

2. Tested saponins

Hederacolchiside A (1) and hederacolchiside A' (2) were isolated from the leaves [5, 6]. α -Hederin (3), β -hederin (4), δ -hederin (5), colchiside 4 (6), colchiside 6 (7), and colchiside 7 (8) were isolated from the berries [4–9].

3. Antifungal assay

The antifungal activity of saponins was evaluated against yeasts and dermatophytes with an agar dilution method as previously described [1]. The tested concentration range was 3.12–100 μ g/ml. The reference antifungal agents were amphotericin B for yeasts and ketoconazole (Janssen pharmaceutical, Beerse, Belgium) for dermatophytes. Minimum Fungicidal Concentration (MFC) was defined as the first concentration showing no visible growth after incubation time.

4. Antiprotozoal assay

The protozoocidal activities against *Leishmania infantum* promastigotes (MHOM/FR/78/LEM75 strain) and *Trichomonas vaginalis* (TVR87 strain) were evaluated as previously described [10]. Regarding *Leishmania*, the inhibitory concentration 50 (IC₅₀) was evaluated with pentamidine as reference compound. The trichomonocidal activity (lethal dose 100, LD₁₀₀) was evaluated with metronidazole as reference compound.

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Flavonoids of *Crataegus stevenii*

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Several species of the genus *Crataegus*, commonly called Hawthorn, have been studied for the therapy of heart diseases, deficiency of the coronary blood supply and arrhythmias. Flavonoids and oligomeric procyanidins are the main constituents responsible for the biological activity of *Crataegus* species [1]. *Crataegus stevenii* Pojark. is one of the 17 species of *Crataegus* growing in Turkey [2]. This report is a part of series on the chemical investigations of *Crataegus* species from Turkey [3]. We report the isolation and characterization of 7 flavonoids from the leaves, flowers and fruits of *C. stevenii* Pojark.; a lipophilic flavonoid scutellarein 4',7-dimethylether has been isolated for the first time from the *Crataegus* sp. Since the isolation of the compounds do not give the absolute flavonoid quantity, the quantitation of the flavonoids from the leaves, flowers and fruits of *C. stevenii* was done according to DAB 10 [4]. These results are also given in Table 1. According to DAB the *Crataegus* leaves and flowers should have minimally 0.7% flavonoid content, so 1.39% flavonoids found in the flowers and 0.89% in the leaves are relatively high for *Crataegus* species. Hyperoside is the main compound in all parts of *C. stevenii*.

Table: Quantity of the flavonoids isolated from *Crataegus stevenii*

Compounds	Material		
	Leaves (500 g)	Flowers (250 g)	Fruits (220 g)
Scutellarein	–	35 mg (D)	13 mg (D)
4',7-dimethylether			
Apigenin	73 mg (D)	64 mg (D)	16 mg (D)
Quercetin	520 mg (D, E)	120 mg (D)	63 mg (D)
Apigenin	92 mg (D, E)	–	–
7-O-glucoside			
Hyperoside	2095 mg (E)	750 mg (D, E)	122 mg (E)
Vitexin	98 mg (E)	70 mg (E)	–
4'-O-rhamnoside			
Vitexin	125 mg (E)	82 mg (E)	–
2''-O-rhamnoside			
*Quantitation %	0.89	1.39	0.33

* Quantitation shows the amount of total flavonoids calculated of hyperoside, according to DAB 10

Experimental

1. Plant material

Leaves, flowers and fruits of *C. stevenii* were collected near Afyon in Turkey. The plant material was identified by Prof. Dr. Kerim Alpınar and voucher specimens have been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 62472).

2. Extraction, isolation and identification

The dried and powdered leaves, flowers and fruits of *C. stevenii* were extracted in a Soxhlet apparatus first with petroleum ether and then with ethanol. The petroleum ether extract was concentrated (A) and then extracted with 60% ethanol. The aqueous extract was concentrated and extracted with chloroform (B). The ethanol extract was concentrated, diluted with water and extracted with benzene (C), chloroform (D) and ethyl acetate (E) successively.

For the purification of flavonoids silicagel column chromatography, preparative TLC, and PC were applied. The structures of all the isolated com-

pounds were determined by comparison with authentic samples (TLC, UV, IR). Flavone O- and C-glucosides were subjected to acid and FeCl₃ hydrolysis, respectively.

Detailed information of the isolation procedure and copies of the original spectra are obtainable from the author of correspondence.

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