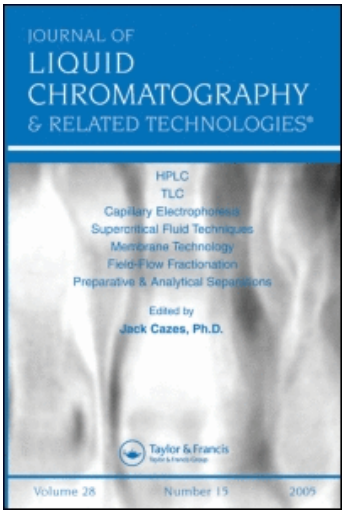

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatographic Investigations of Phenolic Acids and Coumarins in the Leaves and Flowers of Some Species of the Genus Althaea

J. Gudej[a]; M. L. Bieganowska[b]
[a] Department of Pharmacognosy, Institute of Technology and Chemistry of Drugs Medical Academy, łódź., Poland [b] Department of Inorganic, Analytical Chemistry Medical Academy, Lublin, Poland

To cite this Article Gudej, J. and Bieganowska, M. L.(1990) 'Chromatographic Investigations of Phenolic Acids and Coumarins in the Leaves and Flowers of Some Species of the Genus Althaea', Journal of Liquid Chromatography & Related Technologies, 13: 20, 4081 — 4092
To link to this Article: DOI: 10.1080/01483919008049590
URL: http://dx.doi.org/10.1080/01483919008049590

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(20), 4081-4092 (1990)

CHROMATOGRAPHIC INVESTIGATIONS OF PHENOLIC ACIDS AND COUMARINS IN THE LEAVES AND FLOWERS OF SOME SPECIES OF THE GENUS ALTHAEA

J. GUDEJ[1] AND M. L. BIEGANOWSKA[2]
[1]*Department of Pharmacognosy*
Institute of Technology and Chemistry of Drugs
Medical Academy
Muszyńskiego 1
90-151 Łódź, Poland
[2]*Department of Inorganic and Analytical Chemistry*
Medical Academy
Staszica 6
20-081 Lublin, Poland

ABSTRACT

Phenolic acids and coumarins in the leaves and flowers of A.officinalis L., A.armeniaca Ten., A. cannabina L., A.narbonensis Pourr., and A.broussonetiifolia Iljin were investigated by means of high-performance liquid chromatography with reversed-phase systems and paper chromatography. In all the investigated materials only scopoletin was found. The same phenolic acids were identified in all the materials. The contents of phenolic acids was higher in the flowers than in the leaves of the investigated species.

INTRODUCTION

The genus Althaea comprises several species growing in Europe (1,2). In Poland the only known and grown medicinal plant is the marsh-mallow, Althaea officinalis L.(3). The leaves, flowers and roots of the marsh-mallow are used as antiinflammatory and mucilaginous drugs. In the USSR other Althaea species are considered equally valuable medically 4 . A known chemical component of A.officinalis L. and A.armeniaca Ten. is mucilage (5-8), while the data concerning the presence of phenolic acids and coumarins in the leaves of A.officinalis L. are scarce 9,10 . For several years investigations of polyphenolic compounds in A. officinalis L. have been carried out in the Department of Pharmacognosy, Medical Academy, Łódź. Flavonoid compounds have been isolated and phenolic acids and coumarins have been identified chromatographically(11-13).

In the recent years high-performance liquid chromatography has been used for the investigation of phenolic acids and coumarins (14-20).

In the present report the investigation of phenolic acids and coumarins in the leaves and flowers of A. officinalis L., A.armeniaca Ten., A.cannabina L., A. narbonensis Pourr., and A.broussonetiifolia Iljin by means of high-performance liquid chromatography and two-dimensional paperchromatography has been described.

EXPERIMENTAL

1. Plant materials

Plants were cultivated in the Garden of Medical Plants in Łódź, Poland, from seeds provided by various botanic gardens: Althaea officinalis L. - Stuttgart, A.armeniaca Teb. - Zagreb, A.cannabina L. - Frankfurt, Vienna, A.narbonensis Pourr. and A.broussonetiifolia Iljin - Vacratot. The leaves were collected before blossoming (begining of July, 1988) and flowers in the end of July 1988. Vouchers specimens are deposited in the Herbarium of the Department of Pharmacognosy, Medical Academy of Łódź, Poland.

2. Extraction

The dried material (20g) was thoroughly extracted with chloroform in Soxhlet apparatus. The chloroform extract was, after distilling off the solvent, etched with 100 ml of boiling water and left in room temperature for 24 hours. After filtering off the balast substances the aqueous extract was again extracted with chloroform (5 x 100 ml). After distilling off the chloroform the remains were dissolved in 5 ml of methanol - coumarin fraction (E_c). The material after chloroform extraction was again extracted with boiling methanol (5 x 200 ml). From methanol extracts methanol was completely distilled off (vacuum evaporator), and the remains were etched with 100 ml of boiling water

and left at room temperature for 24 hours. After the separation of balast substances, the aqueous solution was extracted with ethyl ether (5 x 100 ml). The ether extracts were condensed partially evaporated to the volume of 50 ml and extracted with 5% $NaHCO_3$ solution (5 x 20 ml). The bicarbonate extracts were acidified with 10% HCl to pH about 3 and again extracted with ethyl ether (5 x 5 ml). The purified ether extracts, after evaporation of the solvent, were dissolved in 10 ml of methanol - phenolic acids fraction (E_a). The extracts so prepared were directly investigated by means of paper chromatography and HPLC.

3. Chromatography

For chromatography the reagents (E.Merck) with the degree of purity for chromatographic analysis were used. The analysis of plant extracts by means of isocratic HPLC was performed with the use of a 302 liquid chromatograph (produced by the Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw), equipped with a 200 ml syringe pump, 5 µl sample injector valve, and an UV 254 nm detector. The separation was carried out on a stainless steel column (250 x 4 mm) packed with 10 µm LiChrosorb RP-18. Coumarins (E_c) were separated by means of mobile phase composed of 30% aqueous methanol with addition of 2.5% acetic acid. Phenolic acids (E_a) were separated by means of mobile phase

Table 1. Rf values of phenolic acids and scopoletin identified by means of two-dimensional paper chromatography in the leaves and flowers of the genus Althaea

No	Compound	Rf	
		I	II
1	Protocatechuic acid	0.06	0.52
2	p-Hydroxyphenylacetic acid	0.37	0.83
3	p-Hydroxybenzoic acid	0.42	0.66
4	Vanillic acid	0.77	0.57
5	Caffeic acid	0.10	0.29, 0.71
6	Syringic acid	0.71	0.51
7	p-Coumaric acid	0.46	0.39, 0.78
8	Ferulic acid	0.67	0.25, 0.74
9	Sinapic acid	0.67	0.15, 0.67
10	Salicylic acid	0.91	0.47
11	Scopoletin	0.42	0.30

composed of methanol - 80% formic acid - water 20:3:77 . Mobile phase flow rate was 1.2 ml min^{-1}. The separations were carried out at room temperature.

Two-dimensional paper chromatography was carried out on Whatman 1 (33 x 33 cm) paper. In dimension I mobile phase composed of benzene - acetic acid - water (6:7:3) was used, while in dimension II - sodium formate - 80% formic acid - water (10:1:200). Spots on chromatograms were localized in UV light before and after ammonia vapours application, and in daylight after sprinkling with 1% $FeCl_3$, 0.5% diazo-sulphanilic acid in 10% Na_2CO_3 or with 0.3% diazo-p-nitroaniline.

RESULTS AND DISCUSSION

Phenolic acids and coumarins in the leaves and flowers of five species of the genus Althaea: A. officinalis L., A.armeniaca Ten., A.cannabina L., A.narbonensis Pourr., and A.broussonetiifolia Iljin, have been investigated by means of paper chromatography and HPLC.

Rf values of the components identified by means of two-dimensional paper chromatography are given in Table 1. The separation of coumarin fraction (E_c) with HPLC is presented on chromatograms - Figures 1 and 2, and phenolic acids (E_a) on Figures 3 and 4.

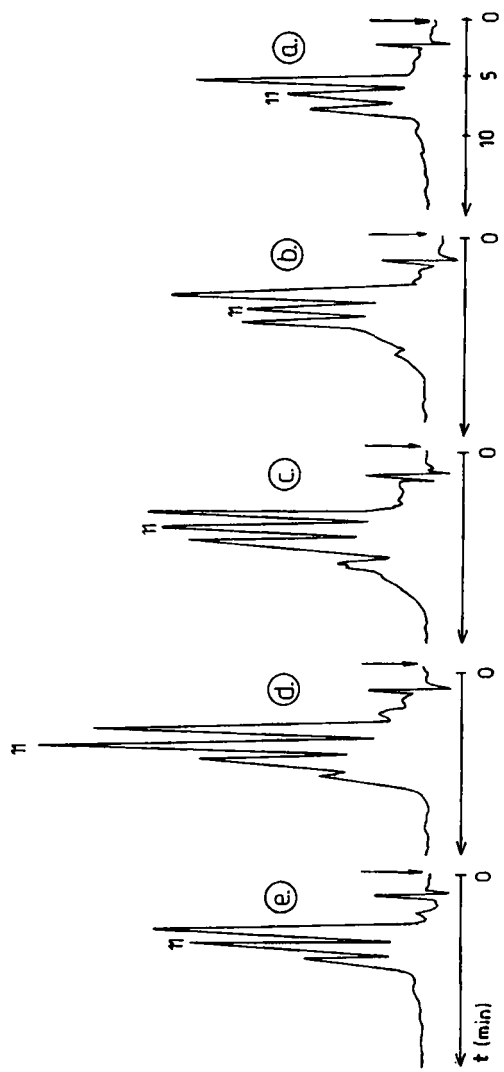

Figure 1

Chromatograms (HPLC) of the chloroform extracts (E_c) from the leaves (a) Althaea officinalis, (b) A. armeniaca, (c) A.cannabina, (d) A.narbonensis, (e) A. broussonetiifolia. For the identification of the solutes see Table 1.

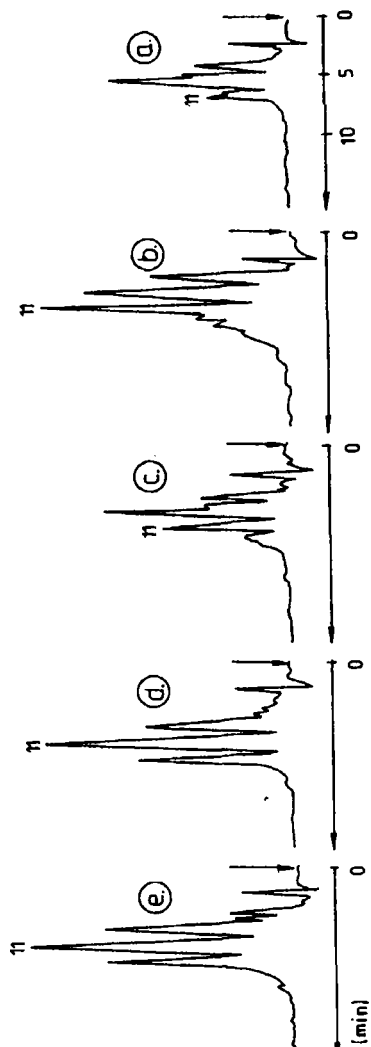

Figure 2

Chromatograms (HPLC) of the chloroform extracts (E_C) from the flowers (a) Althaea officinalis, (b) A. armeniaca, (c) A.cannabina, (d) A.narbonensis, (e) A. brousonetiifolia. For the identification of the solutes see Table 1.

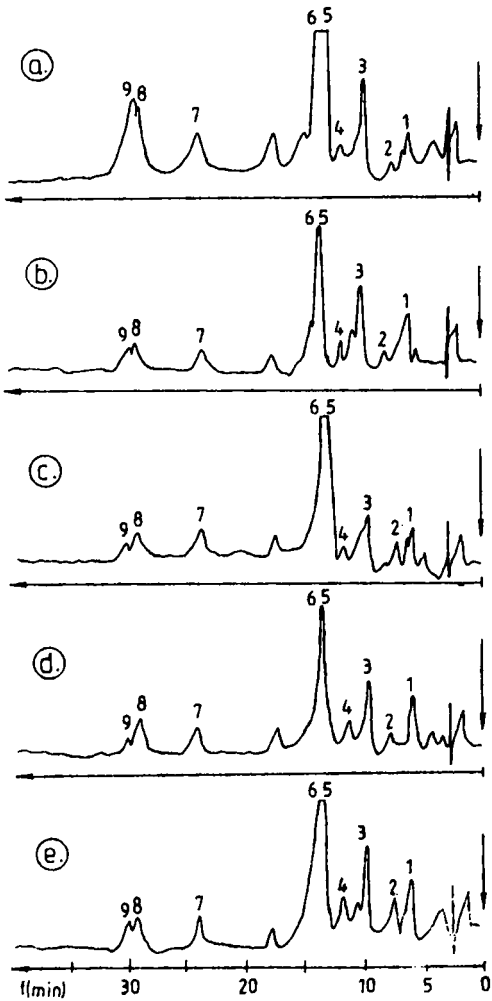

Figure 3

Chromatograms (HCPL) of the phenolic acids fraction (E_a) from the leaves (a) Althaea officinalis, (b) A. armeniaca, (c) A.cannabina, (d) A.narbonensis, (e) A. brousonetiifolia. For the identification of the solutes see Table 1.

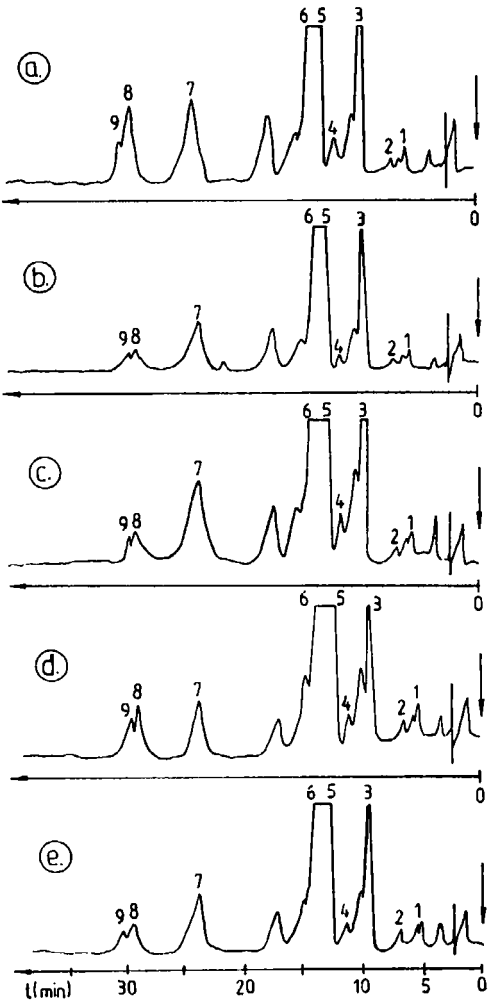

Figure 4

Chromatograms (HPLC) pf the phenolic acids fraction (E_a) from the flowers (a) Althaea officinalis, (b) A. armeniaca, (c) A.cannabina, (d) A.narbonensis, (e) A. brousonetiifolia. For the identification of the solutes see Table 1.

In all the investigated materials only scopoletin was found in coumarin fraction, while esculetin, esculin, 4-methylesculetin, herniarin and umbelliferon were absent. In all the investigated materials the following phenolic acids were identified: protocatechuic, p-hydroxyphenylacetic, p-hydroxybenzoic, vanillic, syringic, salicylic, caffeic, p-coumaric, ferulic and sinapic.

The presence of salicylic acid could not be shown by means of HPLC (weak detection at 254 nm). Syringic and caffeic acids could not be separated, either.

Despite qualitative investigations it can be seen (by the dimensions of spots and the height of peaks) that the contents of phenolic acids in the flowers of the investigated plants is much higher than in the leaves.

REFERENCES

1. Flora USSR, vol 15, Moscow, 1950.
2. Flora Europea, vol 2, Cambridge, 1958.
3. Szafer, Wł., Kulczyński, St., Pawłowski, B., Rośliny polskie, PWN, Warsaw, 1953.
4. Czikow, P., Łaptiew, J., Rośliny lecznicze i bogate w witaminy, PWRiL, Warsaw,1987.
5. Franz, G., Planta Med., 14, 90, 1966.
6. Franz, G., Chladek, M., Pharmazie, 28, 128, 1973.
7. Tomoda, M., Shimazu, N., Suzuki, H., Taksan, T., Chem.Pharm.Bull., 28, 2277, 1981.
8. Rosik, J., Kardosova, A., Toman, R., Capek, P., Cesk.Farm., 33, 68, 1984.

9. Herman, K., Arch.Pharm., 292, 325, 1959.
10. Koleva, M., Ninow, S., Daskalow, V., Ilieva, S., Farmatsiya, 36, 15, 1986.
11. Gudej, J., Acta Polon.Pharm., 42, 192, 1985.
12. Gudej, J., Acta Polon.Pharm., 44, 369, 1987.
13. Gudej, J., Acta Polon.Pharm., 45, 338, 1988.
14. Hartley, R.D., Buchan, H., J.Chromatogr., 180, 139, 1979.
15. Charpentier, B.A., Cowles, J.R., J.Chromatogr., 208, 132, 1981.
16. Casteele, K.V., Geiger, H., Van Sumere, Ch.F., J.Chromatogr., 258, 111, 1983.
17. Olivier, B., Balansard, G., Maillard, C., Vidol, E., J.Pharm.Belg., 40, 173, 1985.
18. Głowniak, K., Bieganowska, M.L., J.Liq.Chromatogr., 8, 2927, 1985.
19. Pussayanawin, V., Vetzel, D.L., J.Chromatogr., 391, 243, 1987.
20. Wawrzynowicz, T., Wąsmundzka-Hajnos, M., Bieganowska, M.L., Chromatographia, 28, 161, 1989.