# External Flavonoids of 12 Species of *North* American Eupatorieae (Asteraceae)

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*Eupatorium ssp., Brickellia eupatorioides, Asteraceae – Eupatorieae, Lipophilic Exudate, Flavonoid Aglycones*

The flavonoid aglycones excreted by and deposited on aerial parts of 12 species of tribe Eupatorieae, including 10 species of *Eupatorium* (narrowly defined), *Conoclinium coelestinum*, and *Brickellia eupatorioides*, have been analyzed. The flavonoid pattern of *Eupatorium* is dominated by 6-O-methylated flavones, and there are relatively few differences between species including those of section *Verticillata*. In contrast, both *Conoclinium* and *Brickellia* were found to have significantly different flavonoid patterns. In *Conoclinium*, this included more highly O-methylated flavones, including the relatively rare compound 5,7,4'-triOH-6,3',5'-triOMe flavone. In *B. eupatorioides* this consisted of O-methylated flavonols that are a feature previously reported to be characteristic of *Brickellia* and thus support the placement of the species in this genus.

## Introduction

In an earlier paper, one of us has reported external flavonoids found in *Eupatorium cannabinum* (Stevens *et al.*, 1995). In this only European representative of the genus, the amount of exudate in general and the amount of flavonoids in particular is extremely low. Flavonoid aglycones have been reported previously for some 35 species, but in none of the reports their localization in/on the plant received attention. We have now carried out a thorough analysis of the exudated flavonoids accumulated externally on 10 species of *Eupatorium*, as well as two other representatives of the tribe Eupatorieae from eastern North America, *Conoclinium coelestinum* and *Brickellia eupatorioides*.

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## Material and Methods

Aerial parts including inflorescences were collected in the field and air-dried. Vouchers have been deposited at the University of Tennessee Herbarium (TENN). The amounts of dry leaf material used in this study varied between some 40 and 280 g. The average amount of exudate recovered was 2.7% of the dry weight (minimum *E. purpureum* – 0.9%, maximum *E. altissimum*: 7.1%). The collection data are as follows.

*Brickellia eupatorioides* (L.) Shinn. (= *Kuhnia eupatorioides* L.) Tennessee, Loudon County, along Friendsville road, near Centerville Store. E. E. Schilling 95–12.

*Conoclinium* coelestinum (L.) DC. [= *Eupatorium* coelestinum L.] – Tennessee, Knox Co., roadside along Hardin Valley Rd., near intersection with Byington-Solway road. E. E. Schilling 95–17.

*E. album* L. Tennessee, Knox County, field along Couch Mill Road, E. E. Schilling 95–6.

*E. altissimum* L. Tennessee, Loudon County, along Friendsville road, near Centerville Store. E. E. Schilling 95–13.

*E. dubium* Willd. ex Poiret North Carolina, Tyrell County, ditch near Columbia, E. E. Schilling 95–4.

*E. fistulosum* J. Barratt Tennessee, Knox County, ditch along Oak Ridge Highway, Schilling 95–3.

*E. hyssopifolium* L. Tennessee, Knox County, field along Couch Mill Road, E. E. Schilling 95–9.

*E. maculatum* L. New York, Tompkins County, lakeside area, Cornell University campus. E. E. Schilling 95–16.

*E. purpureum* L. Tennessee, Blount County, side of Chilhowee Mountain south of Maryville, Schilling 95–14.

*E. rotundifolium* L. Tennessee, Anderson County, Haw Ridge park, trail along ridge top, E. E. Schilling 95–11.

*E. serotinum* Michaux Tennessee, Anderson County, Haw Ridge park, trail along ridge top, E. E. Schilling 95–10.

*E. sessilifolium* L. Tennessee, Knox County, field along Couch Mill Road, Schilling 95–5.

Dried plant material was briefly rinsed with acetone to dissolve externally accumulated lipophilic material. The concentrated exudate was defatted (MeOH, -10°; centrifugation) and passed over Sephadex LH-20, eluted with MeOH, to separate the flavonoids from the dominating terpenoids.

When necessary, the flavonoid portions were column chromatographed over polyamide SC-6 or over silica, eluted with toluene and increasing amounts of methyl ethyl ketone and methanol. The flavonoids were either identified directly by TLC comparisons with markers, or they were analyzed by spectroscopic methods. For TLC on silica gel we used the solvents a) toluene – MeCOEt 9:1 and b) toluene – dioxane – HOAc 18:5:1; for TLC on polyamide DC-11 we used the solvents c) toluene-petrol$_{100-140°}$-MeCOEt-MeOH, 12:6:2:1, d) toluene – dioxane – MeOH 8:1:1, and e) toluene-MeCOEt-MeOH 12:5:3, respectively. Chromatograms were viewed under UV before and after spraying with "Naturstoffreagenz A". Terpenoids were visualized by spraying silica plates with $MnCl_2$ reagent, followed by heating (Jork *et al.*, 1989). Flavonoid aglycones were identified by direct comparisons with markers and/ or by their spectral data. NMR spectra were recorded in DMSO-$d_6$ at 400 MHz ($^1$H) and 100 MHz ($^{13}$C) on a Bruker ARX-400 instrument.

The following NMR data were measured for 5,7,4'-trihydroxy-6,3',5'-trimethoxy flavone. $^1$H NMR (DMSO-$d_6$) δ (ppm) 13.7 (s, 5-OH), 10.63 (s, OH), 9.33 (s, OH), 7.33 (s, H-2'/ H-6'), 6.97 (s, H-3), 6.67 (s, H-8), 3.89 (s, 3'-OMe/5'-OMe), 3.77 (s, 6-OMe). 13C NMR δ (ppm): 163.7 (C-2), 103.1 (C-3), 182.2 (C-4), 152.7* (C-5), 131.3 (C-6), 157.2 (C-7), 94.4 (C-8), 152.4* (C-9), 104.1 (C-10), 120.4 (C-1'), 104.4 (C-2'), 148.3 (C-3'), 139.9 (C-4'), 148.2 (C-5'), 104.4 (C-6'), 60.0 (6-OMe), 56.4 (3'-OMe), 56.4 (5'-OMe).

## Results and Discussion

Externally accumulated flavonoid aglycones were found in all the plants studied. Scutellarein-6-methyl ether, luteolin, the 6-methyl and the 6,3'-dimethyl ethers of 6-hydroxyluteolin were found

Table I. Flavonoid aglycones on 12 species of Eupatorieae. 1, *Eupatorium album;* 2, *E. altissimum;* 3, *E. hyssopifolium;* 4, *E. rotundifolium;* 5, *E. serotinum;* 6, *E. sessilifolium;* 7, *E. dubium;* 8, *E. fistulosum;* 9, *E. maculatum;* 10, *E. purpureum;* 11, *Conoclinium coelestinum;* 12, *Brickellia eupatorioides.*

| OH-Subst. | Me-Subst. | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Flavonols** | | | | | | | | | | | | | |
| Kaempferol | | | | | | X | | | | | | | X |
| Kaempferol | 3,7,4'-Me | | | | | | | | | | | | X |
| 6-OH-Kae | 6-Me | | X | | | X | | | | | | | |
| 6-OH-Kae | 3,6-Me | | X | | | | | | | | | | X |
| 6-OH-Kae | 3,6,7-Me | | | | | | | | | | | | X |
| 6-OH-Kae | 3,6,4'-Me | | | | | | | | | | | | X |
| 6-OH-Kae | 3,6,7,4'-Me | | | | | | | | | X | | | X |
| Quercetin | | X | | X | X | X | X | | X | | | | |
| Quercetin | 3-Me | | | X | | X | | | | | | | |
| Quercetin | 3,7,3',4'-Me | | | | | | | | | | | | X |
| Quercetaget. | 3,6,7-Me | | | | | | | | | | | | X |
| Quercetaget. | 3,6,7,3',4'-Me | | | | | | | | | | | | X |
| **Flavones** | | | | | | | | | | | | | |
| Apigenin | 7-Me | | | | | X | | | | | | | |
| Scutellarein | 6-Me | X | | X | X | X | X | X | X | X | X | | |
| Scutellarein | 6,7-Me | | X | | | | | X | | | | | |
| Scutellarein | 6,4'-Me | | | X | | X | | | | | | | |
| Scutellarein | 6,7,4'-Me | | X | | | | | | | | | | |
| Luteolin | | X | X | X | X | | X | X | X | X | X | X | |
| Luteolin | 3'-Me | | | | | | | | | | | X | |
| Luteolin | 4'-Me | | X | | | | | | | | | | |
| 6-OH-Lut | 6-Me | X | X | X | X | X | X | X | X | X | X | | |
| 6-OH-Lut | 6,7-Me | | X | | X | | | | | | | | |
| 6-OH-Lut | 6,3'-Me | X | | X | X | | X | X | | X | X | X | X |
| 6-OH-Lut | 6,7,4'-Me | | X | | | | | | | | | | |
| 6-OH-Lut | 6,7,3',4'-Me | | X | | | | | | | | | | |
| 6-OH-Lut | 5,6,7,3',4'-Me | | | | | | | | | | | X | |
| 5,7,4'-OH- | 6,3',5'-OMe | | | | | | | | | | | X | |
| **Flavanone** | | | | | | | | | | | | | |
| Eriodictyol | | X | | X | X | X | | | X | | | | |

in almost every species, though not necessarily as major flavonoids. *In E. altissimum,* the 6,7,4'-triMe and the 6,7,3',4'-tetraMe of 6-hydroxyluteolin were obtained in crystalline form; 6-methoxyluteolin was isolated as major product from *E. hyssopifolium*, while scutellarein-6,4'-diMe was dominating in *E. serotinum*. About half of the species also exhibited quercetin and eriodictyol as minor exudate flavonoids. The complete results are presented in Table I.

The species of *Eupatorium* as narrowly defined by King and Robinson (1970; 1987) produced relatively homogeneous assemblages of 6-O-methylated flavones. The species of *Eupatorium* section *Verticillata* ("Joe-Pye Weeds"), which were earlier segregated as a distinct genus *Eupatoriadelphus,* did not exhibit any distinctive compounds relative to other species of *Eupatorium*. *E. altissimum* and *E. serotinum* both produced a number of compounds not observed in other *Eupatorium* species. The remaining species, *E. album, E. hyssopifolium, E. rotundifolium,* and *E. sessilifolium*, are part of a large species complex in which hybridization and apomixis are common (Montgomery and Fairbrothers, 1970), and lack of differentiation of flavonoids is comparable to the morphological overlap that blurs the distinction of many of the species boundaries. Similar assemblages of compounds have been reported fór other species of *Eupatorium* (Kupchan *et al.*, 1969; Herz *et al.*, 1981; Herz and Kulanthaivel, 1982; Stevens *et al.,* 1995).

The flavonoid data provide further support for the segregation of *Conoclinium* from *Eupatorium*, and suggest a possible relationship of the genus to *Ageratum*. Some taxonomists still treat *C.* coelestinum as part of a large *Eupatorium* (e.g. Cronquist 1981), but considerable evidence has accumulated to support the proposal of King and Robinson (1970) to restrict *Eupatorium* to a relatively small number of primarily north temperate species. The external flavonoids of *C. coelestinum* included flavones with a high degree of O-methylation (Table I; Le Van and Pham, 1979). Similar, highly O-methylated compounds are present in species of *Ageratum* (Adesogan and Okunade, 1979; Quijano *et al.*, 1980, 1982a,b). Molecular studies have also suggested that there may be a close relationship between *Ageratum* and *Conoclinium* (Schilling, unpublished data). 5,7,4'-trihydroxy-6,3',4'-trimethoxy flavone (6-methoxy tricin) is a rather rare flavone. It was first reported from *Conoclinium coelestinum* (Herz *et al.,* 1980), later from several *Artemisia* species, from *Carphochaete bigelovii* (Meurer and Mabry, 1987), from *Conoclinium greggii* (Martinez-Vazquez *et al.,* 1993) (all Asteraceae) and, strangely enough, from *Cleome droserifolia* (Capparaceae) (Sharaf *et al.,* 1992).

The presence of a diversity of methoxylated flavonols is a feature that appears to support placement of *B. eupatorioides* within *Brickellia*. This species is often treated as part of the segregate genus *Kuhnia,* based on its plumose rather than merely barbellate pappus bristles. King and Robinson (1987) follow Shinners (1971) in merging the two, and suggest that the species of *Kuhnia* form a lineage that, although monophyletic, is part of a larger group of comparatively herbaceous species of *Brickellia*. Over 95 species of *Brickellia* have been investigated to some degree for flavonoids (Mabry *et al.,* 1981), and the presence of O-methylated flavonols (in conjunction with other compounds) appears to be characteristic of the genus (Ahmed *et al.,* 1986, 1988; Timmermann *et al.,* 1979, 1981; Ulubelen *et al.*, 1980; Mues *et al.*, 1979; Rosler *et al.,* 1984; Li *et al.*, 1986). Bohlmann *et al.* (1982) in a paper on diterpenes of *B. eupatorioides* (mispelled as *B. eupatoriedes*) report the presence of a single flavonoid, xanthomicrol (5,4'-dihydroxy-6,7,8-trimethoxy flavone), which was not found in the present study.

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