were between 0.39% and 3.12%. Against fungi, the essential oil was active in a wider range of concentrations between 1.56 and 12.50%. Against molds, the essential oil had a fungicidal effect at higher concentrations for *Aspergillus niger* (50–80%), while the fungistatically effective concentrations for *A. flavus* were somewhat lower (6.25–59%). Significantly lower concentrations (0.39–3.12%) had an effect on *Aspergillus ochraceus*. The essential oil had a strong effect on the dermatophytes (Microsporum gypseum and Trihophyton mentagraphytes) ranged between 1.56 and 6.25%. In case of *Epidermophyton floccosum*, the active concentrations were 25% (MIC) and 50% (MMC).

Experimental

Tanacetum parthenium was collected during the flowering stage in June and July of 1995 in Hrv. Zagorje in Croatia. The flowers were dried and obtained by hydrodistillation according to Ph. Jug. IV [10]. The oil was diluted in pentane before being analyzed by GC and GC/MS. GC analysis of the essential oil was performed on a Pye Unicam PU 4550 equipped with FID and a 25 m × 0.32 mm fused silica column with CP-Sil-8 CB (SE 54-CB). Conditions of the instrument: Injection port and detector kept at 250 °C, oven was programed 1 min at 60 °C, then 4 °C/min to 220 °C; carrier gas (hydrogen) flow rate was 0.5 ml/min. Quantitative data were obtained with the assistance of a PU 4810 microprocessor after normalization of the peaks. Components were identified using retention times, and also by comparing obtained MS with those stored in a Data Library. MS (70 eV) were taken using Kratos MS 25 GC/MS instrument coupled with a data station.

The antimicrobial activity of *T. parthenium* essential oil was determined by the agar diffusion and broth dilution methods. Mueller-Hinton agar was used as the bacteria-breeding diffusion method, and Sabouraud's agar for the fungi, molds and dermatophytes. The broth dilution method was applied using nutrient broth and Sabouraud medium [11]. Suspensions of the vegetative forms of microorganisms was used as inoculum. In the case of molds and dermatophytes suspensions of their spores were applied. The spores of the molds and the dermatophytes were prepared with the required volume of the physiological solution. Agar was inoculated with 1 ml of inoculum (10^6–10^7 colony forming units of microorganisms).

Different concentrations of the essential oil (1 µl) were applied to the medium (6 mm diameter wells). The inoculated medium, together with the applied essential oil, was refrigerated for 1 h at 4 °C. The plates were than incubated at either 37 °C for bacteria (24 h), or 25 °C for fungi (2–7 d). After incubation the inhibition zones were measured. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) were determined by the broth dilution method. Dilutions of the essential oil between 0.09 and 80% were prepared. Suspensions of microorganisms were added to test tubes containing sample and control test medium. MIC_s and MMC_s were determined after incubation.

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Received January 22, 1997
Accepted March 15, 1997

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Pharmazie **52** (1997) 11

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Isolation and identification of flavonoid aglycones from some taxa of Sect. *Marginati* of genus *Thymus*

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Many of the wildly growing species of genus *Thymus* L. that appear in Macedonian flora belong the Sect. *Marginati* (A. Kerner) A. Kerner [1]. Most of the taxa are characteristic of the Balkan peninsula representing certain endemisms. Few of them are spread widely throughout the whole territory of Macedonia (*T. tosevii* ssp. *tosevii* Vel. var. *tosevii*, var. *degeneii* Ronn., var. *longifrons* Ronn., *T. longidens* var. *lanicaulis* (Ronn.) while a few other are spread in smaller areas (*T. albanus* H. Braun, *T. balcanus* Borb.) and a certain number of them represent Macedonian endemism (*T. alsarensis* Ronn., *T. oemianus* Ronn. et Soška) [1].

In order to investigate the possible pharmacological interest of some Macedonian *Thymus* taxa, we started an extensive study of the chemical constituents of Sect. *Marginati*. Recently, we reported a modified UV spectrophotometric method for determination of total flavonoids in *Thymus* taxa [2]. The aim of this study was the isolation and identification of free flavonoid aglycones. A distribution of flavonoid aglycones and related phenolic acids in thirteen taxa of Sect. *Marginati* was also observed.

Column chromatography followed by preparative TLC was used to isolate five flavonoid aglycones from diethyl ether extracts. These compounds were the flavones apigenin, luteolin and diosmetin and the flavanones, naringenin and eriodyctiol. They were identified on the basis of data obtained from UV spectra measurements with usual shift reagents [3], TLC comparison with authentic samples, HPLC-DAD analysis as well as m.p. measurements (Table 1). Related phenolic acids, rosmarinic and caffeic, were identified in diethyl ether extracts by HPLC-DAD. Distribution of the flavonoid aglycones and the phenolic acids in thirteen *Thymus* taxa is given in Table 2. In five of them (*T. tosevii* ssp. *tosevii*, var. *tosevii*, var. *longifrons*, var. *degenii*, *T. tosevii* ssp. *substriatus*, *T. longidens* var. *lanicaulis*), belonging to the Sect. *Marginati* Subsection *Verticillati*, apigenin, luteolin, naringenin and eriodyctiol were found to be the only isolated flavonoid aglycones from the diethyl ether extracts. Luteolin was the principal constituent.

T. longidens var. *dassareticus* Ronn. belonging to the same subsection, contained diosmetin instead of luteolin. From *T. alsarensis* and *T. macedonicus* (Deg. et Ur.) Ronn., only apigenin and luteolin were isolated and identified. Using HPLC-DAD, it was possible to identify eriodyctiol in the diethyl ether extracts. Both phenolic acids were identified in each of the above mentioned taxa. Taxa belonged to the Sect. *Marginati* Subsection *Marginati*: *T. moesiacus* Vel., *T. albanus* and *T. balcanus*, contained only flavones apigenin and luteolin, followed by caffeic acid. In *T. jankae* var. *jankae* Čel. and *T. jankae* var. *pantotrichus* Ronn., from the same subsection, higher

18:5:1; CL-1: 30% HOAc; detection: UV light (λ-254 nm and λ-366 nm), 5% solution of $AlCl_3$ in CH_3OH.

HPLC: A column (250 × 4.6 mm) filled with RP-C-18 stationary phase (5 μm) was used. For injection of sample sol solutions a manual loop valve injector (20 μm) was employed. The separation of different components was performed using H_2O/HCOOH (99.9:0.1)/CH_3CN at a flow rate of 0.8 ml/min, starting with 10% CH_3CN for the first 5 min, followed by a gradient to 35% of CH_3CN after 10 min. UV detection was carried out at 254, 286 and 360 nm. Data were compared with those for authentic samples.

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Received December 23, 1996
Accepted February 26, 1997

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