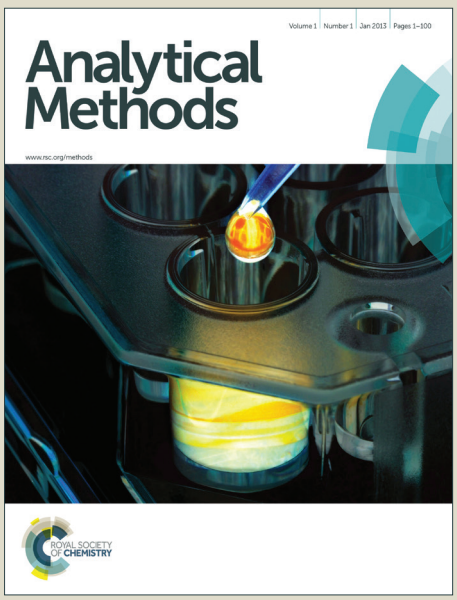
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

DETERMINATION OF ELEVEN FLAVONOIDS IN CHAMOMILE AND LINDEN EXTRACTS BY CAPILLARY ELECTROPHORESIS

Senem ŞANLI[1] and Craig LUNTE[2*]

[1]Department of Chemistry, Faculty of Science and Arts, Usak University, Usak, 64100, TURKEY

[2]Ralph N. Adams Institute for Bioanalytical Chemistry, Department of Chemistry, University of Kansas, Lawrence, KS, 66045, USA

Abstract

A sensitive, dependable, simple and rapid method based on capillary electrophoresis with diode array detection (CE–DAD) was developed for identification and determination of eleven flavonoids (apigetrin, naringin, naringenin, catechin, galangin, apigenin, luteolin, quercetin, myricetin, kaempferol, and kaempferide) in Chamomile and Linden flower extracts. Several parameters which influence the separation were investigated to determine the optimum conditions. At room temperature, the eleven flavonoids could be well separated within 11 min. in a 55cm length capillary at a separation voltage of 24 kV with 40 mM borate buffer (pH 8.9). Under optimum conditions, linearity was achieved within the concentration ranges of 2.5-100.0 μM for all analytes except myricetin and luteolin (5.0-100.0 μM) with correlation coefficients ≥ 0.999. The method was successfully applied to the determination of flavonoids in plant samples such as Chamomile and Linden flowers with satisfactory recoveries.

Keywords: Flavonoids, Chamomile, Linden Flower, Capillary Electrophoresis

1 Introduction

Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings and are classified according to substitutions. Flavonoids are further divided into sub-classes: flavones, flavanones, flavonols, isoflavanones, anthocyanidins and flavan-3-ol derivatives: catechins.[1] Flavonoids have been widely used as therapeutics due to their spasmolytic, antiphlogistic, antiallergic, and diuretic properties.[2] It has been proposed that these activities are a result of the flavonoids oxygen radical scavenging capacity.[3] Flavonols and flavones are of particular interest as they provide both antioxidant and free radical scavenging activity to foods.[4] In addition, epidemiological studies indicate that their consumption result in a reduced risk of cancer and cardiovascular diseases.[5-7]

The structures of the eleven flavonoids used in this study and frequently found in plants, are listed in Table 1. Most of the compounds possess similar structure and chemical characteristics in addition with naringin and apigetrin which are flavanone and flavone glycosides, respectively.

Extracts from linden flowers (*Tilia cordata*), have been used in folk medicine to induce sweating with colds and influenza, as well as to treat nervous tension, anxiety, insomnia, high blood pressure, arteriosclerosis, migraines and digestive upsets.[8] Chamomile extracts (*Matricaria chamomilla*) are used as anti-inflammatory, anti-emetic, bactericide, fungicide and spasmolytic.[9-10] In this work, we have successively developed a sensitive, dependable, simple and rapid method for the determination of eleven flavonoids (apigetrin, naringin, naringenin, catechin, galangin, apigenin, luteolin, quercetin, myricetin, kaempferol, and kaempferide, Table 1) in plant extracts.

So far, analysis of flavonoids has been accomplished predominately by high performance liquid chromatography (HPLC)[11-14] and gas chromatography (GC)[15,16]. There have been capillary electrophoretic and micellar electrokinetic chromatography methods for the separation of selected flavonoids reported[17-30]. Dadakova et al.[18] studied quercetin in plant samples by micellar electrokinetic capillary chromatograph. Fonseca et al.[19] determined ten flavonoids in *Chamomilla recutita* using capillary electrochromatography. Zhang et al.[20] developed a micellar electrokinetic capillary method for determination of several flavonoids in traditional Tibetan herbal tea. Tonin et al.[27] determined flavonoids in neem sample by solvent-modified micellar electrokinetic chromatography. Kocevar et al.[28] developed both a CE and an HPLC method for

determination of five flavonoids in medicinal plants. The main advantages of CE over HPLC are that run times are relatively short and only minimal reagents, mostly of a non-toxic nature, are consumed during analysis, resulting in a more cost effective and environment-friendly technique that both rivals and complements HPLC.

In this paper, we describe a simple capillary electrophoresis method for the simultaneous determination of several flanonoids. The main advantages of this method are a shorter analysis time (11 min) and lower limits of detection relative to previously reported methods.. Analytical conditions established in this study were applied to determine the content of flavonoids in chamomile and linden samples by CE coupled with diode array detection (DAD).

2. Experimental

2.1 Chemicals

Catechin, apigenin, luteolin, quercetin, kaempferol, naringenin, and galangin were obtained from Sigma (St. Louis MO, USA). Kaempferide, myricetin, naringin, and apigetrin (apigenin-7-glucoside) were purchased from Fluka (Switzerland). All flavonoids were used as received without further purification. Methanol (MeOH, HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Sodium tetraborate ($Na_2B_4O_7$), boric acid (H_3BO_3) sodium hydroxide (NaOH) and butylated hydroxytoluen (BHT) were obtained from Sigma (St. Louis MO, USA). Ultrapure water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA).

Stock solutions of flavonoids were prepared by dissolving enough of the studied compound in MeOH (50 mL) to give a 1.00 mM solution. Borate buffer (40 mM) was prepared by mixing Na_2B_4O7 solution with H_3BO_3 (500 mM) in ultrapure water. All reagents were protected from light. Prepared solutions were stored at 4^o C for several days and at -20^o C for long-term storage.

2.2 Apparatus

All separations were performed on a Beckman Coulter P/ACE MDQ Capillary Electrophoresis System with a diode array detector (Palo Alto, CA). A 55 cm × 50 μm i.d. fused silica capillary (Polymicro Technologies, Tucson, AZ) with an effective length of 45 cm was used for the separation.

A Fisher Scientific AB 15 pH/ion analyser with Fisher Scientific Acument combination pH electrode was used. The pH measurements were performed in triplicate to ensure stability and reproducibility of the potentiometric system.

2.3 Procedures

The separation capillary was activated in the first use by flushing sequentially with MeOH (5 min), 1.0 M HCl (2 min), water (2 min), 1.0 M NaOH (20 min), water (2 min), and then with buffer (20 min). The capillary was conditioned daily by washing with 1.0 M NaOH (20 min), water (2 min) and finally with the running buffer (20 min). Between each analysis, the capillary was flushed with 1.0 M sodium hydroxide (3 min), water (2 min) and then the running buffer (3 min). Prior to use, buffer solutions were filtered through 0.25 μm membrane filters (Millipore, Bedford, MA) and degassed by ultrasonication for approximately 10 min. Buffer, containing 40 mM $Na_2B_4O_7$, was adjusted to pH 8.9 with 0.5 M H_3BO_3. The Applied voltage was 24 kV, pressure injection was at 10 psi for 5 s, and the detection was performed at 210 nm.

2.4 Sample preparation

Chamomile and Linden samples were collected from the Aegean part of Turkey. They were dried in the air. Approximately 0.5 g of each sample was ground into powder in a mortar and accurately weighed. The extraction and hydrolysis procedure of Proestos et al.[31] was used with some modifications. To each weighed sample were added 25 mL methanol, 15 mL water and 0.04 g BHT. For hydrolysis, 10 mL 6 M HCl was added. The extraction mixture was refluxed in a water bath at 80 ^{0}C for 1.5 hours in the dark. After cooling, 5 mL of this solution was put in a tube and vacuumed until dryness. The residue was dissolved with buffer. Before the injection, samples were filtered through 0.25 μm membrane filter. All sample solutions were stored in the dark.

3 Result and Discussion

3.1 Effects of pH value and the Buffer Concentration

The mechanism of separation in CE is based on differences between the charge-to-size ratios of the analytes. The molecules of the flavonoids investigated have at least one ionizing phenolic hydroxyl group; ionization of this group is one of the main factors determining the

electrophoretic mobility of these flavonoids.[32] pK_a values reported in the literature for water solutions are shown in Table 1. [33-39]

Borate buffer was employed as the buffer in this work because borate can chelate with the analytes to form more soluble complex anions.[40] The pH dependence of the migration time was investigated over the pH range of 8.7–9.1. The migration times of all analytes increase with increasing pH except mycretin. At pH 8.7, the myricetin peak was split into two smaller peaks, while at pH 9.1, myricetin and apigenin were not separated. The R_s values for myricetin and apigenin are given in Table 2. The optimum pH was determined to be pH 8.9 based on the good resolution, selectivity, and peak shapes of the studied compounds within a relatively short analysis time (11 min). Flavonoids are also more susceptible to oxidative degradation at higher pH and longer separation times.

In addition to pH, the concentration of borate buffer is also an important parameter for the separation. The effect of the borate buffer concentration on migration time was also studied. When the borate buffer concentration increased, migration times increased. Good resolution was achieved with the shortest separation time using 40 mM borate.

3.2 Effects of Separation Voltage

The influence of the separation voltage on the migration time of the analyte was also studied. The results indicated that increasing the separation voltage gave shorter migration time for all studied compounds, but also increased the baseline noise and decreased the resolution of the analytes. However, too low of a separation voltage increased the analysis time considerably and caused peak broadening. The optimal voltage was determined by performing runs at increasing voltages (20, 24 and 26 kV) until deterioration in resolution was noted. Separation of galangin and kaempferol cannot be achieved when the separation voltage was 26 kV or greater. The R_s values for galangin and kaempferol are given in Table 2. The optimum separation voltage was found to be 24 kV, at which good separation can be obtained for all of the analytes.

From the above experiments the optimum conditions for separation of all eleven flavonoids was determined to be; 40 mM borate, pH 8.9 with an applied voltage of 24 kV. A typical electropherogram for a standard solution of the eleven analytes is shown in Fig. 1; it is apparent that satisfactory separation can be achieved within 11 min.

As shown in Fig. 1, the type and position of substituents can strongly effect migration times of the flavonoids. Naringin has a disaccharide substituent, apigetrin has a monosaccharide substituent, while the rest of the flavonoids studied are aglycones. Under the separation conditions utilized, the flavonoids are negatively charged and their electrophoretic mobility is against the electroosmotic flow and away from the detector end of the capillary. Therefore naringin elutes first because of a slower electrophorectic mobility due to the large size of the disaccharide group. Likewise, apigetrin elutes next because of the size of its monosaccharide substituent. The flavonoid aglycones are similar in size but vary in pK_a values. Catechin is only partially charged at the separation pH and therefore has the slowest electrophoretic mobility of the flavonoid aglycones and is thus the first of this group to elute. Luteolin and quercetin are dianions at the separation pH and therefore elute last as they have the fastest electrophoretic mobility. The rest of the flavonoid aglycones studied elute between catechin and luteolin based on a combination of their pK_a values and sizes.

3.3 Linearity, Repeatability and Detection Limits

A series of the standard mixture solutions of the eleven analytes were tested to determine the validation parameters.[41] The linearity was calculated by plotting the peak area versus concentration of the compounds. The calibration curves were obtained by linear least squares regression. The validation data are reported in Table 3. The method exhibited good linearity based on a correlation coefficient > 0.999 for all compounds studied The LOD and LOQ were calculated as LOD = 3.3s/m and LOQ = 10s/m, where s is the standard deviation of response and m is the slope of the corresponding calibration curve.[42,43]

In the literature, there is no method for the simultaneous determination of these eleven compounds by capillary electrophoresis for plant samples. The MEKC method of Dadakova et al.[18] provided an LOC of 0.5 μg mL^{-1} with an analysis time of 20 minutes. Fonseca et al.[19] reported an LOD of 35 μg mL^{-1}. The CE method of Kocevar et al.[28] required an analysis time of 18 minutes. The method described above provides a combination of faster analysis time and improved limits of detection.

3.4 Sample Analysis and Recovery

The validated methodology was applied to quantification of the flavonoids in Chamomile and Linden samples. Typical electropherogram of Chamomile and Linden extracts are shown in Fig 2 and Fig 3. Peaks were identified by standard addition and comparison of absorbance spectra. As an example the absorbance spectra of a quercetin standard and from the peak identified as quercetin in a chamomile extract are shown in Fig 3. All peak identities were determined by co-elution with standards and comparision of absorbance spectra with standard spectra. The contents of the two plant extracts were quite different. In Chamomile extracts, three of the studied compounds were detected (catechin kaempferol and quercetin) under the optimum conditions. The contents of quercetin were highest in Chamomile extracts. In Linden extracts, catechin, kaempferol, apigenin and myricetin were detected with catechin occurring in the highest concentration. The concentration of the analytes in the samples were calculated using their peak areas from the calibration curve obtained under the same conditions.

Accurate amounts of analytes were added to sample and recovery values were obtained using their peak area from the calibration curve. Each sample was analyzed in triplicate. Content and recovery analysis were given in Table 4 for chamomile and linden extracts, respectively. The recoveries for these compounds were between 91.0 % and 100.2 %. The recovery results showed that this method is suitable for real sample analysis.

4 Conclusions

Although several methods have been reported for determination of the different types of flavonoids, to the best of our knowledge, there are no methods yet for simultaneous determination of the eleven flavonoids in this study from the extracts of the herbal plants. In this work, simple, fast and effective CE-DAD method has been applied to simultaneously determine flavonoids in Chamomile and Linden extracts without using organic modifier. Also, this method has been found to be reproducible and highly sensitive. The proposed method promises to be applicable to the identification and determination of the eleven flavonoids in other plant extracts as well.

5 Acknowledgments

This work was supported by the Higher Education Council of Turkey (YOK).

References

1 C. A. Rice-Evans, N. Miller, Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In C. A. Rice-Evans, L. Packer, Flavonoids in health and disease, New York: Marcel Dekker Inc. 1997, p. 199 – 219.

2 G. Cao, E. Sofic, R. L. Prior, *Free Radic. Biol. Med.,* 1997, **5**, 749-760.

3 H. M. Merken, G. R. Beecher, *J. Chromatogr. A.,* 2000, **897**, 177-184.

4 F. Shahidi, P. K. Wanasundara, *Crit. Rev. Food Sci. Nutr.,* 1992, **32**, 67-103.

5 L. Wattenburg, *Cancer Res.,* 1985, **45**, 1-8.

6 L. Wattenburg, *Proc. Nutr. Soc.,* 1990, **49**, 173-180.

7 H. Wei, L. E. Tye, D. F. Bresnick, *Birt Cancer Res.,* 1990, **50**, p. 499-502.

8 M. Coleta, M. G. Campos, M. D. Cotrim, et al. Pharmacopsychiatry 34 (suppl 1): S20–1, 2001.

9 N. G. Bisset, Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis, CRC Press, Boca Raton, FL, 1994, p. 332.

10 World Health Organization. Monographs on Selected Medicinal Plants, vol. 1: Flos Chamomillae. WHO, Geneva, 1999, p. 86.

11 A. Obmann, S. Purevsuren, M. Zehl, C. Kletter, G. Reznicek, S. Narantuya, S. Glasl, *Phytochem. Anal.,* 2012, **23**, 254–259.

12 T. Z. Niu, Y. W. Zhang, Y. L. Bao, Y. Wu, C. L. Yu , L. G. Sun, J. W. Yi, Y. X. Huang, Y. X. Li, *J. Pharm. Biomed Anal*., 2013, **76**, 44–48.

13 M. Francisco, D. A. Moreno, M. E. Cartea, F. Ferreres, C. G. Viguera, P. Velasco. *J. Chromatogr. A.*, 2009, **1216**, 6611–6619.

14 G. H. Shui, L. P. Leong, *J. Chromatogr. A* 2002, **977**, 89–96.

15 C. S. Creaser, M. R. Koupai-byzani, G. R. Stephenson, *J. Chromatogr.,* 1989, **478**, 415-420.

16 R. Christov, V. Bankova, *J. Chromatogr.,* 1992, **623**, 182-185.

17 Z. K. Shihabi, T. Kute, H. Y. Garcia, M. Hinsdale, *J. Chromatogr. A.*, 1994, **680**, 181-185.

18 E. Dadakova, E. Prochazkova, M. Krizek, *Electrophoresis,* 2001, **22**, 1573-1578.

19 F. N. Fonseca, M. F. M. Tavares, C. Horváth, *J. Chromatogr. A.*, 2007, **1154**, 390-399.

20 Y. Zhang, J. Chen, X. M. Ma, Y. P. Shi, *J. Pharm. Biomed. Anal.* 2007, **45,** 742-746.

21 X. J Chen, H. Ji, Y. T. Wang, S. P. Li, *J. Sep. Sci.* 2008, **31,** 881-887.

22 M. N. Luo, H. W. Lu, H. Q. Ma, *J. Pharm. Biomed. Anal.* 2007, **44,** 881-886.

23 Y. Lua, C. Jiaa, Q Yao, H. Zhong, M. C. Breadmore, *J. Chromatogr. A.*, 2013, **1319,** 160–165.

24 M. E. Yue, T. F. Jiang, Y. P. Shi, *Talanta* 2004, **62,** 695–699.

25 S. Zhang, S. Dong, L. Chi, P. He, Q. Wang, Y. Fang, *Talanta* 2008, **76**, 780-784.

26 S. P. Wang, K. J. Huang, *J. Chromatogr. A.*, 2004, **1032,** 273– 279.

27 F. G. Tonin, A. V. Jager, G. A. Micke, J. P. S. Farah, M. F. M. Tavares, *Electrophoresis* 2005, 26, 3387–3396.

28 N. Kocevar, I. Glavac, R. Injac, S. Kreft, *J. Pharm. Biomed. Anal.* 2008, 46, 609–614.

29 T. F. Jiang, X. Du, Y. P. Shi, *Chromatographia*, 2004, 59, 255-258.

30 G. Chen, H. Zhang, J. Ye, *Anal Chim Acta*, 2004, 20, 69-76.

31 C. Proestos, N. Chorianopoulos, G. J. E. Nychas, M. Komaitis, *J. Agric. Food Chem.*, 2005, 53, 1190-1195.

32 P. H. Morin, J.C. Archambault, P. Andre, M. Dreux, E. Gaydou, *J Chromatogr A.*, 1997 791, 289–297.

33 H. Serra, T. Mendes, M. R. Bronzea, A. L. Simplicio, *Bioorg. Med. Chem.*, 2008, **16**, 4009–4018.

34 E. A. González, M. A. Nazareno, C. D. Borsarelli, *J Chem Soc Perkin Trans.*, 2002, **2**, 2052-2056.

35 I. Kron, Z. Pudychová-Chovanová, B. Veliká, J. Guzy, P. Perjési, *Monatsh. Chem*., 2012, **143**, 13-17.

36 M. Tungjai, W. Poompimon, C. Loetchutinat, S. Kothan, N. Dechsupa, S. Mankhetkorn, *The Open Drug Delivery Journal*, 2008, **2**, 10-19.

37 S. Ramešová, R. Sokolová, I. Degano, J. Bulíčková, J. Zabka, M. Gál, *Anal Bioanal Chem.*, 2012, **402,** 975-82.

38 M. E. Arrington, Superoxide Dismutase Inhibitor Screening and Characterization Using 19F NMR. Thesis, Department of Chemistry and Physics, Western Carolina University, 2010. http://libres.uncg.edu/ir/wcu/f/Arrington2010.pdf.

39 http://www.hmdb.ca/metabolites/HMDB37441

40 S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann, H. Michael, *Anal Chem.*, 1991, **63**, 1541–1546.

41 CH, Topic Q2A validation of analytical procedures methodology PMP/ICH/281/95.

42 C. M. Riley, T. W. Rosanske, Development and Validation of Analytical Methods, Elsevier, New York, 1996.

43 M. E. Swartz, I. S. Krull, Analytical Method Development and Validation, Marcel Dekker Inc., New York, 1997.

Figure Captions

Fig 1. Electropherogram of eleven flavonoids under optimum conditions. Running buffer 40 mM sodium tetraborate solution (pH 8.9); uncoated fused silica capillary 55 cm (45 cm to detector) x 50 µm i.d.; applied voltage 24 kV; detection UV absorbance at 210 nm; pressure injection, 10 psi for 5 s; ambient temperature. Peaks represent 1: Naringin, 2: Apigetrin, 3: Catechin, 4: Naringenin, 5: Kaempferide, 6: Galangin, 7: Kaempferol, 8: Apigenin, 9: Mycretin, 10: Luteolin, 11: Quercetin (Each compounds are 0.1 mM)

Fig 2. Typical electropherogram of a chamomile extract. 1: Catechin; 2: Kaempferol; 3: Quercetin

Fig 3. Typical electropherogram of a linden extract. 1: Catechin; 2: Kaempferol; 3: Apigenin; 4: Myricetin

Fig 1.

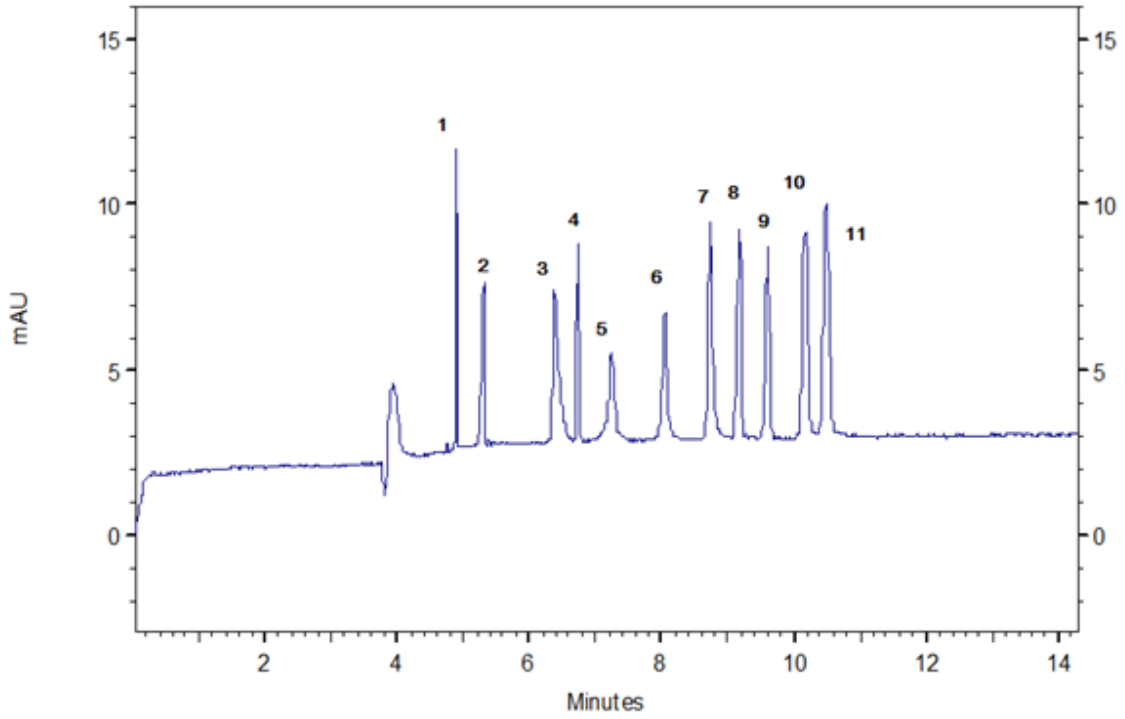


Fig 2.

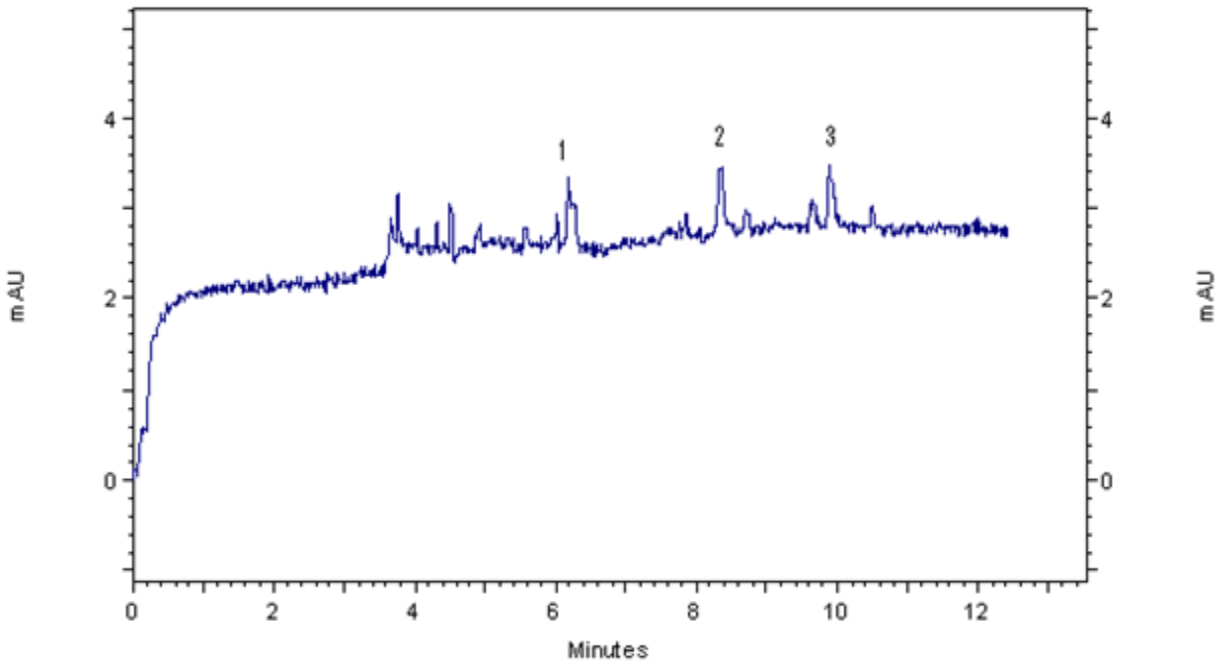


Fig 3.

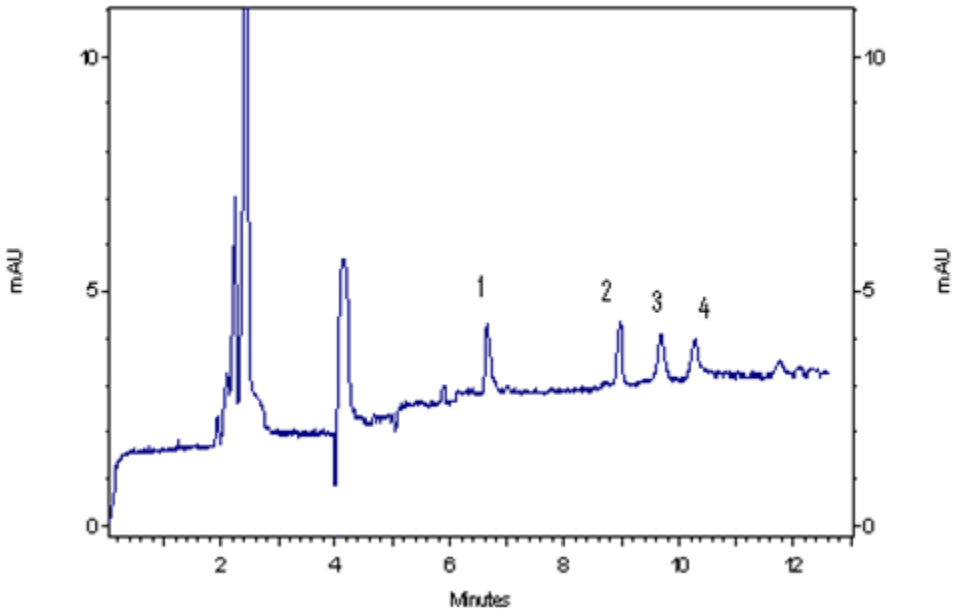
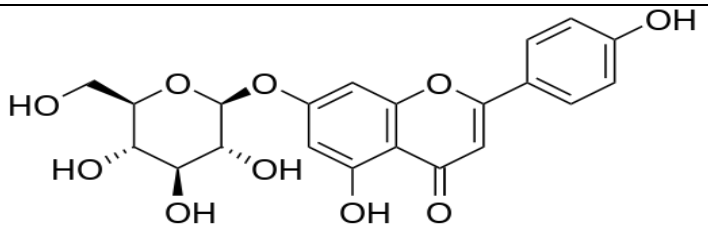
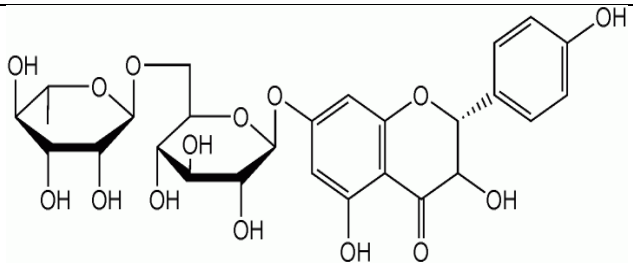
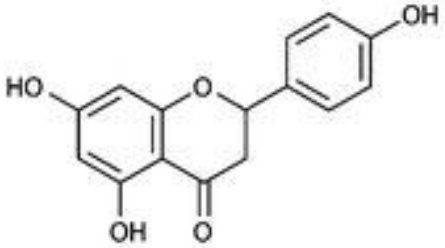
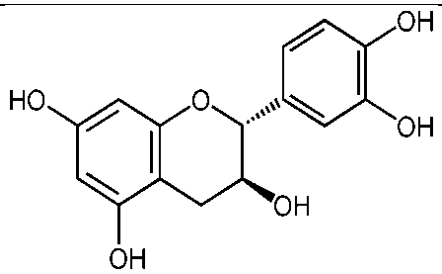
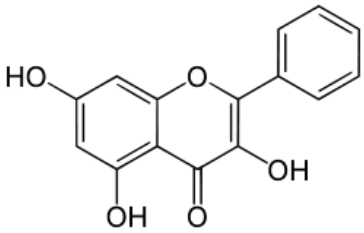
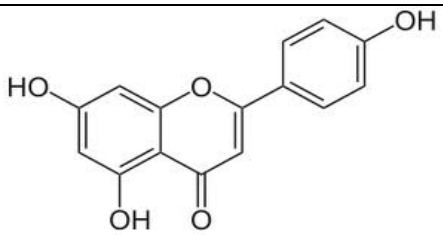
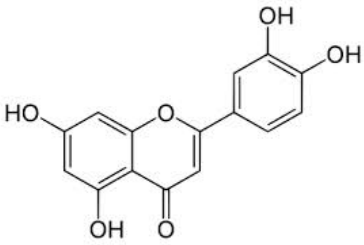
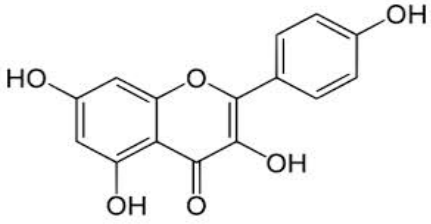
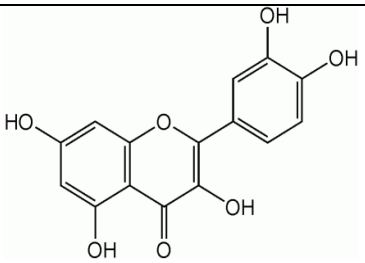


Table 1.

Compounds	Chemical Structure
Apigetrin (Apigenin-7-glucoside) CAS No : 578-74-5	 $C_{21}H_{20}O_{10}$ MW: 432.38 g mol^{-1}
Naringin (4',5,7-Trihydroxyflavanone-7-rutinoside) pK_{a1} = 10.2 ± 0.1 [33]; 9.04 [34] pK_{a2} > 11.50 [33] CAS No : 10236-47-2	 $C_{27}H_{32}O_{14}$ MW: 580.53 g mol^{-1}
Naringenin pK_{a1} = 6.8 ± 0.1 [33]; 7.08 ± 0.12 [35] pK_{a2} = 10.4 ± 0.2 [33] pK_{a2} > 11.50 [33] CAS No : 480-41-1	 $C_{15}H_{12}O_5$ MW: 272.26 g mol^{-1}
Catechin pK_a = 8.6 [36] CAS No : 154-23-4	 $C_{15}H_{14}O_6$ MW: 290.27 g mol^{-1}

Galangin pK_{a1} = 7.19 pK_{a2} = 9.44 [36] CAS No : 548-83-4	 $C_{15}H_{10}O_5$ MW: 270.24 g mol^{-1}
Apigenin pK_a = 7.86 [36] CAS No : 520-36-5	 $C_{15}H_{10}O_5$ MW: 270.24 g mol^{-1}
Luteolin pK_{a1} = 5.99 ± 0.32 pK_{a2} = 8.40 ± 0.42 [37] CAS No : 491-70-3	 $C_{15}H_{10}O_6$ MW: 286.24 g mol^{-1}
Kaempferol pK_a = 7.89 [36] CAS No : 520-18-3	 $C_{15}H_{10}O_6$ MW: 286.24 g mol^{-1}
Quercetin pK_{a1} = 5.87 ± 0.14 pK_{a2} = 8.48 ± 0.09 [37]; 7.76 [36] CAS No : 117-39-5	 $C_{15}H_{10}O_7$ MW: 302.24 g mol^{-1}

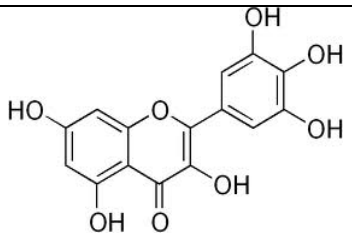
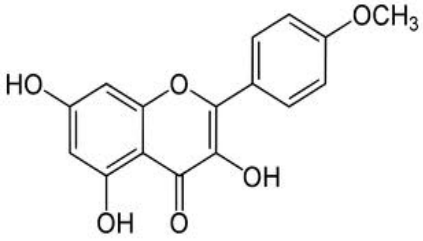
Myricetin pK_a = 6.5 [38] CAS No : 529-44-2	 $C_{15}H_{10}O_8$ MW: 318.24 g mol^{-1}
Kaempferide pK_a = 6.44 [39] CAS No : 491-54-3	 $C_{16}H_{12}O_6$ MW: 300.26 g mol^{-1}

Table 2. R_s values for kaempferol –galangin and apigenin-myricetin

	Voltage			pH		
Compounds	**20**	**24**	**26**	**8.7**	**8.9**	**9.1**
Kaempferol-galangin	2.844	3.195	2.835	1.821	3.981	5.862
Apigenin-Myricetin	3.520	3.523	3.832	4.950	3.596	1.917

Table 3. Statistical evaluation of the calibration data of studied flavonoids by CE–DAD.

Compounds	Naringin	Apigetrin	Catechin	Naringenin	Kaemp-feride	Galangin	Kaemp-ferol	Apigenin	Myricetin	Luteolin	Quercetin
Linear Range (µM)	2.5-100	2.5-100	5-100	2.5-100	2.5-100	2.5-100	2.5-100	2.5-100	5-100	5-100	2.5-100
Correlation Coefficient	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
LOD (µM)	0.143	0.298	0.181	0.098	0.298	0.346	0.5640	0.611	2.019	0.966	1.434
LOQ (µM)	0.434	0.904	0.550	0.297	0.904	1.049	1.712	1.851	6.731	2.929	4.346
Migration Time	4.770	5.226	6.226	6.578	7.109	7.785	8.360	8.819	9.351	9.806	10.097
%RSD of Migration	0.334	1.011	0.557	0.072	1.031	0.287	0.414	0.707	0.315	0.467	0.307

Table 4. Determination results of recovery in this method with dried Chamomile[a] and Linden[b] extracts (n = 3)

Compounds	Original content of sample (μg/g)	RSD (%)	Added amount (μg/g)	Found (μg/g)	Recovery (%)	RSD (%)
Catechin	43.36[a]	0.6	34.80	77.19	97.2	2.0
	44.69[b]	1.2	58.00	101.69	98.3	0.7
Kaempferol	28.44[a]	0.5	22.90	51.39	100.2	1.8
	26.75[b]	0.3	74.42	100.80	99.5	1.2
Quercetin	25.05[a]	1.2	12.09	36.68	96.2	1.8
Apigenin	36.90[b]	0.2	87.07	116.16	91.0	0.4
Mycretin	35.68[b]	0.6	50.92	83.62	94.2	1.6