC, the linear correlation over the same range of concentration was $y = 18.35x - 92.61$, $R^2 = 0.9999$ for sanguinarine and $y = 14.91x - 29.97$, $R^2 = 0.9999$ for chelerythrine. The detection limit for HPLC analysis for both alkaloids was 3 μM. The RSDs% for the concentration were 5.9% sanguinarine for and 4.6% for chelerythrine. Both methods were employed for determination of sanguinarine and chelerythrine content in plant products (Fig. 3) and showed similar results with mutual correlation of $y = 0.9943x + 0.0009$, $R^2 = 0.9999$ ($n = 16$). The results from both CE and HPLC were comparable, but CE offered a shorter analysis time without pre-treatment of samples and lower cost.

Isoguanosine and berberine in the extract of the traditional Chinese medicinal herb showed the potential of anticancer activity [98]. Analysis of both compounds was achieved within 14 min by CZE using sodium citrate (pH 2.7; 100 mM) as the running buffer. The separation voltage was 12 kV and the detection wavelength was at 254 nm. The concentration required for the quantitation of both compounds was in the range of 0.1–50 μg/ml. The reproducibility of the method calculated from the RSDs% of the migration time for isoguanosine and berberine were 0.22 and 1.32% and the RSDs% of the peak area were 2.8 and 3.2%, respectively. This method was rapid, simple and required only small sample volume without the use of organic solvent in the running buffer.

Non-aqueous CE is a novel mode of CE with enhanced selectivity [131]. The technique is useful for analyses of hydrophobic compounds and compounds which is readily absorbed on the capillary wall [131]. Types of organic solvents greatly influence the selectivity of small molecules with closely related mass to charge ratio [132,133]. Li et al. [48] employed non-aqueous CE for the determination of berberine in *Rhizoma coptidis* and its

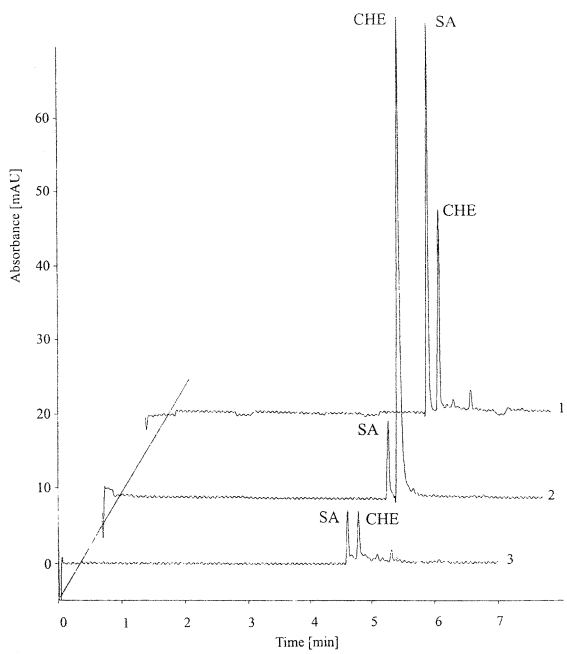

Fig. 3. Capillary electropherograms of *M. cordata* (1) and *Dicranostigma lactucoides* (2) QBA fractions, and of a 1:1 diluted oral rinse (3) SA, sanguinarine; CHE, chelerythrine. Experimental conditions: electrolyte consisting of acetonitrile–phosphate Tris-buffer (pH 2.5; 50 mM) (50:50, v/v); capillary 75 cm (45 cm to UV detector) × 75 μ I.D.; applied voltage 30 kV; temperature 25 °C; UV absorbance at 260 nm; injection vacuum 0.3 s. Reprinted from Ref. [97] with permission from Elsevier Science.

preparations. The optimized electrolyte was sodium acetate (75 mM) containing acetic acid (1 M) in methanol. The linearity of the technique was in the range of 25–200 μg/ml and the reproducibility determined from the RSD% was $< 2\%$. Non-aqueous CE was also used for analysis of ten quinolizidine alkaloids from extracts of traditional Chinese herbal drugs, e.g. *Sophora flavescens* Ait (Kushen), *S. alopecuroides* L. (Kudouzi or Kugancao) and *S. tonkinensis Gapneo* (Shandougen) [50]. The alkaloids were matrine, sophocarpine, oxymatrine, oxysophocarpine, sophoridine, cytisine, sophoramine, aloperine, lehmannine and dauricine. The ten alkaloids were well separated within 18 min using the background electrolyte containing tetrahydrofuran–acetic acid–ammonium acetate (50 mM) (10:0.5:89.5, v/v/v) in methanol. The linearity of the method was over a range of 2.51–50.1 μg/ml for sophoridine and sophocarpine, 2.71–54.2 μg/ml for matrine, 3.30–65.9 μg/ml for oxymatrine and 3.10–62.0 μg/ml for oxysophocarpine. The recovery was 98.0–101.3% for five alkaloids with RSDs% of 1.03–2.68% and the detection limit for all alkaloids were in within 0.93–2.31 μg/ml.

The structural related indole alkaloids psilocybin and baeocystin in hallucinogenic mushrooms *Psilocybe semilanceata* are zwitterionic compounds that are difficult to separate by MEKC either using cationic or anionic micelles [52]. However, analysis of the two indole alkaloids was successfully obtained by derivatization with propyl chloroformate prior CZE at pH 7.2 [52]. The selected pH was used in order to enhance the electrophoretic mobility of the analytes.

CZE was proven to be a powerful tool for monitoring the major alkaloid in tobacco such as nicotine, and minor alkaloids, nornicotine, anabasine and anatabine. Analysis of these compounds is of great importance for the Bureau of Alcohol, Tobacco and Firearms (ATF)-regulated products. Ralapati [112] demonstrated that the optimum conditions were sodium phosphate buffer (pH 2.5; 25 mM); capillary 44 cm × 100 μ I.D.; applied voltage 10 kV; temperature 20 °C; UV absorbance at 260 nm; injection hydrodynamically 2 s. At a higher pH (pH 6.9), nicotine appeared as monoprotonated compound and migrated with the higher electrophoretic mobility ($t_m = 2.99$ min) than at a lower pH (pH 2.5, $t_m = 26.63$ min). The peak area counts at the higher pH (7464) was greater than at the lower pH (4422), however, the UV absorbance was maximized at the lower pH. Thus, a lower pH was favorable. Calibration curve over a range of 1.724–17.24 μg/ml showed a correlation coefficient of 0.9915. Migration time precision of nicotine at two different concentrations, 1.724 and 17.24 μg/ml, calculated from coefficient variation was 0.69 and 1.09%, respectively. For peak area count, the values were 9.04 and 4.29, respectively. The method was employed for determination of nicotine in beedi, a form of cigarette found in India, cigarette, cigar, pipe tobacco, roll-your-own (RYO) tobacco, chewing tobacco and snuff. Samples were prepared by extracting about 100-mg tobacco with 10 ml of deionized water, shaking for 1 h and filtering through a 0.2 μm filter prior injection. The nicotine contents found in beedi, cigarette, cigar, habana and herbal filter were 3.49, 1.75, 1.39, 1.70 and 0.00%, respectively. In all samples, nicotine appeared as a single peak around 6.0–6.4 min and was well separated from the other peaks. In another study, anionic surfactant, 100 mM SDS, was recommended in the running electrolyte to enhance the separation of nicotine, nornicotine, myosmine, anatabine and anabasine extracted from tobacco [111].

Ori et al. [134] established a rapid and simple method for the determination of tropan alkaloids (hyoscyamine and scopolamine) from scopolia extract. MEKC was employed for the analysis of the two alkaloids on a fused silica capillary (56 cm × 75 μm I.D.) containing acetonitrile–sodium borate (pH 10; 20 mM) and SDS (100 mM) (3:97, v/v). The applied voltage was 15 kV and the on-column detection was at 210 nm. The alkaloids were separated within 40 min with good linearity in the range of 4–12 μg/ml ($R^2 = 0.9970$) for hyoscyamine and 390–1150 ng/ml ($R^2 = 0.9976$) for scopolamine.

CE interfaced with MS was efficient for analysis of various classes of alkaloids including monoterpenoid indole alkaloids, protoberberines/benzophenanthridine, beta-carboline alkaloids and isoquinlines. Unger et al. [116] reported the

simultaneous separation of a mixture of fifteen indole alkaloids and biogenic amines. The optimum conditions were the electrolyte containing acetonitrile–ammonium acetate buffer (pH 3.1; 100 mM) (50:50, v/v); capillary 55 cm (50 cm to detector) × 50 μm I.D.; applied voltage 15 kV; temperature 25 °C; UV absorbance at 200 nm; injection 345 mbar s. The electrolyte was selected since it was volatile, which is compatible for CE-MS analysis. The conditions provided baseline separation all analytes except the epimeric indoles, serpentine and alstonine. This condition was successfully carried out with minor modifications of separation voltage to 18 kV and column temperature to 15 °C, for separation of nine alkaloids of a group of ten protoberberines/benzophenanthridines. The protoberberine, which were sanguinarine, berberine and coptisine, migrated first and were well separated between 11.5 and 12.5 min. The second group, which consisted of the methoxylated protoberberine-type, palmatine, benzophenanthridine chelidonine and the isomeric protoberberines columbamine and jatrorrhizine, migrated around 14 min. The latter two co-migrated due to their closely related structures. The only difference was the position of the phenolic hydroxyl group at ring D resulting in similar electrophoretic mobilities. The tetrahydroprotoberberine-type, stylopine and canadine migrated around 15 min, while the dihydroxylated alkaloid, scoulerine migrated at 16.5 min. Additionally, the method provided baseline separation of β-carboline alkaloids, norharmane, harmane, harmaline, harmine, harmalol and harmol, between 10.78 and 13.60 min. Substituents on the alkaloids showed great effect on the electrophoretic mobility and migration order, which was the methoxylated harmaline and harmine followed by the hydroxylated harmalol and harmol. Comparing of harmaline vs. harmine and harmalol and harmol, the more basic compound, harmaline and harmalol showed lower migration time. Opium alkaloids including thebaine, codeine, papaverine, morphine, narcotine and narceine, were also determined under the same conditions. The migration order also depended on the structure-mobility relationship. The lipophilic, methoxylated thebaine migrated first, followed by the monohydorxylated codeine, then the dihydorxylated morphine.

The developed method was transferred to CE-MS analysis with minor modifications in order to avoid contamination of the MS, to decrease the ionic strength of the electrolyte and to minimize the electrical current generated between the inlet vial (+ 30 kV) and the sector-MS electrospray tip (+ 8 kV). The optimum CE-MS conditions for separation of indole-type alkaloids were acetonitrile–ammonium acetate buffer (pH 4; 80 mM) (50:50, v/v); capillary 75 cm (20 cm to UV detector) × 50 μm I.D.; applied voltage 22 kV; temperature 20 °C; injection 1.73 bar s. The electrospray (ES) conditions were sheat liquid, methanol–water–acetic acid (89:10:1), 1 μl/min; U_{ES} 3.5 kV. MS scanning speed 5 dec/s, mass range m/z 100–900. Thirteen of the fifteen indole alkaloids were completely separated, whereas vincristine and raufloridine were not resolved from each other. The CE-MS analysis of isoquinoline alkaloids from opium was performed using the same conditions, except that the MS scanning speed of 3 dec/s and mass range m/z of 150–1000. The ES-MS spectra were characterized by signal-to-noise ratios of 20:1 (morphine) up to more than 100:1 (papaverine), a dominating signal corresponding to $[M + H]^+$ and alkaloid cluster ions such as $[2M + H]^+$, $[2M + Na]^+$ and $[2M + K]^+$. Results from CE-UV and CE-MS analysis showed that the reconstructed total ion current (RIC) provided a smaller signal-to-noise ratio than the UV signal. The presence of dead volume in the ES-MS caused the back-diffusion of analyte ions into the sheath flow and decreased the separation efficiency, which can be compensated by using the single-ion extraction from the full scan RIC data. This study showed that CE coupling with MS provided specific information on identification of alkaloids by combinations of electrophoretic mobility and the molecular weight.

3.3. Terpenoids

Terpenoids can be divided into subgroups according to their structures (Fig. 4). Analysis of terpenoids by CE has been recognized only a few years ago. Most of its applications are determina-

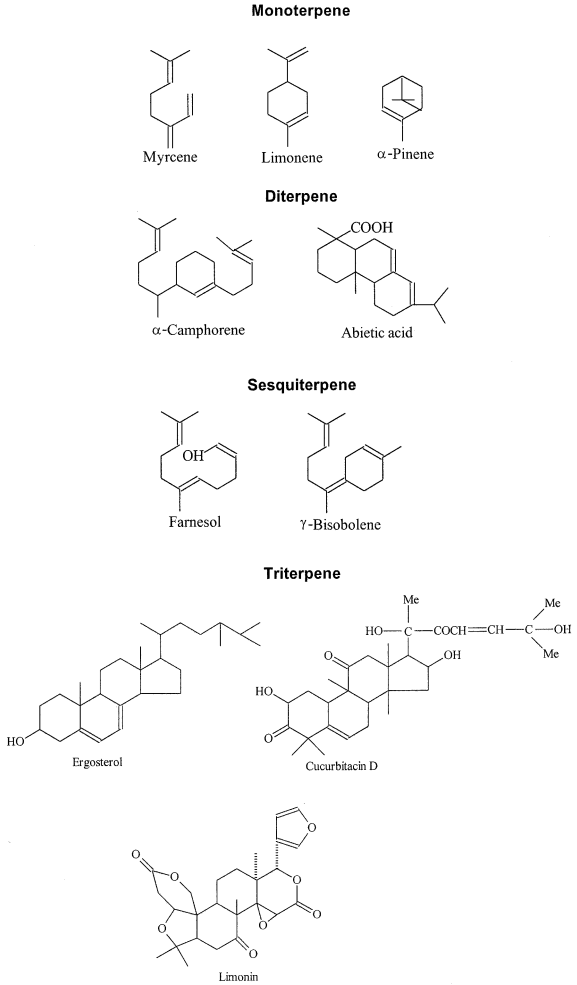

Fig. 4. Structures of various terpenoids.

tion of monoterpenes, diterpenes and triterpenes. Gahm et al. [117] studied the CZE analysis using sulfated β-cyclodextrins and native α-cyclodextrin as chiral additives for the chiral separation of neutral, cyclic and bicyclic monoterpene. The analytes included α-pinene, β-pinene, camphene and limonene. The conditions were sodium phosphate buffer (pH 3.3; 10 mM) containing sulfated β-cyclodextrins (6.5 mM) and α-cyclodextrin (7.5 mM); capillary 57.6 cm (50 cm to detector) × 75 μm I.D.; applied voltage 20 kV (negative polarity); temperature 20 ± 2 °C; UV absorbance at 214 nm; injection, hydrostatic 2 or 4 s. The enantiomeric separation was not obtained, when only sulfated β-cyclodextrin was used as a chiral additive. This was due to the negatively charged of the additive, which acted as non-specific carriers to the anodic site of the capillary. Addition of α-cyclodextrin to the electrolyte enhanced the differences of enantiomer mobilities, which resulted in the enantiomeric separation of the terpenes (Fig. 5). The resolution of enantiomeric α-pinene, β-pinene, camphene and limonene were 25, 12, 12 and 4, respectively. Results also indicated that the hydrophobic-driven complexation between α-pinene and sulfated β-cyclodextrin was important for enantiomeric separation. The order of relative binding strength of monoterpenes to sulfated β-

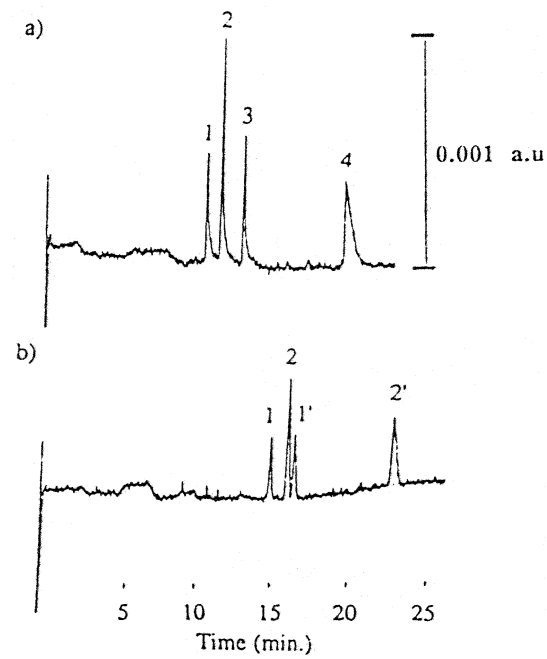

Fig. 5. (a) Electropherogram of the seperation of tetraline (1), (+)-camphene (2) and (−)-camphene (4). Peak 3 is an unidentified impurity. Experimental conditions: electrolyte consisting of sodium phosphate buffer (pH 3.3; 10 mM) containing sulfated β-cyclodextrins (6.5 mM) and α-cyclodextrin (10 mM); capillary 57.6 cm (50 cm to detector) × 75 μm I.D.; applied voltage 20 kV (negative polarity); temperature 20 ± 2 °C; UV absorbance at 214 nm; injection, hydrostatic 2 or 4 s. (b) Electropherograms of the seperation of (−)-limonene (1), (+)-β-pinene (2), (+)-limonene (1′) and (−)-β-pinene (2′). Experimental conditions: same as (a) except α-cyclodextrin (15.1 mM). Reprinted from Ref. [117] with permission from Elsevier Science.

cyclodextrins and α-cyclodextrin was α-pinene > β-pinene ≈ camphene > limonene.

Monoterpene glycosides (paeoniflorin and oxypaeo niflorin) as well as gallic acid and its derivatives (methyl gallate and tannic acid) in *Paeonia radix* were analyzed by CZE [118]. The conditions were borate buffer (pH 10.5; 100 mM); capillary 80 cm (65 cm to detector) × 75 μm I.D.; applied voltage 20 kV; UV absorbance at 254 nm; injection hydrodynamic 5 s per 5 cm. The migration times were 8.14, 10.94, 15.57 and 31.71 min for paeoniflorin, oxypaeoniflorin, methyl gallate and gallic acid, respectively. Tannic acid appeared as broaden multiple peaks between 20.5 and 24.5 min. Paeoniflorin and oxypaeoniflorin are the active ingredients in *P. radix*, which were quantified by the developed method using 3,4-dimethoxycinnamic acid as an internal standard. Linearity of both compounds was achieved in a range of 2–20 ng/nl. For paeoniflorin ($n = 8$), the RSDs% were 2.2 and 3.0% at the concentration of 5 and 20 ng/nl, respectively. For oxypaeoniflorin, the RSDs% were 4.2 and 2.8%, respectively. The temperature control was not applied for the study causing the high variation in migration time, 4.6% for paeoniflorin and 5.1% for oxypaeoniflorin.

3.4. Phenolic acids

Phenolic acids are naturally occurring plant secondary metabolites, which exist as ester or alcohol depending of the plant genera and parts used. For example, cinnamic acid and benzoic acid derivative are available in higher plants, phenolic choline ester are found in cruciferous seed material and phenolic acids are present as esters of malate and carbohydrates in vegetative plant parts.

CZE and MEKC are applicable for analysis of various phenolic acids. For instance, gallic acid and its methyl ether derivatives were separated in the electrolyte containing sodium borate (6 mM) sodium phosphate (10 mM) (pH 8.8) and SDS (100 mM) with the voltage of 18 kV (negative polarity) and the detection wavelength at 215 nm [78]. Under these conditions, hydrophobic interactions and electrophoretic mobility played an important role for separation of anionic solutes.

Bjergegaard et al. [73] determined various benzoic acid derivatives (e.g. salicylic acid, 4-hydroxybenzoic acid and isovanillic acid) and cinnamic acid derivatives (e.g. coumaric acid, ferulic acid, isferulic acid and sinapic acid) by MEKC. The conditions were borate (18 mM) phosphate (30 mM) buffer (pH 7.0) containing cetyltrimethylammonium bromide (CTAB) (50 mM); capillary 67.5 cm (44.5 cm to UV detector) × 50 μm I.D.; applied voltage 20 kV; temperature 40 °C; UV absorbance at 280 nm; injection vacuum 1 s. Selectivity of the separation was based on the differential partitioning of phenolic acids between the aqueous phase and micellar phase formed by CTAB. All phenolic acids as well as the structural isomers such as ferulic vs. isoferulic acid and 4-hydroxybenzoic vs. salicylic acid and *cis–trans* isomers of cinnamic acid derivative sinapic, ferulic and coumaric acid were well separated within 20 min. The migration order was *p*-hydroxybenzoic acid, isovanillic acid, *trans*-sinapic acid, *trans*-ferulic acid, *cis*-sinapic acid, *trans p*-coumaric acid, *cis*-ferulic acid, *cis p*-coumaric acid, isoferulic acid and salicylic acid, respectively.

3.5. Quinones

Sakodinskaya et al. [127] employed MEKC for the separation of HQ and some of its derivatives, hydroquinone monomethyl ether (MHQ), hydroquinone dimethyl ether (DMHQQ), hydroquinone monopropyl ether (PHQ), hydroquinone monobenzyl ether (BHQ) and hydroquinone monophenyl ether (PhHQ). The optimum conditions were methanol–sodium borate buffer (pH 9.5; 10 mM) containing SDS (75 mM) (10:90, v/v); capillary 50 cm (38 cm to detector) × 75 μm I.D.; applied voltage 10 kV; temperature 30 °C, UV absorbance at 254 nm; injection, hydrodynamic 6 s per 10 cm. Higher concentration of SDS caused the longer migration time for phenyl and benzyl ether. Increasing of the voltage reduced the migration time for all compounds due to the higher EOF. However, the peaks were in close proximity with the negative peak of methanol causing difficulty in quantitative analysis. Increasing of pH enhanced the differences of charge-to-mass ratio and differences in hydropho-

bicity, therefore the separation was improved. Stability of the analytes, however, decreased at high pH due to the rapid oxidation. Adding of organic solvent improved the selectivity of the separation and 10% of methanol was optimized. The linearity of HQ was obtained over the range of 5×10^{-5}–8×10^{-4} M with the correlation coefficient of 0.9997. Precision of the method calculated from the RSD% of peak area ration of HQ/caffeine (as an internal standard) was 1.8% ($n = 6$).

Comparative study of CE and HPLC on the separation of anthraquinone-1-sulphonate and its related impurities, anthraquinone-2-sulphonate, anthraquinone-1, 8-sulphonate and anthraquinone-1, 5-sulphonate were investigated by Williams et al. [128]. The optimum conditions for CE were buffer consisting of boric acid (8 mM) sodium borate buffer (pH 10; 50 mM); capillary 72 cm (50 cm to detector) × 50 μm I.D.; applied voltage 20 kV; temperature 30 °C, UV absorbance at 254 nm; injection 1 s vacuum. For HPLC, the conditions were acetonitrile–water (60:40, v/v) containing 0.8% w/v CTAB; column 25 cm × 4.5 mm ODS Hypersil; flow rate 1 ml/min; temperature 40 °C; detection UV absorbance at 254 nm; injection 10 μl. Separation from both techniques was obtained between 14 and 17 min and the efficiency calculated from number of theoretical plate was 1.2×10^5–6.7×10^4 for CE and 1.8×10^4–2.6×10^3 for HPLC. Anthraquinone-1-sulphonate was better resolved from anthraquinone-2-sulphonate by HPLC than by CE, but CE provide better peak shape for all compounds. The linearity of anthraquinone-1-sulphonate calculated from peak area was achieved over the range of 0–350 μg/ml with the correlation coefficients of 0.99995 and 0.99999 for HPLC and CE, respectively. The limit of detection for anthraquinone-1-sulphonate was 0.7 μg/ml for CE and 0.006 μg/ml for HPLC, while the mass detection limits were 2.8 and 56 pg, respectively. The excellent mass detection limit of CE was due to the small injection volume (0.004 μl), compared with HPLC (10 μl). Precision of the method calculated from the RSD of peak area was 0.5% by HPLC and 2% by CE. The reproducibility of CE was limited by injection, which can be improved by the multiple injections or the used of an internal standard.

Glatz et al. [126] determined pyrroquinonline quinone (PQQ) by CZE. Results showed that a buffer consisting of β-alanine-HCl (pH 3.0; 50 mM,); capillary 64.5 cm (56 cm to detector × 50 μm I.D. with extended light path; applied voltage 25 kV (negative polarity); temperature 25 °C; UV absorbance at 249 nm and injection 50 mbar for 6 s was optimized for the separation. The conditions provided a sharp peak of PQQ with a short migration time (3.2 min) and a stable baseline. Linearity of PQQ calculated from peak area was performed in the range of 5–500 μM and the correlation coefficient was 0.9998. Detection limit of the CZE method was in the range of 0.1–0.2 μM at a signal to noise ratio of 3, comparing to 0.05–0.08 μM using HPLC and fluorescence detection, 0.01 μM using HPLC and amperometric electrochemical detection and 0.1 μM using isotachophoresis and potential gradient and UV detection. Intra-day reproducibility calculated from peak area was 2.5% and the inter-day reproducibility of the migration time was $< 0.18\%$.

3.6. Coumarins

Coumarins are naturally occurring benzopyran derivatives in plants and essential oils. They are widely used as fragrance in perfumes, toothpaste and tobacco products. In addition, coumarins show activity in treatment of various diseases such as brucellosis, burns, rheumatic and cancer. Ochocka et al. [61] demonstrated the separation of coumarin derivatives including 7-methoxycoumarin, coumarin, 7-hydroxycoumarin, 6,7-dihydroxy-coumarin, dihydrocoumarin, coumarinic acid and 4-hydroxycoumarin by CE. The electrophoretic conditions were boric acid (0.2 M) borax (0.05 M) in water (11:9, v/v) (pH 8.5); capillary 58 cm (51 cm to UV detector) × 50 μm I.D.; applied voltage 25 kV; temperature 25 °C; UV absorbance at 280 nm; pneumatic injection 7 s. All coumarins were completely separated except for 7-methoxycoumarin and coumarin, which co-eluted. However, both derivatives are not naturally co-existed in most plants. The method was

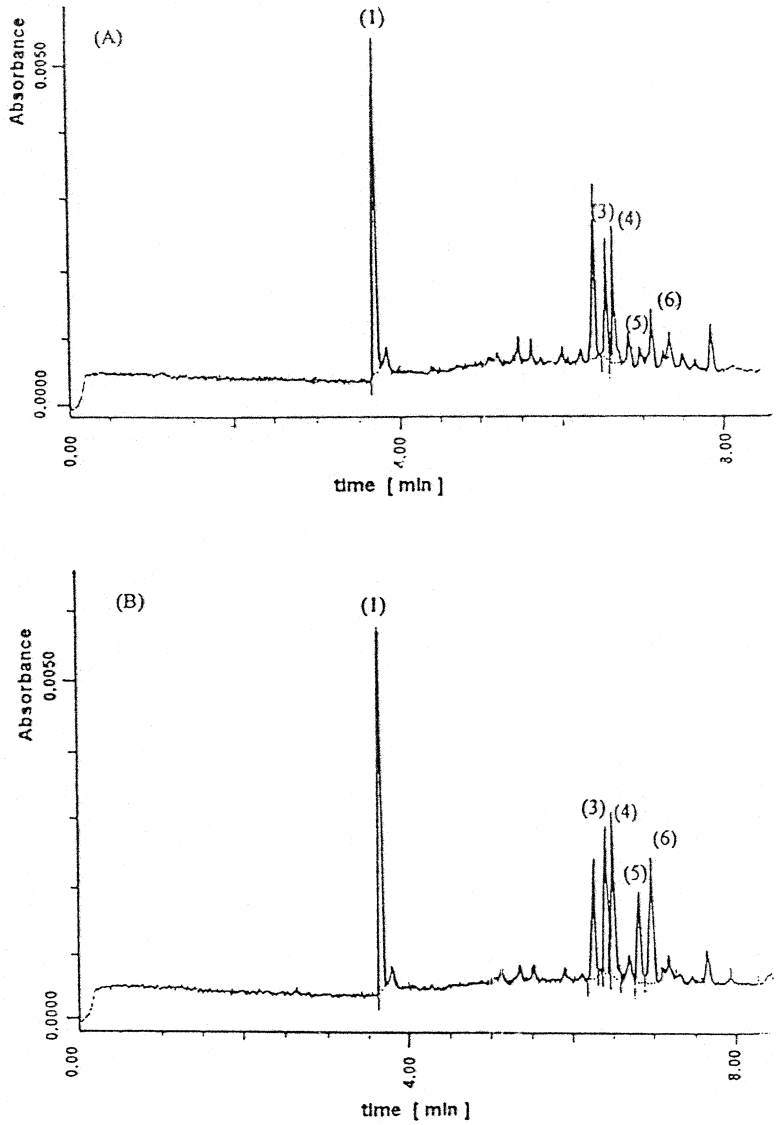

Fig. 6. (A) CE seperation of an extract obtained from aerial parts of *C. segetum* L. Peaks: 1 = 7-methoxycoumarin (herniarin); 3 = 7-hydroxycoumarin (umbelliferone); 4 = 6,7-dihydroxy-coumarin (aesculetin); 5 = dihydrocoumarin; 6 = coumarinic acid. (B) The same extract spiked with standards. Experimental conditions: boric acid (0.2 M) borax (0.05 M) in water (11:9, v/v) (pH 8.5); capillary 58 cm (51 cm to UV detector) × 50 μm I.D.; applied voltage 25 kV; temperature 25 °C; UV absorbance at 280 nm; pneumatic injection 7 s. Reprinted from Ref. [61] with permission from Elsevier Science.

applied for determination of coumarins from aerial and root parts of *Chrysanthemum segetum* L. From CE analysis, 7-methoxycoumarin (26%), 7-hydroxy-coumarin (18%), 6,7-dihydroxycoumarin (12%) dihydrocoumarin (11%) and coumarinic acid (4%) were found in the aerial part (Fig. 6). Whereas, 7-methoxycoumarin (40%), 7-hydroxycoumarin (30%) and dihydrocoumarin (5%) were major coumarins in the root part. The RSDs% of coumarins found in the aerial part were between 0.002 and 1.9, while in the root the values were between 0.4 and 5.6%.

MEKC using SDS and cetyltrimethylammonium chloride (CTAC) micelles at pH 7.0 was employed for the separation of uncharged natural compounds, such as coumarin derivatives or desulphoglucosinolates, and charged solutes, such as phenolic carboxylic acids or glucosinolates [78]. The migration order was 2-coumarone, 3-acetyl coumarin, 7-hydroxy-4-methyl coumarin, 7-methoxy coumarin, 7-methyl coumarine and 5,7-dimethoxy coumarin.

4. Conclusions

CE, namely CZE and MEKC mode, is an important tool for qualitative and quantitative analysis phytochemical substances. Different classes of these compounds including flavonoids, alkaloids, terpenoids, phenolic acid, quinones and coumarins, have been analyzed by this technique. Analysis of these compounds by CE are usually confirmed or compared with the well-established HPLC. Results from both techniques are mostly comparable. For instance, CE and HPLC provided the similar linearity and precision for the analysis of sixteen tea ingredients except that CE sensitivity was five times lower. In the case of sanguinarine and chelerythrine alkaloids, the linearity, precision and limits of detection by both methods showed no significant differences. Linearity of antraquinone-1-sulphonate by CE and HPLC was excellent, but the precision and detection limits by HPLC were better than by CE. Precision of CE can be improved be multiple injections or uses of internal standards. Comparing with other technique, CE offer advantages in terms of high efficiency, simplicity, low solvent consumption and short analysis times. CE coupled with ultraviolet (diode-array), electrochemical, and MS detectors greatly enhance the capability of this technique. A major drawback of CE is low sensitivity, which can be overcome by using extended light path capillary tubes or newly design detection cells. Method development and validation are required prior applying CE procedures for analysis of phytochemical substances. Method development involves optimization of factors effecting separation conditions, for example types, pHs and concentrations of electrolytes, types and concentrations of surfactants and organic modifiers, temperatures and applied voltages. Several parameters (e.g. accuracy, precision, limit of detection, limit of quantitation, linearity and robustness) should be investigated in CE method validation.

It is very important to emphasis that, CE will not eventually replace HPLC in the phytochemical analysis. HPLC remains useful and is a method of choice for analysis many natural products. CE, on the other hand, can be an alternative where analysis requires higher efficiency or resolution than HPLC, for example in cases of phenolic polymers, bioflavonoids and alkaloids. Additionally, the number of studies describing CE procedures for analysis of other plant metabolites is rapidly increasing. It can be concluded that CE will become a powerful technique for resolving of complex phytochemical substances.

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