Chromatographic Investigations of Flavonoid Compounds in the Leaves and Flowers of Some Species of the Genus *Althaea*

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Key Words

Column liquid chromatography
Paper chromatography
Flavonoids
Genus *Althaea*

Summary

The flavonoids present in the leaves and flowers of *Althaea armeniaca Ten., A. cannabina L., A. narbonensis Pourr.* and *A. broussonetiifolia Iljin* were investigated and compared to the flavonoids present in the leaves and flowers of *A. officinalis L.* The investigations were carried out by high-performance liquid chromatography and two-dimensional paper chromatography. The same flavonoids were found in the flowers of all the investigated species while differences could be noted in the flavonoid composition of the leaves. Both the qualitative and quantitative difference was the greatest in the flavonoids of the leaves of *A. cannabina L.*

Introduction

In our previous paper [1] we have reported on the investigations of phenolic acids and coumarins in the leaves and flowers of some species of the genus *Althaea* by HPLC and paper chromatography. HPLC is often applied in the investigation of flavonoid compounds in plant material [27]. The present report is a continuation of our previous investigations of the polyphenolic compounds in the genus *Althaea* [810]. Here we are investigating the flavonoids present in the leaves and flowers of *Althaea armeniaca Ten., A. cannabina L., A. narbonensis Pourr.* and *A. broussonetiifolia Iljin* as compared to the flavonoids present in the leaves and flowers of *A. officinalis L.* (marshmallow) by HPLC and two-dimensional paper chromatography.

Experimental

Plant materials

The plant materials have been described in our previous report [1].

Extraction

After extraction with chloroform, the material (leaves, flowers) (10 g) was extracted with boiling methanol (5 × 200 ml). The solvent was distilled off completely from the combined methanol extracts, the greasy residues were diluted in 50 ml of water and after separation of the ballast substances, extracted with ethyl ether (E_I), ethyl acetate (E_{II}), and from the leaves additionally with n-butanol (E_{III}). After distilling off the solvents the residues were diluted with 50 ml of methanol, except for the acetate extracts from the leaves (E_{II}), which were diluted with 20 ml of methanol.

Chromatographic Analysis

The HPLC analysis of plant extracts was performed with the use of Model 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 × 4 mm) packed with 10 µm LiChrosorb RP-18. Methanol-acetic acid-water mixtures were used as mobile phase, at the following volume ratio:

Ethyl ether extracts (E_I):	50 : 3 : 47
Ethyl acetate extracts (E_{II}):	40 : 3 : 57
Butanol extracts (E_{III}):	70 : 3 : 27

The flow rate was 1.2 ml/min. The separations were carried out at room temperature. Two-dimensional paper chromatography was carried out on Whatman 1 (33 × 33 cm) paper. In dimension I mobile phase composed of n-Butanol-acetic acid-water (4 : 1 : 5) was used as the mobile phase in dimension I and 15 % aqueous acetic acid in dimension II. Spots of flavonoids were localized in UV light, after spraying with 1 % methanolic solution of $AlCl_3$.

0009-5893/90/9 0333-04 $ 03.00/0

Table I Flavonoids identified in the leaves and flowers of the genus *Althaea*, using two-dimensional paper chromatography

No	Compound	RF		Presence in material									
				A. officinalis		*A. armeniaca*		*A. cannabina*		*A. narbonensis*		*A. broussonetiifolia*	
		I	II	L	F	L	F	L	F	L	F	L	F
1	Dihydrokaempferol 4′-O-glucoside	0.56	0.64		+++		+++		+++		+++		+++
2	Naringenin 4′-O-glucoside	0.71	0.58		+		+		+		+		+
3	Quercetin 4′-O-glucoside	0.56	0.40	+	+	+	+	++	+	+	+	+	+
4	Kaempferol 3-O-glucoside	0.70	0.51	+	+	+	+	+	+	+	+	+	+
5	Tiliroside	0.90	0.34	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
6	Hypolaetin 8-0-gentiobioside	0.19	0.18	+++	x	++	x	x	x	++	x	++	x
7	Hypolaetin 8-0-gentiobioside	0.50	0.14	x		x				x		x	
8	Hypolaetin 4′-methyl ether 8-0-glucoside	0.52	0.22	x		x				x		x	
9	Hypolaetin 4′-methyl ether 8-0-glucoside-3′-sulphate	0.27	0.60	+		+				x		x	

Abbreviation: L – Leaves, F – Flowers, x – Trace amounts, +, ++, +++ increasing amounts

Results and Discussion

In order to separate the set of flavonoids in the investigated materials by isocratic HPLC fractioning of the methanol extracts was necessary, as the separation of the whole set of flavonoids was impossible because of marked differences in the polarity of these compounds.

The results of paper chromatography investigations are presented in Table I. HPLC separation of the extracts from various materials are presented in the chromatograms shown in Figures 1–5.

The flowers of all the investigated species of marshmallow contained qualitatively identical sets of flavonoids. The presence of tiliroside kaempferol 3-0-glucoside, quercetin 3-0-glucoside, dihydrokaempferol 4′-O-glucoside, naringenin 4′-O-glucoside and trace amounts of hypolaetin 8-0-gentiobioside has been confirmed. The dominating compounds in the flowers were tiliroside and dihydrokaempferol 4′-O-glucoside.

In the leaves both qualitative and quantitative differences have been observed in the flavonoid content. The set of flavonoids closest to that of *A.*

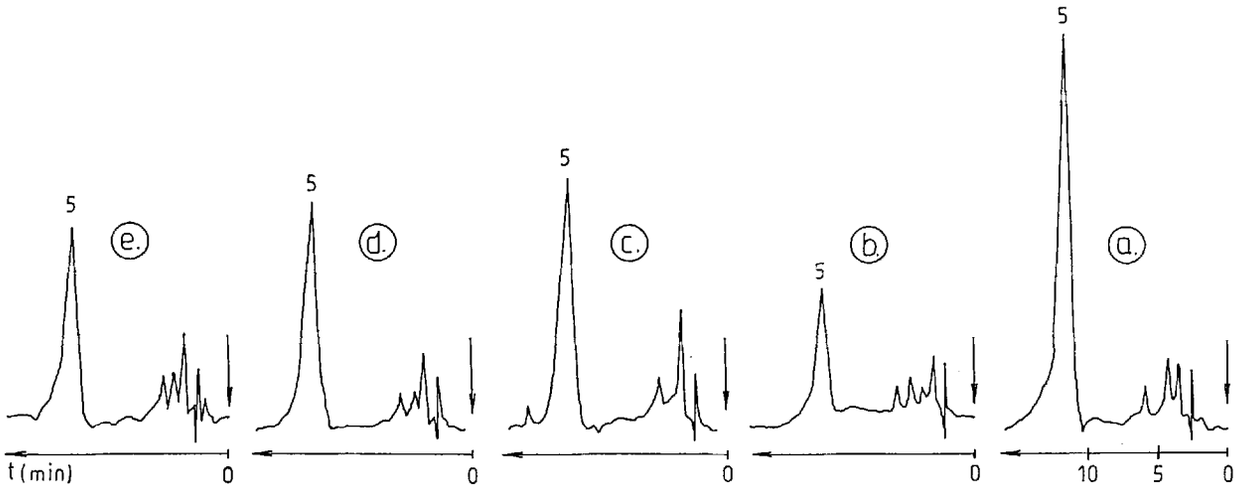

Figure 1
Chromatograms (HPLC) of the ethyl ether extracts (E_I) from the leaves of (a) *Althaea officinalis*, (b) *A. armeniaca*, (c) *A. cannabina*, (d) *A. narbonensis*, and (e) *A. broussonetiifolia*. For the identification of the solutes see Table I.

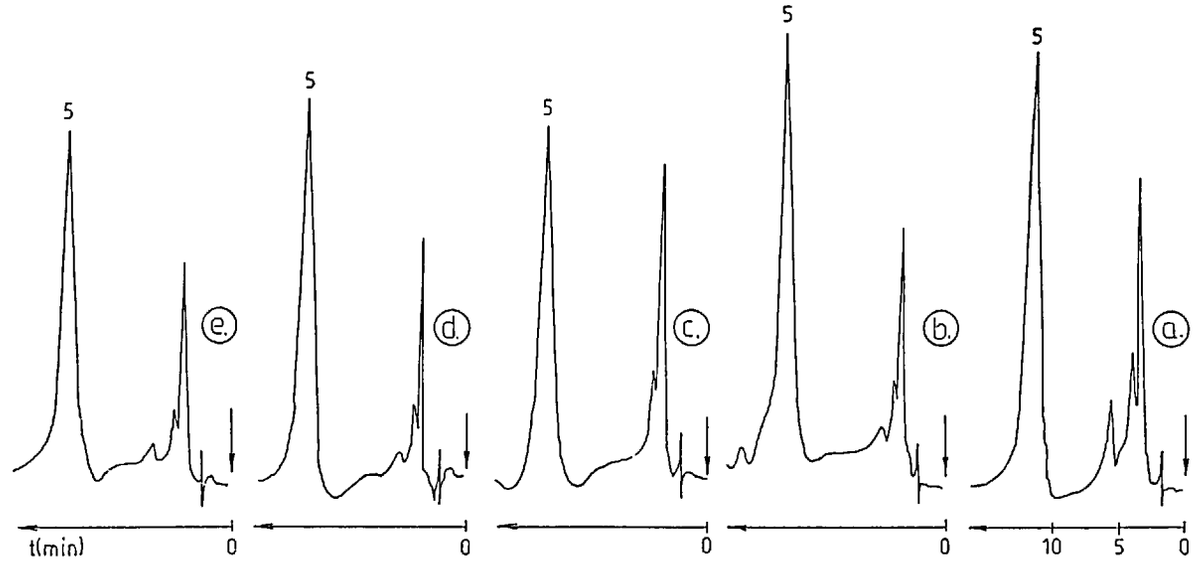

Figure 2
Chromatograms (HPLC) of the ethyl ether extracts (E_I) from the flowers of (a) *Althaea officinalis*, (b) *A. armeniaca*, (c) *A. cannabina*, (d) *A. narbonensis*, and (e) *A. broussonetiifolia*. For the identification of the solutes see Table I.

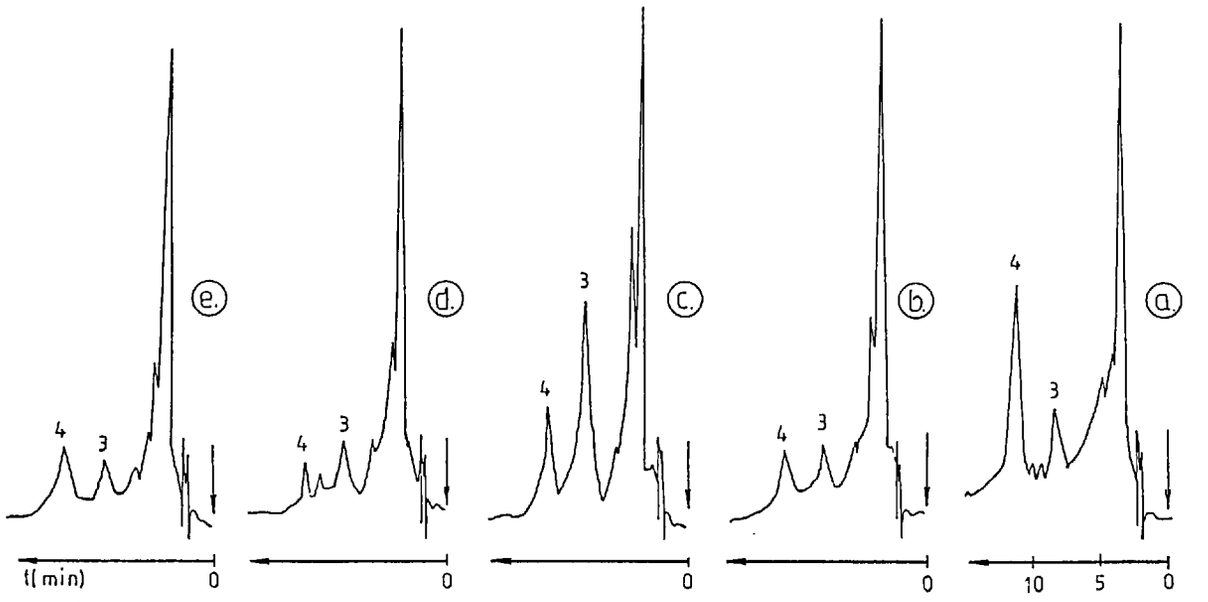

Figure 3
Chromatograms (HPLC) of the ethyl acetate extracts (E_{II}) from the leaves of (a) *Althaea officinalis*, (b) *A. armeniaca*, (c) *A. cannabina*, (d) *A. narbonensis*, and (e) *A. broussonetiifolia*. For the identification of the solutes see Table I.

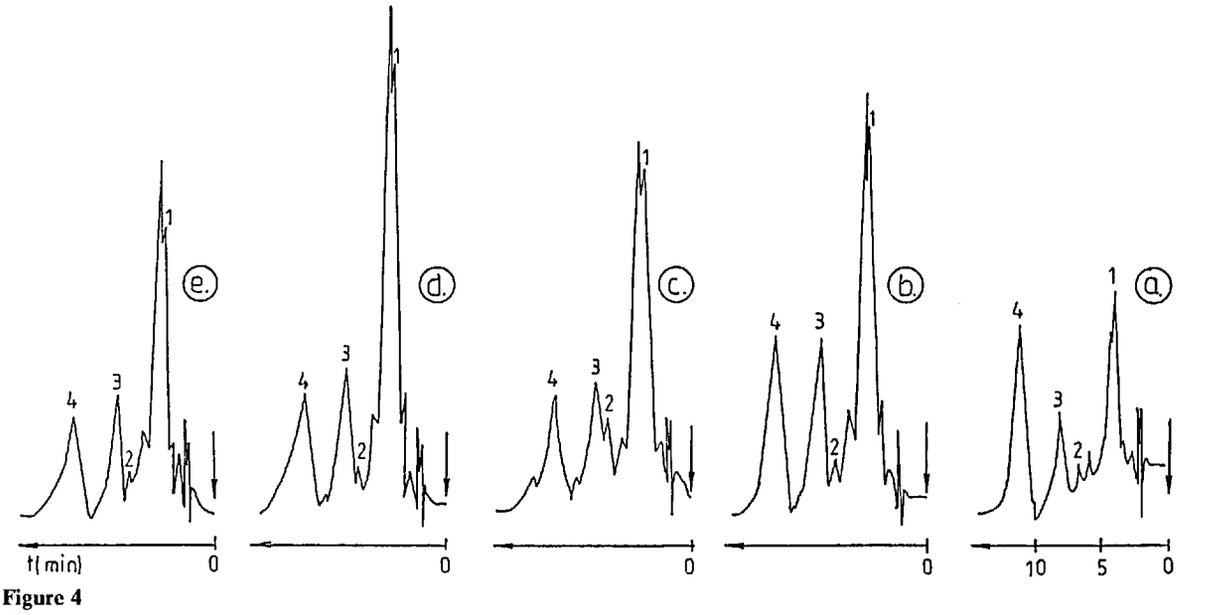

Figure 4
Chromatograms (HPLC) of the ethyl acetate extracts (E_{II}) from the flowers of (a) *Althaea officinalis*, (b) *A. armeniaca*, (c) *A. cannabina*, (d) *A. narbonensis*, and (e) *A. broussonetiifolia*. For the identification of the solutes see Table I.

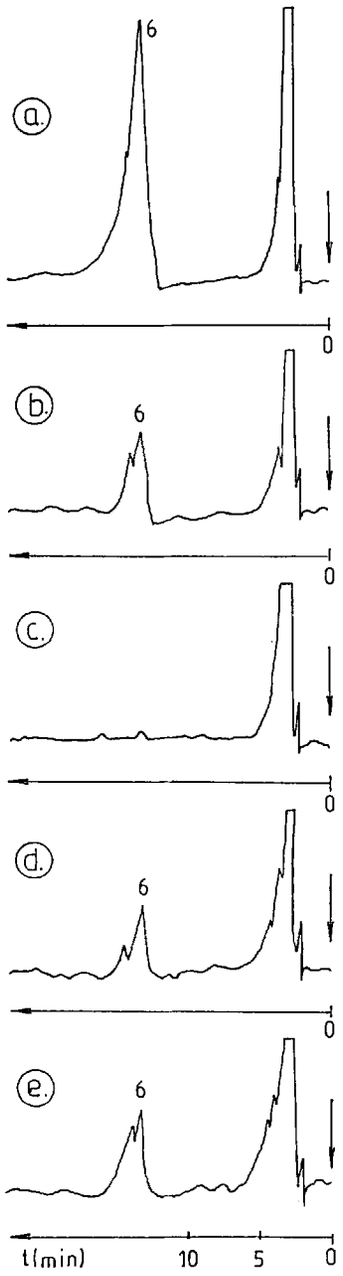

Figure 5
Chromatograms (HPLC) of the n-butanol extracts (E_{III}) from the leaves of (a) *Althaea officinalis*, (b) *A. armeniaca*, (c) *A. cannabina*, (d) *A. narbonensis*, and (c) *A. broussonetiifolia*. For the identification of the solutes see Table I.

officinalis L. was the set of leave flavonoids of *A. armeniaca Ten.* The most different set both qualitatively and quantitatively, was observed for the leave flavonoids of *A. cannabina L.* The dominting flavonoids in the leaves of *A. officinalis L.*, *A. armeniaca Ten.*, *A. narbonensis Pourr.*, and *A. broussonetiifolia Iljin* were tiliroside and hypolaetin 8-O-gentiobioside, while in the leaves of *A. cannabina L* tiliroside and quercetin 3-O-glucoside were the dominating compounds. According to recent literature data some of flavonoids found in the investigated materials show pronounced antiinflammatory activity, as e.g. tiliroside [11] and hypolaetin 8-O-glucoside and its aglycone [12–16]. They may be a supporting factor for the antiinflammatory effect of the mucilage present in the investigated materials.

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Received: Juni 27, 1990
Accepted: July 27, 1990
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