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FLAVONOIDS AND TOCOPHEROLS FROM *Paronychia kapela*

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Paronychia kapela (Hacq.) Kerner (*Caryophyllaceae*) (sin. *P. chionaea*) is a weed widespread in Southern and Western Europe, in particular in Spain, Italy, and Croatia, where it's also known as "Sanguinaria." As a medicinal one, this plant is used, as an infusion, in the folk medicinal tradition of the above-mentioned countries as a diuretic, hypotensive, antirheumatic, and depurative agent and in the treatment of infections of the respiratory and urinary apparatus, to cure renal calculosis, and topically as a liniment for contusions, wounds, and burns [1]. No other therapeutic applications nor phytochemical studies about this plant are cited in the literature, and this represents the first phytochemical study about this plant. In the present communication we wish to report the results obtained on the qualitative and quantitative analysis of flavonoids and tocopherols extracted from *P. kapela* grown in central Italy.

The plant was collected in June 2002 in Monte Vettoretto (Pian Grande di Castelluccio, Umbria, Italy) and air dried. The plant was identified by Luigi Menghini and Rita Pagiotti and a voucher specimen is deposited at Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali of University of Perugia, Italy.

Carrying out the analysis of tocopherols, 70 g of triturated dried aerial parts of *P. kapela* were extracted for 12 h with *n*-hexane (500 mL) in a Soxhlet apparatus and the corresponding solution evaporated to dryness; 1.25 g (1.78% of the dry weight) of the *n*-hexane extract were obtained and analyzed by GC/MS spectrometry, using a Hewlett Packard 6890 gas chromatograph equipped with a 12.5 m × 0.25 mm MetSil column coupled to HP ChemStation Software. The carrier gas was helium at a pressure of 3.5 kg/cm^2 and the column temperature was programmed from 50°C to 270°C at 10°C/min. The chromatogram was obtained using a reporting integrator and the composition recorded as a percent area. Mass spectra were obtained from a GC-MS system, operating in the EI mode at 70 eV, equipped with a 12.5 m × 0.25 mm MetSil column and an HP 5973 Mass Selective Detector, using the same chromatographic conditions reported above. The column was connected to the mass spectrometer ion source *via* an open split interface heated at 250°C. Identification of chemical constituents was based on a comparison of mass spectra obtained from commercially available samples and from the Nist98 Mass Spectral Database. The chemical composition of the apolar fraction of *P. kapela* is reported in Table 1. The main components of the *n*-hexane extract are long chain hydrocarbons, long chain alcohols and esters, phytol, and notably tocopherols, which represent about 15% of the total extract. Other components contained in low percentage were triterpenes, steroids, waxes, and fatty acids.

Carrying out an analysis of flavonoids, 2 g of finely triturated sample (flowers, leaves, seeds, and roots) from *P. kapela* were extracted with 100 mL of a 7:3 methanol/water mixture at room temperature for 48 h. Each extract was filtered, concentrated, washed with chloroform (3 × 20 mL), stirred with 15 mL of 4N HCl at 85°C for 45 min, and finally extracted with ethyl acetate (3 × 20 mL). The collected organic phases were dried on Na_2SO_4 and evaporated to dryness. The residue was dissolved in methanol (2 mL) and analyzed by HPLC. Analysis of flavonoids was carried out on all parts of the plant using an HPLC apparatus equipped with a 250 × 4 mm (∅ = 5 μm) RP18 column and coupled to a UV detector (λ = 340 nm). Eluents were 3% aqueous acetic acid (A) and acetonitrile (B) using three ramps (A/B 84:16 from 0 to 20 min, A/B 70:30 from 20 to 22 min, A/B 84:16 from 22 to 30 min) along the elution time. The chromatogram was obtained using a reporting integrator and the composition recorded as a percent area. Identification of chemical constituents was based on a comparison of retention times with those of commercially available pure samples.

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TABLE 1. Percentage Chemical Composition of *n*-Hexane Extract of *P. kapela*

Compound	%
1,19-Eicosadiene	11.77±0.12
1,4-Eicosadiene	7.33±0.09
Phytol	8.47±0.07
1-Eicosanol	6.27±0.07
1-Docosanol acetate	13.99±0.10
Nonacosane	22.44±0.12
Hentriacontane	9.59±0.09
Tocopherols	14.85±0.06

Values expressed as mean of 3 measurement ± S.D.

TABLE 2. Flavonoid Composition of Flowers, Leaves, Seeds and Roots Extracts of *P. kapela*

Compound	Flowers*	Leaves*	Seeds*	Roots*
Chlorogenic acid	3±0.5	2±0.2	70±0.7	1±0.1
Caffeic acid	10±0.9	4±0.2	60±0.8	7±0.2
Vitexin	110±1.2	14±0.3	60±1.2	3±0.2
Rutin	74±1.3	114±1.4	180±1.4	60±0.6
Quercetin-3-D-galactoside	30±0.2	43±0.9	282±1.1	23±0.4
Myricetin	25±0.4	6±0.1	142±1.2	6±0.3
Luteolin	15±1.2	37±0.8	94±0.9	2±0.1
Kaempferol	70±1.2	2±0.2	1805±1.2	1±0.1

*mg/g of the dry weight. Values expressed as mean of 3 measurement ± S.D.

The flavonoid composition of flowers, leaves, seeds, and roots of *P. kapela* is reported in Table 2.

In all samples eight flavonoids were identified as the main components: chlorogenic acid, caffeic acid, vitexin, rutin, quercetin 3-*D*-galactoside, myricetin, luteolin, and kaempferol. As reported in Table 2 vitexin, rutin, and kaempferol were the most abundant components in flowers, rutin is contained in the highest amount in leaves, kaempferol is the most abundant component in seeds, and finally rutin is the main component in roots. However, the flavonoid content in this latter part of *P. kapela* is by far less when compared to flowers, leaves, and seeds.

The methanolic extract of *P. kapela* was preliminarily tested for its *in vitro* antimycotic activity against *Candida albicans, Trichophyton mentagrophytes, Aspergillus flavus,* and *Fusarium culmorum*, but no activity was observed.

In conclusion, from the data reported herein it is evident that *P. kapela* contains a quite high concentration of antioxidant active principles, both lipophilic (tocopherols) and hydrophilic (flavonoids); the latter may account for the reported biological effects exerted by polar extracts from *P. kapela*. This study represent a valid contribution to the ongoing search for antioxidants - containing plants for medical purposes. Studies to further characterize the phytochemical composition and the biological activity of *P. kapela* extracts are now in progress in our laboratories.

REFERENCES

1. For a review on pharmacological uses described so far of extracts from *P. kapela* see http://www.fitoterapia.net/vademecum/plantas/657.html