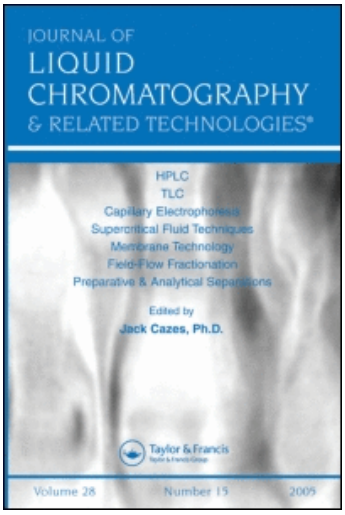

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Comparative HPLC Analysis of Polyphenolic Compounds in Four Species of Galium L.

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COMPARATIVE HPLC ANALYSIS OF POLYPHENOLIC COMPOUNDS IN FOUR SPECIES OF GALIUM L.

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ABSTRACT

Four species of Galium L. (Rubiaceae) were comparatively studied by analytical HPLC for the chlorogenic acid and the flavonoid pattern. The comparison of the HPLC profiles proved a striking correlation of the flavonoid pattern with the classification of the sectios and groups giving a promising feature in the chemotaxonomic study.

INTRODUCTION

Galium is a large and taxonomically difficult genus of the Rubiaceae family, comprising many species with known therapeutic properties [1,2]. In the present work HPLC is used to record a profile of the polyphenolic content of four species of Galium L.. These species are Galium aparine L., G. tricornutum Dandy, G. heldreichii Hal. and G. melanantherum Boiss.. The first two are annuals belonging to the sectio Kolgyda Dumort. (sectio Aparine (D.C.) Griseb). The other two are perennials belonging to the series Erecta Pobed. of the sectio Leiogalium Lebed.. G. aparine is a very variable cosmopolitan

species. G. tricornutum is a european and asiatic species. G. heldreichii and G. melanantherum belong to two different groups of closely related taxa. The first of them belongs to the group of G. mollugo and the second to the G. incurvum group. Both have a relatively small area of distribution: the first is an Aegean element and the second is an endemic of S.E. Greece [3,4].

In the recent years HPLC has been used for the investigation of polyphenolics as an accurate and sensitive technique which gives results rapidly compared to the more classical procedures [5,6]. In the HPLC chromatograms we can notice the difference in the polyphenolic patterns of the species belonging to different sectios and groups.

EXPERIMENTAL

Plant Material

The aerial parts of the above species were collected in June from Attiki. The plant material was dried in a cool dark place and powdered. Voucher specimens are deposited in the laboratory of Pharmacognosy of the University of Athens. 1 g of each specimen was extracted with methanol under reflux for 3 hrs. The extracts were concentrated to 2 ml and adjusted to a volume of 25 ml with methanol.

Chromatography

The above mentioned methanolic solutions were filtered through acrodisc CR 0.45 μm (Gelman) cartridges and 5 μl of the filtrates were injected into HPLC column. The HPLC isocratic analysis was carried out with a Waters Liquid Chromatograph Model 590 equipped with U6K injector, Waters Lambda-Max model 481 variable wavelength detector and Lichrosorb RP-18 column (25 cm × 4.6 mm), 10 μ. The mobile phase was methanol-acetic acid 5% (40-60% by volume). The mobile phase components were degassed in an ultrasonic bath and filtered through a Millipore HA (0.45 μm) membrane filter. The flow rate was 2 ml/min, the UV detector was monitored at 340 nm (0.01 aufs), the chart speed was 1 cm/min and the chromatography run time 10 min.

The interpretation of the peaks was performed by comparison of the retention time with those obtained from reference substances chromatographed under the same conditions. The reference substances were previously isolated and identified with standard procedures from G. melanantherum [7] and G. heldreichii [8].

The HPLC chromatograms of the four species are illustrated in figures 1 and 2.

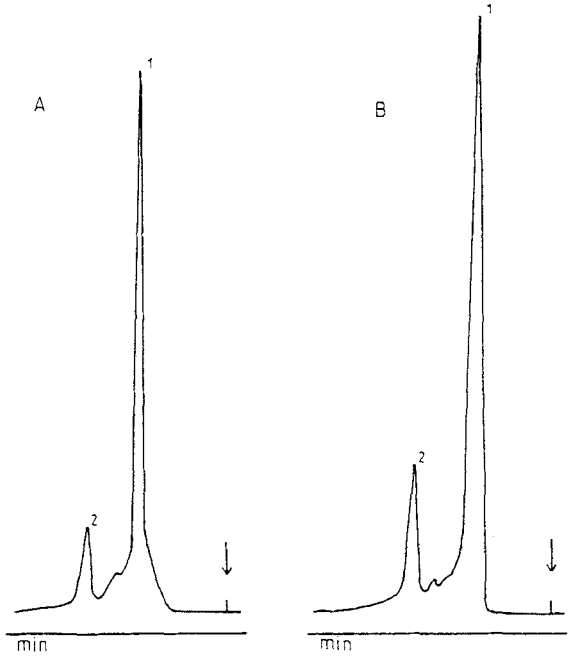

FIGURE 1. HPLC profiles of G. aparine (A) and G. tricornutum (B). 1: chlorogenic acid (t_R 2.08 min), 2: rutin (t_R 3.45 min).

RESULTS AND DISCUSSION

By examining the HPLC profiles we observe that chlorogenic acid is predominant in all species and the differences are noticed in the flavonoid pattern between the sectios. The two species (G. aparine and G. tricornutum) belonging to the sectio Kolgyda Dumort are very poor in flavonoids and they are characterized by the presence of rutin (quercetin-3-rutinoside). In the contrary the two species (G. melanantherum and G. heldreichii) belonging to the sectio Leiogalium Lebed. are richer in flavonoids and they are characterized by the presence of flavone and flavonol mono- and di-glycosides: *luteolin-7-glucoside*, *luteolin-7-diglucoside*, *rutin*, *isoquercitrin* (quercetin-3-glucoside), kaempferol-3-rutinoside. The presence of C-glucosides (orientin: luteolin-8-C-glucoside and vitexin: apigenin-8-C-glucoside) in G. melanantherum contrary to their absence in G. heldreichii is an additional differentiation feature between these species belonging to different groups.

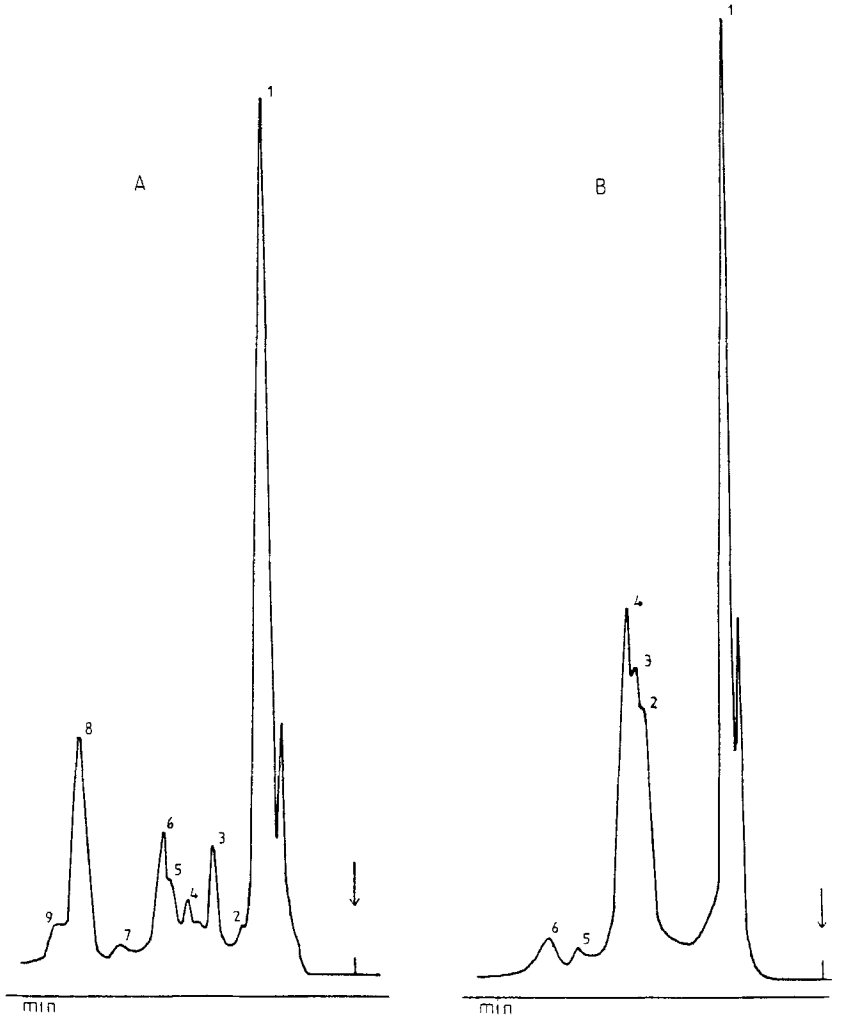

FIGURE 2. HPLC profiles of G. melanantherum (A) and G. heldreichii (B). Peaks of (A) = 1: chlorogenic acid (t_R 2.19 min), 2: orientin (t_R 2.70 min), 3: vitexin (t_R 3.35 min), 4: luteolin-7-diglucoside (t_R 3.99 min), 5: luteolin-7-glucoside (t_R 4.38 min), 6: rutin (t_R 4.50 min), 7: isoquercitrin (t_R 5.65 min), 8: unidentified, 9: kaempferol-3-rutinoside (t_R 7.05 min). Peaks of (B) = 1: chlorogenic acid (t_R 2.18 min), 2: luteolin-7-diglucoside (t_R 3.95 min), 3: luteolin-7-glucoside (t_R 4.18 min), 4: rutin (t_R 4.45 min), 5: isoquercitrin or/and hyperoside (t_R 5.65 min), 6: unidentified.

Isocratic HPLC analysis was chosen because of its reproducible results. The aglyka were not considered because under these conditions of chromatography they possess longer retention times and hence very broad peaks (tailing).

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