# Further Flavonoids and Other Phenolics of *Thymus webbianus* Rouy

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Seven different flavonoids from the aerial parts of *Thymus webbianus* were isolated. They were identified by UV spectra, TLC and HPLC-DAD as luteolin, apigenin, eryodictiol, naringenin, luteolin-7-O-glucoside, apigenin-7-O-glucoside and apigenin-6,8-di-C-glucoside. Other phenolics characterized by HPLC-DAD analysis were: protocatechuic, chlorogenic, syringic, *p*-coumaric and 3,5-dicaffeoylquinic acids.

The genus *Thymus* includes eight sections, seven of which are composed principally of Mediterranean plants, often endowed with antimicrobial and spasmolytic properties. The species that grow in the Iberian Peninsula are characterized by a large number of endemisms. The composition of the essential oil of *Thymus webbianus,* a species inhabiting a very restricted litoral area in Eastern Spain, and its flavonoid aglycone content were previously studied (Zafra-Polo *et al.,* 1988; Blázquez *et al.,* 1990) from a chemotaxonomical point of view.

Our present objective is to complete this study by characterizing the possible presence of other flavonoids and phenolic acids.

Column chromatography followed by preparative TLC was used to isolate seven flavonoid compounds from ethyl acetate and butanol extracts of *Thymus webbianus.* These compounds were two flavones (luteolin and apigenin), two flavanones (eryodictiol and naringenin) and three glycoside flavones (apigenin-6,8-di-C-glucoside, luteolin-7-O-glucoside and apigenin-7-O-glucoside). These compounds were identified on the basis of the coincidence between data from UV spectra measurement with the usual reagent shifts, TLC comparison and HPLC-DAD analysis with authentic samples. Aglycones and sugars were also identified by co-TLC against standards after the usual acid hydrolysis of glycosides.

In the diethylether extract whose aglycone flavones had previously been reported, we have now identified protocatechuic, chlorogenic, syringic, *p*-coumaric and 3,5-dicaffeoylquinic acids on the basis of the HPLC retention times and by reference compound data stored in a computer system.

It should be noted that apigenin and luteolin have been found in both free and glycoside states. In fact, these compounds are the most frequently isolated flavones of the *Thymus* genus. Concerning the glycosides, the flavonoid O-glycosides are common in *Thymus,* as in many other members of the Labiatae family, while C-glycosides are much less frequent. However, mono- and di-C-glycosides of apigenin are extensively described in the *Thymus* genus. For example, the presence of apigenin-6,8-di-C-glucoside (Vicenin-2) is a constant in the majority of the *Thymus* species, but not in other related genera such as *Origanum.* In this work we have isolated this compound from the butanolic extract of *T. webbianus* and identified it before and after hydrolysis acid. *T. webbianus* is therefore another species of the *Thymus* section that corroborates the almost universal distribution of Vicenin-2 in the *Thymus* genus (Husain and Markham, 1981).

In relation to the flavanone content, there are few species in which these compounds are described. The flavanones naringenin and eryodictiol are simultaneously present only in *T. piperella* (*Piperella* section) (Barberán et al., 1985). Up to now only the flavanone naringenin has been described in *T. vulgaris* (*Thymus* section). In the *Thymus webbianus* species, which belongs to this section and is analyzed here, both flavanones were isolated. On the other hand, eryodictiol, considered rare in the *Thymus* genus and in the Labiatae family, appears here in large amounts than naringenin.

Another fact related to *T. piperella* is the isolation of 7,4′-dimethylapigenin in the *Thymus*

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*webbianus*, whereas until now it had only been described in species belonging to *Piperella* section. Nevertheless, *Thymus webbianus* differed significantly from this section. Genkwanin, cirsimaritin and the methoxylated flavone thymonin, for example, were not found in the extensively studied *Thymus piperella* species and were isolated in *Thymus webbianus*, as occurs in other species belonging to *Thymus* section (Blázquez *et al.*, 1990).

Finally, we have found considerable amounts of phenolic acids. These compounds are rarely studied in the *Thymus* species and, in fact, in the *Thymus* section have only been extensively studied in *T. carnosus* (Marhuenda *et al.*, 1987). In the present study, we also found *p*-coumaric and syringic acids as this last species. However, we also characterized three other phenolic acids, one of which – 3,5-dicaffeoylquinic – has not been described before in species of the *Thymus* genus.

## Materials and Methods

### *Plant material*

The aerial parts of *Thymus webbianus* Rouy (Lamiaceae) was collected at the flowering stage in March, 1986, at Peñón de Ifach (Alicante, Spain). A voucher specimen was deposited in the herbarium of the Department of Botany, Faculty of Pharmacy, University of Valencia.

### *Extraction and separation*

Air-dried leaves and stems (100 g) of *T. webbianus* were cut-off, crushed and extracted with 70% aqueous MeOH (5×2 l). Liquors were combined and the methanol was removed under reduced pressure. The aqueous fraction was treated successively with diethylether, ethyl acetate and butanol yielding 2.65, 1.60 and 5.50% extracts respectively.

### *HPLC analysis*

HPLC-DAD was carried out on a Merck-Hitachi HPLC system (L-6200 pump) equipped with a photodiode array detector (Merck-Hitachi L-3000) with a reversed phase column Lichrospher RP-18 (12.5×0.7 cm) (particle size 5 μm).

The separation of different compounds was performed using water–TFA (99.95:0.05) as the first solvent (A) and MeOH–TFA (99.95:0.05) as the second one (B), at a flow rate of 1 ml/min starting with 10% B and increasing B to 20% at 5 min, 50% at 45 min and 80% at 55 min. Column pressure was 60–80 bar and the UV detector was set at 280 nm. Filtered samples of extracts (10 mg/ml) in MeOH were applied to the column by mean of a 20 μl loop valve. Data were compared with authentic samples. Identification of isolated flavonoids was carried out by UV spectra on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer.

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