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Original Article

Separation of Methanolic Leaf Extracts of Three *Rutaceous* Plants by Capillary Electrophoresis and High-Performance Liquid Chromatography Methods

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ABSTRACT

Introduction: In the phytochemical analysis of various multi component mixtures, Capillary electrophoresis (CE) method was considered as the best alternative tool due to high efficiency resolution separations, low solvent consumption and less maintenance costs. **Materials and Methods:** This study uses CE and high-performance liquid chromatography (HPLC) methods to evaluate the major components of methanolic leaf extract of *Rutaceous* plants. And also, to assess the purity of the components in isolated fractions and most importantly for the qualitative identification of the individual components of extracts based upon the relative retention time and UV profile. **Results:** The separation of the compounds in HPLC was attained by using ACE-5-C_{18} (250 x 4.6 mm) with a flow rate of 1.5 ml/min, with the UV detection at 254 nm and CE was equipped with a UV diode-array detector and bare, fused silica capillaries (56 cm 50 µm) with micro extended light path were used for the separations. **Conclusion:** Our study reveals that CE technique is a simple, economical and convenient method and shown high-resolution for multi-components found in plant extracts compared with HPLC profiles.

Keywords: HPLC, Capillary Electrophoresis, Relative Retention Time, *Rutaceae*.

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INTRODUCTION

Medicinal plants are the major sources for finding and identification of new compounds which may be having different biological activities. Based upon the traditional healers claim, some species of *Rutaceae* family demonstrated wide variety of medicinal properties in different parts of the world.[1] *Clausena excavate* is one of the important plant of *Rutaceae* family available in many continents[2] provides good source for isolation and identification of number of compounds and tested for various biological activities. It was observed that various parts of the plant namely roots, leaves, stems, barks, fruits, flowers and twigs are used for medicinal purpose, some of them are used as for food.[3]

Phytochemical survey reveals that wide range of secondary metabolites alkaloids,[4] Coumarins,[5] Pyrano-coumarins,[6] limnoids,[7] essential oils,[8] insecticides[9] were isolated from the different species of *Rutaceous* plants. Pharmacological survey reveals that researchers around the world identified anticancer,[10] antibacterial,[7] antifungal,[7] anti-HIV,[11] immunomodulatory,[12] antinociceptive,[13] antimalarial,[14] insecticidal[9] activities of extracts and isolated compounds. However, some of the species of *Rutaceae* family namely *Tetractomia roxbhughiana* and *Glycosmis calcicola* are not completely elucidated and this forms the basis of our study.

High Performance Liquid Chromatography (HPLC)is used for the separation, identification and quantification of different secondary metabolites in crude plant extracts, however method development and subsequent isolation procedures are found to be expensive.[15] In the phytochemical analysis of various multi component mixtures Capillary electrophoresis (CE) method was considered as the best alternative tool due to high efficiency resolution separations, low solvent consumption and less maintenance costs.[16]

The present investigation was aimed at identification of major constituents of three *Rutaceous* plants using CE and HPLC methods and comparison of the resolution profiles of two methods and also weigh advantages and disadvantages of each method.

CE has been used for a long time as a highly efficient and alternative technique to HPLC in areas such as pharmaceutical analysis. It is widely used to separate a complex array of large and small molecules like protein, peptides, urine metabolites

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etc. According to literature reports[17] This technique is used for analysing extracts of leaves, bark, roots and marine organisms. Phytochemical survey shows that CE and Micellar electro kinetic chromatography (MEKC) techniques were used for the separation of wide range of diversified chemical compounds like saponins,[18] coumarins,[19] alkaloids, flavonoids,[20] isoflavonoids, steroids from different plant sources. Seven coumarin derivatives were resolved with MEKC using borate / phosphate buffer at pH 7.[21] Flavonoids are widely spread in most of the plant families, separation of a mixture of sulphated flavanoids and flavonoids, (-)-epicatechin sulphate (ECS), (+)-catechin sulphate (CS), quercetin sulphate (QS), Apigenin sulphate (AS), and 6, 2, 3-flavonoid sulphate (FS) was reported.[20] And there were many researchers reported the separation of saponins by using capillary zone electrophoresis[18,22,23] in different conditions. Using CE in both its capillary zone electrophoresis (CZE) and MEKC modes allows the analyst to resolve both ionic (by CZE) and neutral (by MEKC) compounds on the same capillary using buffers with or without an organic modifier, a micelle or cyclodextrin.[21,24]

The present investigation was aimed to use the capillary electrophoretic technique to get high-resolution profiling of extracts and in particular to assess the purity of "semi-purified" or preparative LC-purified fractions of extracts. And compare the resolution profile of the HPLC and CE methods.

MATERIALS AND METHODS

Plant material

The leaves of Rutaceous plants *Clausena excavate, Tetractomia roxbhughiana* and *Glycosmis calcicola* were collected from the northern regions of Malaysia and their identity and authentication was done by Prof. Bowen, Department of Pharmacognosy, University of Sunderland, and a specimen sample was deposited in the herbarium. The plant materials were dried well under shade and powdered using an electric blender.

Preparation of crude extract

The extraction of the powdered leaf material was successively carried out with petroleum ether, hexane, chloroform, dichloromethane, ethanol, and water. In each case 500 ml of the solvent used for extraction. After each successive extraction, and before the extraction with another solvent, the powdered material was air dried and weighed. Maximum extractive value is achieved with methanol. The methanolic leaf extract was used for further fractionation by HPLC and CE. Samples were prepared by dissolving 1 mg of the dried extract in 1 mL of HPLC grade methanol and passed through 0.45 m Acrodisc syringe filters (Pall Corporation, Ann Arbor, MI, USA). Sample injections were freshly prepared by dissolving a small amount of the stock solution with methanol before injection.[25]

HPLC Analysis

The chromatographic conditions were reported in our previous work.[26,27] Briefly, the HPLC system consisted of a Shimadzu LC-10 AD pump, Shimadzu SPD-6AV variable wavelength UV/Vis detector, and using low dead volume connections, and A1-450 Dionex integrator (Dyson Instruments, Hettonle-Hole, Tyne and Wear, UK). Injections were made manually using a Rheodyne 7125-injection valve (Anachem, Luton, Beds., UK) fitted with a 20 µl, 50 µl, 1 mL and 2 ml loops for analytical, semi-preparative and preparative scale up, respectively.[26] Separations were performed on reverse phase columns on analytical scale, ACE-5-C_{18} (250×4.6 mm I.D.), semi-preparative scale ACE-5-C_{18} (250×7 mm I.D.) and preparative scale ACE-5-C_{18} (250×22 mm I.D) were used for the above studies.[28] Mobile phases were prepared using HPLC grade methanol, HPLC far-UV grade (Sigma-Aldrich, Poole, and Dorset UK). Water was distilled and doubly de-ionized using and Elgostat option 3 (Vivendi Water Systems, High Wycombe, Bucks., UK), [Methanol-water (20:80, v/v)].[26]

Capillary electrophoresis analysis

In the electrophoresis experiments, buffers were prepared using sodium dihydrogen phosphate and sodium borate (purchased from Sigma-Aldrich, Poole, Dorset, UK) and deionised double distilled water. Sodium tetraborate (Sigma-Aldrich), sodium dodecyl sulphate (Sigma-Aldrich), sulphated -cyclodextrin (Sigma-Aldrich), NaOH, sodium cholate (Sigma-Aldrich), were used in the preparation of run buffers.

Capillary electrophoresis was carried out on an Agilent CE system, which was equipped with a UV diode-array detector.[29-30] Bare, fused silica capillaries (56 cm 50 µm) microextended light path were used for the separations. Sample and buffer solutions were passed through 0.45 m Acrodisc syringe filters (Pall Corporation, Ann Arbor, MI, USA) prior to use. In capillary electrophoresis, the medium consists of a solution of buffer salts adjusted to a desired pH. This buffer solution is contained within a fused silica capillary. When a current is applied, cations in the middle are migrated towards the cathode. These hydrated cations will drag aqueous buffer with them towards cathode. Then the uncharged species move at the same speed as the electro osmotic force, positively charged ions move faster and negatively charged ions move slower. CE system comprised of f two buffer reservoirs, a detector, a capillary with a transparent detection window, and a high voltage power supply. Commonly the sample is introduced into the capillary by hydrodynamic or electro kinetic means. The separation voltage applied was 14 *kv* and the temperature was maintained at 25°C. This involves either applying a small positive pressure in the inlet or a potential difference across the capillary.

Preparation of buffers

Cholate/ SCD buffer (Hanna-Brown, M *et al*, 2004) (B1): 0.1 M sodium cholate and 6.25 mM sulphated -cyclodextrin with each 20 mM sodium dihydrogen phosphate and sodium tetraborate (20 mM MEKC); SDS/SCD (B2): Electrolyte comprised of 25 mM sodium borate, 75 mM SDS and 6.25 mM sulphated -cyclodextrin at pH adjusted to 9.50 with 1M NaOH after the addition of SDS and sulphated -cyclodextrin; Cholate buffer (B3): Sodium cholate MEKC system consists of 20 mM Sodium tetra borate at pH 8.9 with 0.2 M sodium cholate; SDS buffer: 20 mM SDS in 200 mM sodium tetraborate at pH 9.1, temperature 25°C, voltage 14 kV.

RESULTS AND DISCUSSION

Generally, plant extracts contain complex multi-component mixtures. To resolve these, it was planned to use high-resolution separation techniques like CE as well as the LC approaches so as to better assess the number of phytoconstituents in isolated fractions, the purity of components and also ultimately qualitative identification of the individual constituents based upon the relative retention times and more importantly with UV profile. This technique is very convenient and has more resolving power, which is quite essential for analyzing multi-component mixtures usually found in plants.[1] Due to its high resolving power, it can overcome the problems of non-baseline resolution associated with HPLC as shown in Figure 1-3. The main disadvantage of using CE analysis was that it could not be used for preparative work but doing capillary electrophoresis preparative work had never remotely been a consideration.

The main problem encountered using HPLC of plant extracts and fractions was that with the higher loads, the resolution achieved with the micro injections could not be seen in the higher loads due to the complex nature of plant extracts and multi-component mixtures. It was clearly desirable to search for a high-resolution technique so that it could be used to resolve all the components of plant extracts and also to assess the purity of the components. With the selection of different MEKC buffers, the first aim was to know how the buffers performed when changes in experimental variables were made. It was observed that in the case of cholate buffer good resolution was obtained but the main disadvantage was the long migration times. However, the initial aim was to establish resolution so that, after achieving this, higher voltages would then be applied to shorten the migration times of the components (Figure 3).

Based on the investigation it was concluded that the cholate buffer as shown in Table 1 gave better resolution and peak sensitivity whereas, at the other end of the scale, SDS buffer blocked the capillaries. Monitoring of reaction purity was assessed by capillary electrophoresis and the electropherograms were shown in Figure 4-6.

Initial work on plant extracts was performed by running standard mixtures of typical plant metabolites for the comparison of these buffers with respect to the migration of different classes of plant

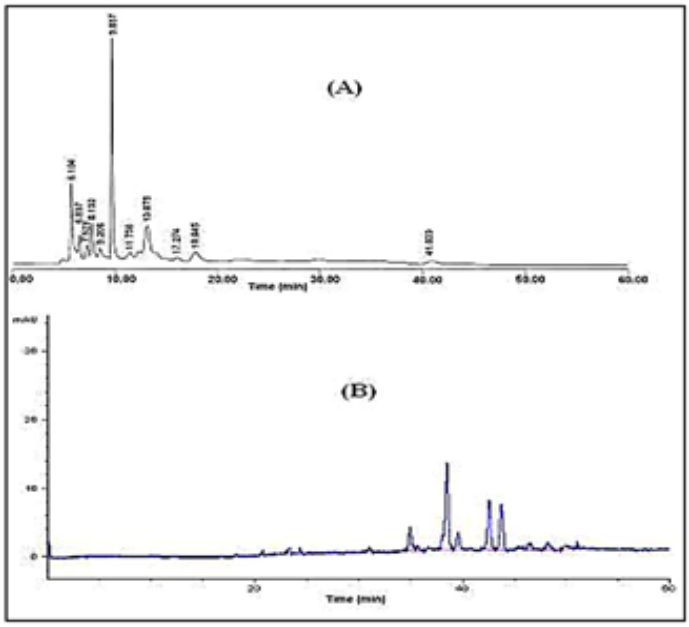

Figure 1: (A) RP-HPLC profile of methanolic leaf extract of *Tetractomia roxburghiana* with a mobile phase of methanol-water (20:80, v/v); C_{18} 250 X 4.6 mm; concentration of 1 mg/ml, flow rate: 1.5 ml/min; max: 254 nm. (B) Electropherogram of methanol leaf extract of *Tetractomia roxburghiana* Capillary: (56 cm 50 µm), Voltage 14 kV, Temp 25°C. Run buffer: Sodium cholate MEKC system consists of 20 mM sodium tetraborate at pH 8.9 with 0.2 M sodium cholate.

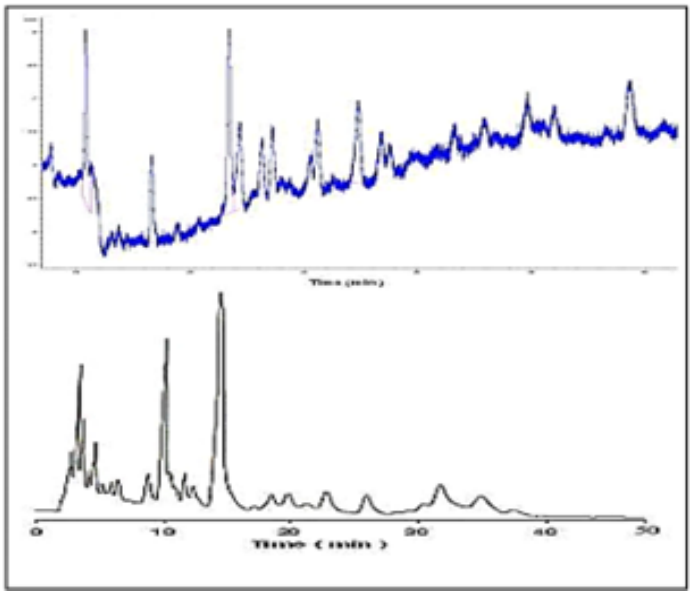

Figure 2: (A) Electropherogram of methanol leaf extract of *Glycosmis calcicola* Capillary: (56 cm 50 µm), Voltage 14 kV, Temp 25°C. Run buffer: Sodiumcholate MEKC system consists of 20 mM sodium tetraborate at pH 8.9 with 0.2 Msodium cholate. (B) Semi-preparative reversed phase HPLC profile of methanolic leaf extract of *Glycosmis calcicola* with a mobile phase of methanol-water (20:80, v/v) C_{18} 250 7 mm; conc. 1. 5 mg/ml, flow rate: 1.5ml/min; max: 245 nm.

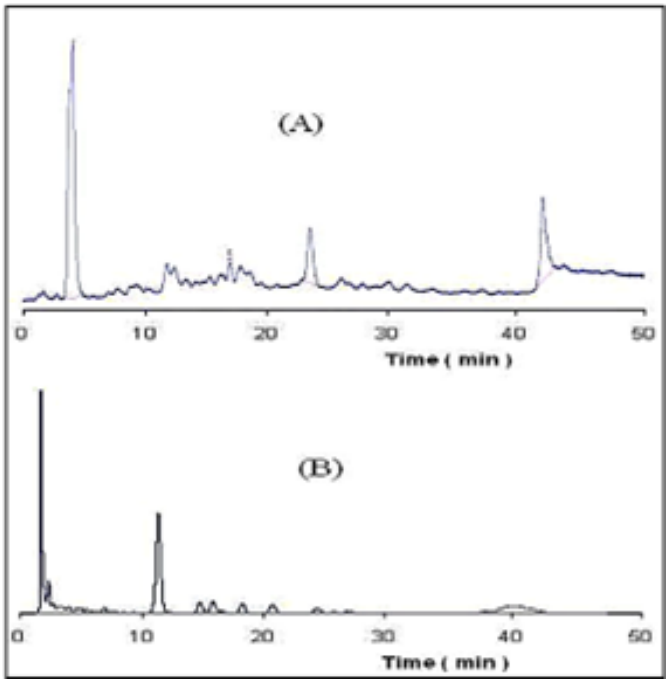

Figure 3: Electropherogram of methanol leaf extract of *Clausena excavata* Capillary: (56 cm 50 µm), Voltage 14 kV, Temp 25°C. Run buffer: Sodiumcholate MEKC system consists of 20 mM sodium tetraborate at pH 8.9 with 0.2 Msodium cholate. (B) Analytical reversed phase HPLC profile of methanolic leaf extract of *Clausena excavata* with a mobile phase of methanol-water (20:80, v/v) C18 250 4.6 mm; conc. 1. 5 mg/ml, flow rate: 1.5ml/min; max: 245 nm.

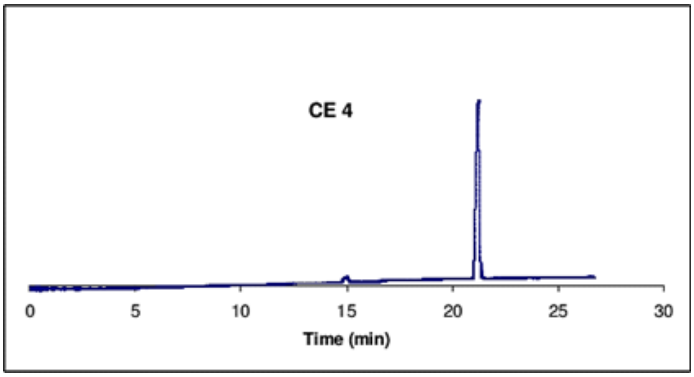

Figure 4: Electropherogram of CE 4 fraction obtained from preparativeHPLC, concentration ranging from 100-200 µg/ml. CE conditions Capillary:(56cm 50 µm), Voltage 14 kV, Temp 25°C. Sodium cholate MEKC system consistsof 20 mM sodium tetraborate at pH 8.9 with 0.2 M sodium cholate.

metabolites. Separation of standard mixtures of flavonoids namely rutin, 2,6-dihydroxyflavoneand quercetin as shown in Figure 7, quinolids, steroids and other mixtures were established and it was observed that, in general, alkaloids came first followed by flavonoids as shown in the (Table 1, illustrations were shown in Figure 8-13). CE separations, using the optimum cholate / SCD buffer, of methanolic leaf extracts of *Rutaceous* plants were carried out and compared with separation with that of the HPLC profile. CE had given higher resolution as expected, but still the non-polar components had not been resolved, particularly the standard steroidal compounds, with MEKC buffers. Nonetheless

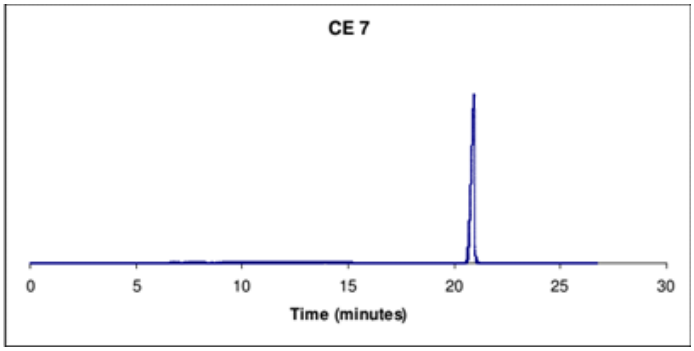

Figure 5: Electropherogram of CE 7 fraction obtained from preparative HPLC, concentration ranging from 100-200 µg/ml. CE conditions Capillary: (56cm 50 µm), Voltage 14 kV, Temp 25°C. Sodium cholate MEKC system consistsof 20 mM sodium tetraborate at pH 8.9 with 0.2 M sodium cholate.

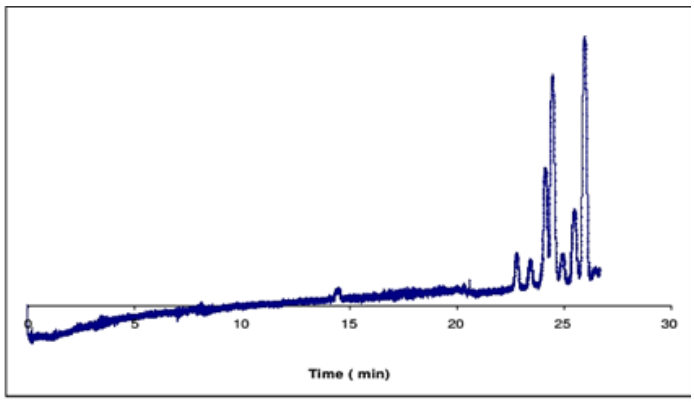

Figure 6: Electropherogram of CE 4 fraction obtained from preparative HPLC, concentration ranging from 100-200 µg/ml. CE conditions Capillary: (56cm 50 µm), Voltage 14 kV, Temp 25°C. Sodium cholate MEKC system consistsof 20 mM sodium tetraborate at pH 8.9 with 0.2 M sodium cholate.

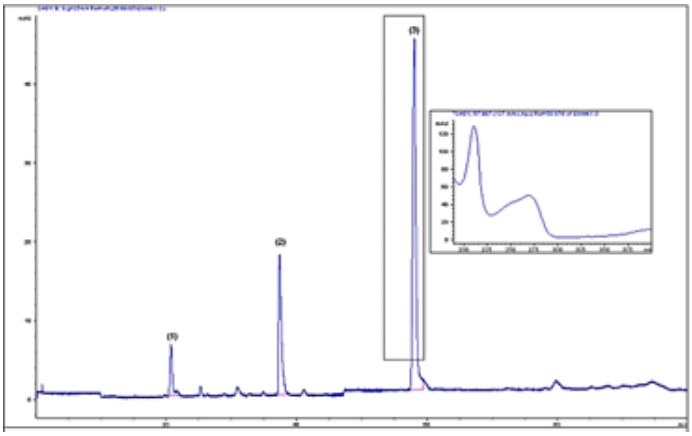

Figure 7: Typical electropherogram of mixture of three flavonoids, rutin, 2, 6-dihydroxy flavone and quercitin. Concentration ranging from 100-200 µg/ml. CE conditions Capillary: (56 cm 50 µm), Voltage 14 kV, Temp 25°C. Run buffer: SDS / SCD buffer. Electrolyte comprised of 25 mM sodium borate, 75 mM SDS and 6.25 mM sulphated -cyclodextrin at pH adjusted to 9.50 with 1M NaOH after the addition of SDS and sulphated –cyclodextrin.

a suitable generic MEKC system, along with some possible alternatives, had been established and this was used to study the composition of other extracts, both semi-purified and purified. CE and MEKC provided high-resolution separations

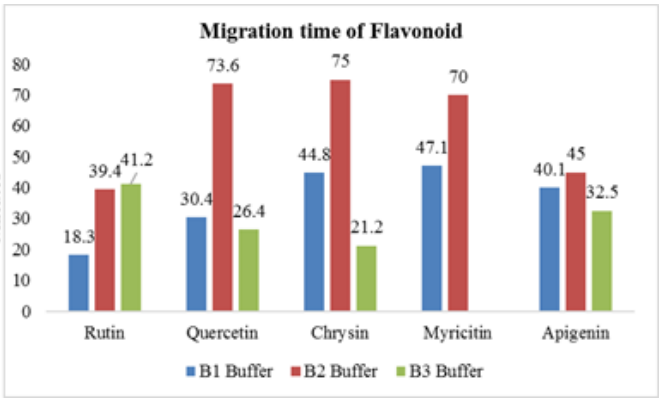

Figure 8: Migration time of Flavonoids.

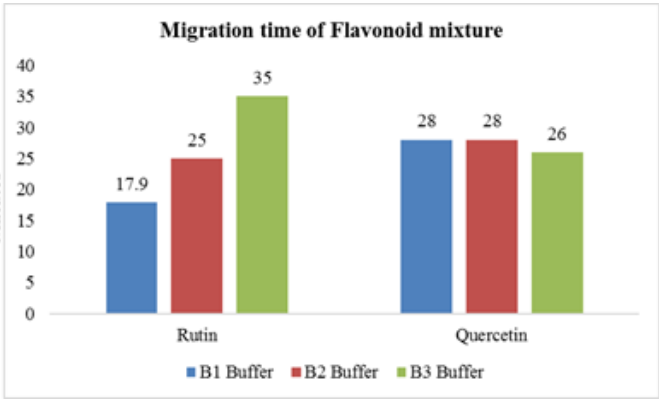

Figure 9: Migration time of Flavonoid mixture.

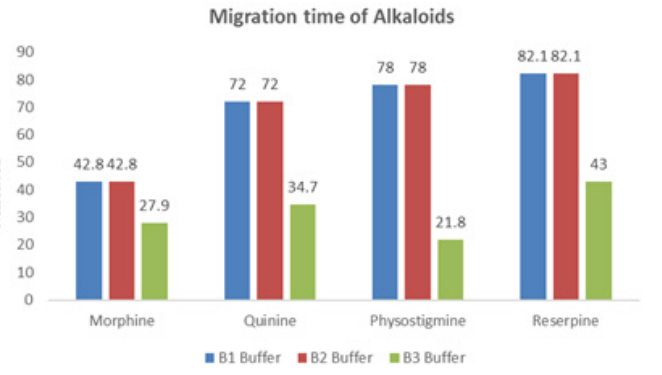

Figure 10: Migration time of Alkaloids.

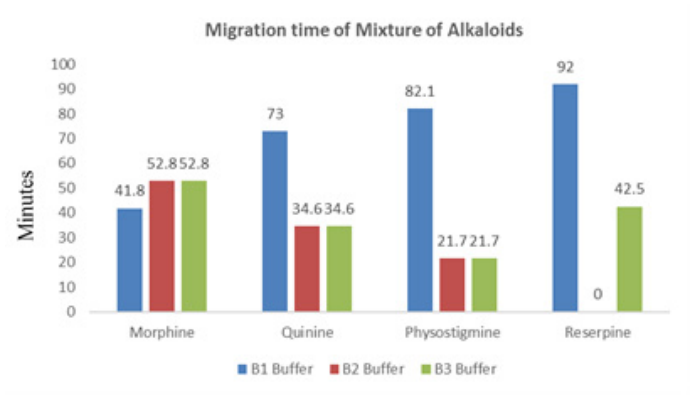

Figure 11: Migration time of mixture of Alkaloids.

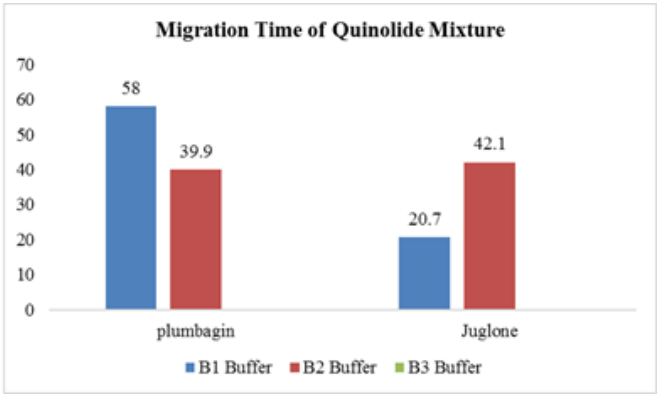

Figure 12: Migration time of Quinolide mixture.

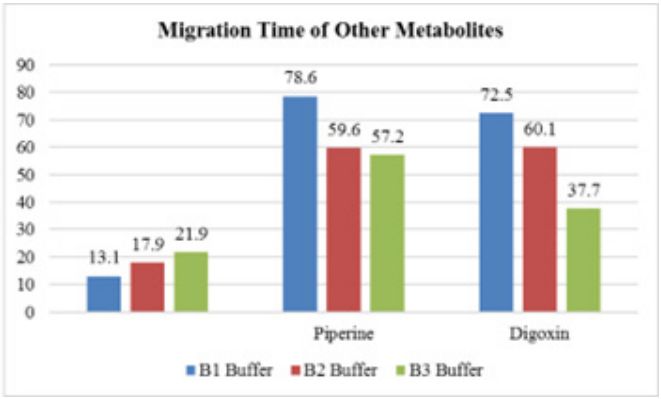

Figure 13: Migration time of other metabolites.

for the methanolic leaf extracts of three *Rutaceous* plants when compared with their HPLC profiles. Although CE with UV photodiode array detection is a good confirmation method of known compounds. CE-MS is a good characterisation method of unknown compounds. At this point capillary electrophoresis method development for identification of plant metabolites is confined to the achievements of high-resolution separations, and establishment of migration times of common known typical plant metabolites have been achieved. In the case of plant metabolites no single buffer is capable of eluting all the classes of compounds in three buffer used (as shown in Figure 1). This area needs further investigation. Alkaloids best resolved in sodium cholate system (B3 buffer), in B2 buffer alkaloids are not resolved properly. The migration times are quite high in three buffers run.

One more problem encountered for the identification of plant metabolites was that UV profiles could not be established for all the eluted peaks; this area still needs to be investigated. However, the combined use of the profiles of HPLC and MEKC that gave anidea about the efficiency of preparative separation. With these CE experiments, it could be said that polar compounds had been easily eluted whereas, non-polarcomponents elution was needed to be further investigated. Further work could be carried out by altering the composition of sulphated beta cyclodextrin in MEKC method, or by looking at non-aqueous CE.

Table 1: Comparison of migration time of plant metabolites in three buffers.

Plant Metabolites		Migration time (Min.)		
		B1 Buffer	B2 Buffer	B3 Buffer
Flavonoid	Rutin	18.3	39.4	41.2
	Quercetin	30.4	73.6	26.4
	Chrysin	44.8	75.0	21.2
	Myricitin	47.1	70.0	No data
	Apigenin	40.1	45.0	32.5
Flavonoid Mixture	Rutin	17.9	25.0	35.0
	Quercetin	28.0	28.0	26.0
Alkaloids	Morphine	42.8	42.8	27.9
	Quinine	72.0	72.0	34.7
	Physostigmine	78.0	78.0	21.8
	Reserpine	82.1	82.1	43.0
Alkaloid mixture	Morphine	41.8	52.8	52.8
	Quinine	73.0	34.6	34.6
	Physostigmine	82.1	21.7	21.7
	Reserpine	92.0	No data	42.5
Steroids	Lanosterol	78.5	26.9	No data
	Stigmasterol	80.0	No data	No data
	Sitosterol	82.0	No data	No data
Quinolide mixture	Plumbagin	58.0	39.9	No Data
	Juglone	20.7	42.1	No Data
Others	Piperine	78.6	59.6	57.2
	Digoxin	72.5	60.1	37.7

CONCLUSION

In conclusion, our results indicate that the high resolving power of CE had been confirmed and better resolution was observed with the semi-purified extracts rather than the crude extracts. However, experimental / instrumental difficulties were encountered more often than in the LC approaches to checking fraction purity. More importantly (a) it was much slower than the equally highly resolving UPLC, and (b) the selectivity of MEKC was similar to that of RP-HPLC rather than orthogonal-like ion-exchange HPLC. MEKC was a useful complimentary approach to the LC approaches. This is the first time we have explored the migration times of typical secondary metabolites found in plants. However further research is required to reduce the migration times of typical plant metabolites and also validation of the method (ICH, 2005) is essential for the quantitative identification of the metabolites

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

UV: Ultra-violet; **HIV:** Human Immunodeficiency Virus; **LC:** Liquid Chromatography; **UPLC:** Ultra-performance liquid chromatography.

SUMMARY

In the current study, the Capillary electrophoresis (CE) method was employed in analysis of methanolic leaf extracts of *Rutaceous* plants. The current method is being considered as one of the best alternative tool due to high efficiency resolution separations, low solvent consumption and less maintenance costs. The separation of the phytoconstituents were achieved with both

HPLC (ACE-5-C18 (250 x 4.6 mm) with a flow rate of 1.5 ml/min, with the UV detection at 254 nm and CE was equipped with a UV diode-array detector and bare, fused silica capillaries (56 cm x50 μm) with micro extended light path were used for the separations. In this study, the results were confirmed that the CE is a useful complimentary alternative to the LC approaches for profiling of plant constituents. This is the first ever approach to explore the separation ability of the technique for typical secondary metabolites found in plants. However further research is required to optimize various separation parameters.

REFERENCES

1. Arbab IA, Abdul AB, Aspollah M, Abdullah R, Abdelwahab SI, Mohan S, *et al*. *Clausena excavata* Burm. f. (*Rutaceae*): A review of its traditional uses, pharmacological and phytochemical properties. J Med Plants Res. 2011 Dec 31;5(33):7177-84.
2. Burkill IH. A dictionary of the economic products of the Malay Peninsula, With contributions by Willam Birtwistle; 1966.
3. Chokeprasert P, Charles AL, Sue KH, Huang TC. Volatile components of the leaves, fruits and seeds of wampee [*Clausena lansium* (Lour.) Skeels]. J Food Compos Anal. 2007 Feb 1;20(1):52-6. doi: 10.1016/j.jfca.2006.07.002.
4. Huang SZ, Luo HR, Ma QY, Peng H, Dai HF, Zhou J, *et al*. Chemical Constituents from the Stems of *Excoecaria acertiflia*. Chem Biodivers. 2014 Sep;11(9):1406-16. doi: 10.1002/cbdv.201400087, PMID 25238081.
5. Kumar R, Saha A, Saha D. A new antifungal coumarin from *Clausena excavata*. Fitoterapia. 2012 Jan 1;83(1):230-3. doi: 10.1016/j.fitote.2011.11.003, PMID 22088496.
6. Kongkathip B, Sutthiprabha S, Yoosook C, Mongkolsook Y, Kongkathip N. Determination of a pyranocoumarin and three carbazole compounds in *Clausena excavata* by RP-HPLC. J Chromatogr Sci. 2010 Jul 1;48(6):445-9. doi: 10.1093/chromsci/48.6.445, PMID 20822658.
7. Sunthitikawinsakul A, Kongkathip N, Kongkathip B, Phonnakhu S, Daly JW, Spande TF, *et al*. Coumarins and carbazoles from *Clausena excavata* exhibited antimycobacterial and antifungal activities. Planta Med. 2003 Feb;69(2):155-7. doi: 10.1055/s-2003-37716, PMID 12624822.
8. Nan P, Hu Y, Zhao J, Feng Y, Zhong Y, Nan P, *et al*. Chemical composition of the essential oils of two Alpinia species from Hainan Island, China. Z Naturforsch C J Biosci. 2004 Apr 1;59(3-4):157-60. doi: 10.1515/znc-2004-3-402, PMID 15241915.
9. Cheng SS, Chang HT, Lin CY, Chen PS, Huang CG, Chen WJ, *et al*. Insecticidal activities of leaf and twig essential oils from *Clausena excavata* against *Aedes aegypti* and *Aedes albopictus* larvae. Pest Manag Sci. 2009 Mar;65(3):339-43. doi: 10.1002/ps.1693, PMID 19115256.
10. Taufiq-Yap YH, Peh TH, Ee GC, Rahmani M, Sukari MA, Ali AM, *et al*. A new cytotoxic carbazole alkaloid from *Clausena excavata*. Nat Prod Res. 2007 Jul 20;21(9):810-3. doi: 10.1080/14786410701258875, PMID 17654285.
11. Kongkathip B, Kongkathip N, Sunthitikawinsakul A, Napaswat C, Yoosook C. Anti-HIV-1 constituents from *Clausena excavata*: Part II. carbazoles and a pyranocoumarin. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. Phytother Res. 2005 Aug;19(8):728-31. doi: 10.1002/ptr.1738, PMID 16177980.
12. Manosroi A, Saraphanchotiwitthaya A, Manosroi J. Immunomodulatory activities of fractions from hot aqueous extract of wood from *Clausena excavata*. Fitoterapia. 2004 Jun 1;75(3-4):302-8. doi: 10.1016/j.fitote.2004.01.009, PMID 15158986.
13. Rahman MT, Alimuzzaman M, Shilpi JA, Hossain MF. Antinociceptive activity of *Clausena excavata* leaves. Fitoterapia. 2002 Dec 1;73(7-8):701-3. doi: 10.1016/s0367-326x(02)00211-3, PMID 12490234.
14. Basir R, Chan KL, Yam MF, Othman F, Abdullah WO, Mohd Moklas MA, *et al*. Antimalarial activity of selected Malaysian medicinal plants. Phytopharmacology. 2012;3(1):82-92.
15. Merken HM, Beecher GR. Measurement of food flavonoids by high-performance liquid chromatography: a review. J Agric Food Chem. 2000 Mar 20;48(3):577-99. doi: 10.1021/jf990872o, PMID 10725120.
16. Carrasco-Pancorbo A, Gómez-Caravaca AM, Cerretani L, Bendini A, Segura-Carretero A, Fernández-Gutiérrez A. Rapid quantification of the phenolic fraction of Spanish virgin olive oils by capillary electrophoresis with UV detection. J Agric Food Chem. 2006 Oct 18;54(21):7984-91. doi: 10.1021/jf0617925, PMID 17031999.
17. Frazier RA, Ames JM, Nursten HE. The development and application of capillary electrophoresis methods for food analysis. ELECTROPHORESIS: An [international journal]. Electrophoresis. 1999 Oct 1;20(15-16):3156-80. doi: 10.1002/(SICI)1522-2683(19991001)20:15/16<3156::AID-ELPS3156>3.0.CO;2-T, PMID 10596823.
18. Oleszek W, Bialy Z. Chromatographic determination of plant saponins—an update (2002-2005). J Chromatogr A. 2006 Apr 21;1112(1-2):78-91. doi: 10.1016/j.chroma.2006.01.037, PMID 16451803.
19. Wang W, Tang J, Wang S, Zhou L, Hu Z. Method development for the determination of coumarin compounds by capillary electrophoresis with indirect laser-induced fluorescence detection. J Chromatogr A. 2007 Apr 27;1148(1):108-14. doi: 10.1016/j.chroma.2006.09.070, PMID 17383667.
20. Dantuluri M, Gunnarsson GT, Riaz M, Nguyen H, Desai UR. Capillary electrophoresis of highly sulfated lavanoids and flavonoids. Anal Biochem. 2005 Jan 15;336(2):316-22. doi: 10.1016/j.ab.2004.10.017, PMID 15620899.
21. Issaq HJ. Capillary electrophoresis of natural products. Electrophoresis. 1997;18(12-13):2438-52. doi: 10.1002/elps.1150181234, PMID 9456058.
22. Li W, Gong S, Wen D, Che B, Liao Y, Liu H, *et al*. Rapid determination of aristolochic acid I and II in Aristolochia plants from different regions by β-cyclodextrin-modified capillary zone electrophoresis. J Chromatogr A. 2004 Sep 17;1049(1-2):211-7. doi: 10.1016/S0021-9673(04)01336-6, PMID 15499935.
23. Cao Y, Wang Y, Ye J. Differentiation of Swertia Mussotii Franch from *Artemisiae capillaris* Herba by capillary electrophoresis with electrochemical detection. J Pharm Biomed Anal. 2005 Sep 1;39(1-2):60-5. doi: 10.1016/j.jpba.2005.03.006, PMID 16085134.
24. Ali MA, Farah MA, Al-Hemaid FM, Abou-Tarboush FM, Al-Anazi KM, Wabaidur SM, *et al*. Assessment of biological activity and UPLC–MS based chromatographic profiling of ethanolic extract of *Ochradenus arabicus*. Saudi J Biol Sci. 2016 Mar 1;23(2):229-36. doi: 10.1016/j.sjbs.2015.02.010, PMID 26981004.
25. Namiesnik J, Gorecki T. Sample preparation for chromatographic analysis of plant material. J Planar hromatogr Mod TLC. 2000;13(6):404-13.
26. Guntupalli C, Ramaiah M, Kumar GS. RP-HPLC analysis and antimicrobial screening of *Clausena excavata* burm. F.(rutaceae). Int J Phytother. 2013;3(2):91-7.
27. Chakravarthi G, Reddy AR, Ramaiah M. Assessment of biological activity and comparison of UPLC and RP-HPLC chromatographic profiles of *Clausena excavata* Burm. F. Pharmacogn J. 2017;9(2):185-91. doi: 10.5530/pj.2017.2.30.
28. Guntupalli C. Phytochemical and Biological Investigations of *Rutaceous* plants; 2008 ([doctoral dissertation]. University of Sunderland).
29. Guillo C, Perrett D, Hanna-Brown M. Validation and further optimisation of a cyclodextrin-modified micellar electrokinetic capillary chromatography method for urine profiling. Chromatographia. 2004 May 1;59(2):S157-64. doi: 10.1365/s10337-004-0218-8.
30. Guideline IH. Validation of analytical procedures: text and methodology Q2. In: International conference on sharmonization, Geneva, Switzerland. Vol. R1; 2005 Nov 10. p. 11-2.

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