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Antifungal and antiprotozoal activities of saponins from *Hedera colchica*

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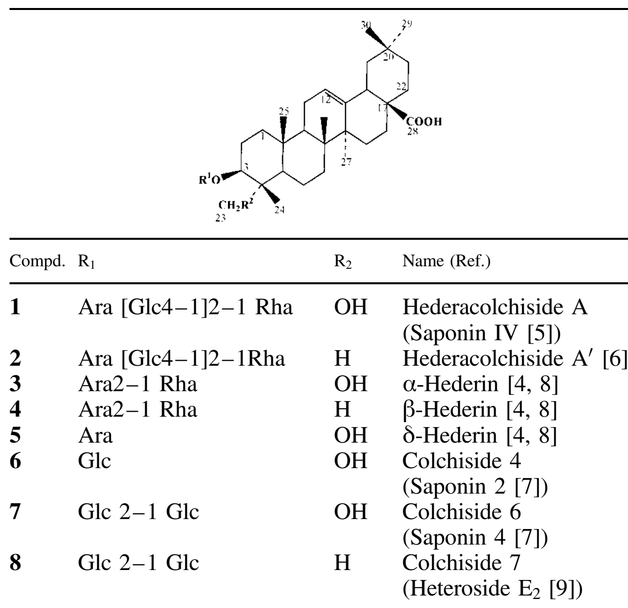
Several triterpenoid saponins from *Hedera helix* L. (Araliaceae), the common ivy, were shown to exhibit activity against yeasts, dermatophytes as well as leishmania [1, 2]. The present study focus on *Hedera colchica* K. Koch, an ivy species endemic in Georgia, which is used in traditional medicine [3]. The antifungal and antiprotozoal activities of 8 monodesmoside saponins isolated and previously characterized from *Hedera colchica* were investigated (**1–8**, Table 1) [4–9].

As shown in Table 2, the best antifungal activity was obtained with **1** and **3**. However, the activity was lower than those of the reference antifungal agents. The most susceptible yeast species was *Candida glabrata*. With regard to structure-activity relationships, these results confirm and extend those of our previous study [1]. Saponins with hederagenin as aglycone (**1**, **3**, **5**, **6**, **7**) were the most active. Their activity is significantly modulated by the number, kind and sequence of sugar residues. A comparison of **1** and **3** shows that the presence of glucose branched on the arabinose residue ($1 \rightarrow 4$) improves the activity against *Candida* sp. strains. With regard to antiprotozoal activity, *Trichomonas vaginalis* was more sensitive to the saponins than *Leishmania* promastigotes. Compounds **1** and **2** were as effective as the reference drug metronidazole against *Trichomonas vaginalis*. Comparing **1**, **2**, **3** and **4**, it is seen that the anti-*Trichomonas* activity was not influenced by the aglycone, unlike for the antifungal activity, and was slightly enhanced by the ($1 \rightarrow 4$) linkage between the glucose and the arabinose residues. The best activity on *Leishmania* promastigotes was obtained with the saponin with oleanolic acid as aglycone, especially with **2**. However, the activity was lower than those of the reference antileishmanial drug pentamidine. A comparison of **1** and **3** shows that the ($1 \rightarrow 4$) linkage between the glucose and the arabinose residues results in a significant decrease in activity. These results show that the structure-activity relationships must be established for each type of microorganisms. This could be related to the differences in structure and the composition of cell envelopes. The biological activities of saponins are mainly linked to their ability to complex with sterols in the plasma membrane, thus resulting in lethal cell permeabilization [4]. For fungi, saponins must pass through the polysaccharidic cell wall first before reaching the target. The glycosilation could thus affect their passage by altering the molecular mass, shape and charge, as it has been discribed for glycosilated secreted proteins [11].

Table 1: Tested saponins from *Hedera colchica*



Compd.	R_1	R_2	Name (Ref.)
1	Ara [Glc4–1]2–1 Rha	OH	Hederacolchiside A (Saponin IV [5])
2	Ara [Glc4–1]2–1Rha	H	Hederacolchiside A′ [6]
3	Ara2–1 Rha	OH	α-Hederin [4, 8]
4	Ara2–1 Rha	H	β-Hederin [4, 8]
5	Ara	OH	δ-Hederin [4, 8]
6	Glc	OH	Colchiside 4 (Saponin 2 [7])
7	Glc 2–1 Glc	OH	Colchiside 6 (Saponin 4 [7])
8	Glc 2–1 Glc	H	Colchiside 7 (Heteroside E_2 [9])

Experimental

1. Plant material

Leaves and Berries of *Hedera colchica* were collected in Bagdati (Georgia) in 1996. A voucher specimen was deposited at the Institute of Pharmacochemistry, Tbilisi, Georgia. (leaves n°: 70996, berries n°: 80197).

Table 2: *In vitro* antifungal and antiprotozoal activities of triterpenoid saponins isolated from *Hedera colchica* given in µg/ml as MFC for fungi, LD_{100} for *Trichomonas vaginalis*, and IC_{50} for *Leishmania infantum*

Organisms	Compound								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9***
Fungi									
Candida albicans Y0109	12.5	–**	25	–	–	–	–	–	1
Candida albicans ATCC 90029	25	–	25	–	–	–	–	–	1
*Candida albicans****	25	–	25	–	–	–	–	–	1
*Candida parapsilosis****	25	–	100	–	–	–	–	–	1
*Candida krusei****	25	–	50	–	–	–	–	–	1
*Candida tropicalis****	12.5	–	25	–	–	–	–	–	1
Candida glabrata ATCC 90030	6.25	25	6.25	50	100	100	–	–	1
Candida kefyr Y0106	12.5	–	12.5	–	–	–	–	–	1
*Trichosporon cutaneum****	12.5	–	100	–	–	–	–	–	1
*Cryptococcus neoformans****	12.5	–	100	–	–	–	–	–	2
Dermatophytes****	12.5	–	12.5	–	–	–	–	–	2
Protozoa									
Trichomonas vaginalis	1	1	2.5	2.5	2.5	5	25	10	1
Leishmania infantum	150	7.5	25	15	25	35	–	–	0.25

* reference compounds; ** no activity in tested concentration range; *** clinical isolates, **** clinical isolates of *Microsporum canis, Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton soudanense*

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 assey

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 μg/ml. The reference antifungal agents were amphotericin B for yeasts and ketoconazole (Janssen pharmaceutica, 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 infanta* promastigotes (MHOM/FR/78/LEM75 strain) and *Trichomonas vaginalis* (TVR87 strain) were evaluated as previously described [10]. Regarding *Leishmania*, the inhibitory concentration 50 (IC_{50}) was evaluated with pentamidine as reference compound. The trichomonacidal activity (lethal dose 100, LD_{100}) 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 4′,7-dimethylether	–	35 mg (D)	13 mg (D)
Apigenin	73 mg (D)	64 mg (D)	16 mg (D)
Quercetin	520 mg (D, E)	120 mg (D)	63 mg (D)
Apigenin 7-O-glucoside	92 mg (D, E)	–	–
Hyperoside	2095 mg (E)	750 mg (D, E)	122 mg (E)
Vitexin 4′-O-rhamnoside	98 mg (E)	70 mg (E)	–
Vitexin 2″-O-rhamnoside	125 mg (E)	82 mg (E)	–
*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 Alpinar 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) succesively.
For the purification of flavonoids silicagel column chromatography, preparative TLC, and PC were applied. The structures of all the isolated com-