STEROIDAL GLYCOSIDES FROM THE SEEDS OF HYOSCYAMUS NIGER L. AND THEIR ANTIFUNGAL ACTIVITY

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Abstract: Phytochemical analysis of the seeds of *Hyocyamus niger L.* (Solonaceae) resulted in the isolation of six steroidal glycosides, two furostanol (**1**, **2**) and four spirostanol saponins (**3, 4, 5, 6**), which were found in this plant for the first time. The structures of these compounds were determined by detailed analysis of their spectral data, including two-dimensional NMR spectroscopy and MS spectroscopy. The antifungal activity of a crude steroidal glycoside extract, fractions of spirostanoles and individual glicosides was investigated *in vitro* against a panel of human pathogenic fungi, yeasts as well as dermatophytes and filamentous species.

Keywords: *Hyoscyamus niger L.*, steroidal glycosides, antifungal activity**.**

INTRODUCTION

The genus *Hyocyamus* belongs to the family Solonaceae and is widely distributed in Europe and Asia [1]. As a weed of cultivation it now grows also in North America and Brazil. The leaves or roots eaten produce maniacal delirium, if nothing worse. It is poisonous in all its parts, and neither drying nor boiling destroys the toxic principle.

Previously this plant never has been studied on steroidal glycosides. The chief constituent of *Hyoscyamus niger L.* (black henbane) was earlier reported by us [2]. The antifungal activity of steroidal saponins, particularly against agricultural pathogens, has been known for a long time [3-6], while other reported activities for this class of compounds include antitumor, hypoglycemic, immunoregulatory, and cardiovascular disease prevention and treatment [7].

Since several steroidal glycosides exhibit fungistatic or fungicidal activity [8,9], extract of seeds has been included in our screening programme for novel antifungal agents of plant origin. Its activity has been investigated *in vitro* against a panel of human pathogenic fungi, yeasts as well as dermatophytes and filamentous species. The antifungal activity of a spirostanoid fraction and of the individual glycosides, has been also evaluated to identify the bioactive compound.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of **1** showed signals for four steroidal methyl groups at δ0.89 (3H, s, Me-19), 0.84 (3H, s, Me-18), 1.02 (3H, d, Me-21), 0.98 (3H, d, Me-27), two methine proton signals at δ 3.68 (1H, m, H-3) and 4.39 (1H, m, H-16) indicative of secondary alcoholic functions, two methylene proton signals at δ3.41 (1H, m, H-26a) and 3.29 (1H, m, H-26b), ascribable to a primary alcoholic function, and four anomeric protons at δ 4.40 (1H, d, J = 7.5 Hz), 4.56 (1H, d, J = 7.5 Hz), 4.69(1H, d, J = 7.5 Hz) and 4.26 (1H, d, J = 7.5 Hz). The ^{13}C NMR spectrum displayed signals ascribable to a hemiketal function at δ 113.8, two secondary carbons bearing alcoholic functions at δ 79.2 and 82.4, and one primary carbon bearing the same functionality at δ 75.9, suggesting the occurrence of a glycoside furostanol skeleton. It was evident from the ^{1}H and ^{13}C NMR data that the sugar chain at C-3 of **1** consisted of three sugar units. The chemical shifts of all the individual protons of the three sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their corresponding carbons could be assigned unambiguously from the HSQC spectrum. These data showed the presence of one β-galactopyranosyl unit (δ 4.40) and two β-glucopyranosyl unit (δ 4.56 and 4.69). A glycosidation shifts were observed for C-4$_{gal}$ (δ 80.3) and for C-2$_{glc}$(δ 85.9). The HMBC spectrum showed key correlation peaks between the proton signal at δ 4.40 (H-1$_{gal}$) and the carbon resonance at δ 79.2 (C-3 of the aglycon), the proton signal at δ 4.56 (H-1$_{glc}$) and the carbon resonance at δ 80.3 (C-4$_{gal}$), the proton signal at δ 4.69 (H-1$_{glcI}$) and the carbon resonance at δ 85.9 (C-2$_{glc}$), the proton signal at δ 4.26 (H-1$_{glcII}$) and the carbon resonance at δ 75.9 (C-26 of the aglycon).

Table 1

¹³C NMR spectral data (300 MHz, CD₃OD) of saponins (1-3)

Carbon	1	2	3
1	37.9	38.3	38.2
2	30.4	30.5	30.6
3	79.2	78.9	79.3
4	34.9	34.8	35.2
5	46.0	46.0	45.6
6	32.9	33.0	32.9
7	32.7	32.7	32.6
8	34.6	34.7	36.3
9	55.7	55.7	55.6
10	36.0	36.0	36.4
11	30.9	22.0	21.8
12	40.8	40.8	40.7
13	55.9	55.9	41.5
14	57.3	57.4	57.4
15	32.4	32.8	32.4
16	82.4	82.2	82.0
17	64.9	64.9	63.5
18	16.5	16.5	16.9
19	12.3	12.8	12.7
20	41.0	41.0	42.5
21	16.0	16.6	14.6
22	113.8	113.8	110.4
23	37.4	37.3	32.5
24	28.9	30.9	29.5
25	34.8	36.4	31.1
26	75.9	75.8	67.4
27	16.8	16.2	16.6
1′	102.7	102.3	102.7
2′	73.0	72.8	73.1
3′	75.3	75.1	75.4
4′	80.3	79.0	79.0
5′	79.3	79.3	75.0
6′	60.6	62.6	60.9
1"	105.0	105.7	105.7
2"	85.9	75.2	75.4
3"	78.0	78.2	78.0
4"	75.6	78.0	71.8
5"	71.6	71.6	78.0
6"	63.0	62.9	63.0
1‴	106.3		
2‴	76.4		
3‴	75.8		
4‴	77.9		
5‴	70.5		
6‴	61.4		
1‴′	104.7	104.1	
2‴′	74.9	74.8	
3‴′	78.4	78.0	
4‴′	71.5	71.4	
5‴′	71.6	71.2	
6‴′	62.8	63.0	

On the basis of this evidence, the structure of compound **1** was established as 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranoside-[(25*R*)- 5α- furostan- 3β, 22α, 26-triol]- 26-*O*-β-D-glucopyranoside.

Comparison of the ^{1}H and ^{13}C NMR spectra of **1** and **2** indicated an identical aglycon moiety. Compound **2** was shown to contain three sugar residues from the HSQC spectrum. The anomeric protons at δ 4.39 (1H, d, *J* = 7.5 Hz), 4.54 (1H, d, *J* = 7.5 Hz) and 4.27 (1H, d, *J* = 7.5 Hz) giving correlations with carbon signals at δ 102.3, 105.7, and 104.1, respectively, were assigned to anomeric protons of β-galactopyranose and two molecules of β-glucopyranose, respectively.

Table 2

In vitro antifungal activity of extract, spirostanoid fraction, individual glicosides, given as minimum fungicidal concentrations (µg/mL)
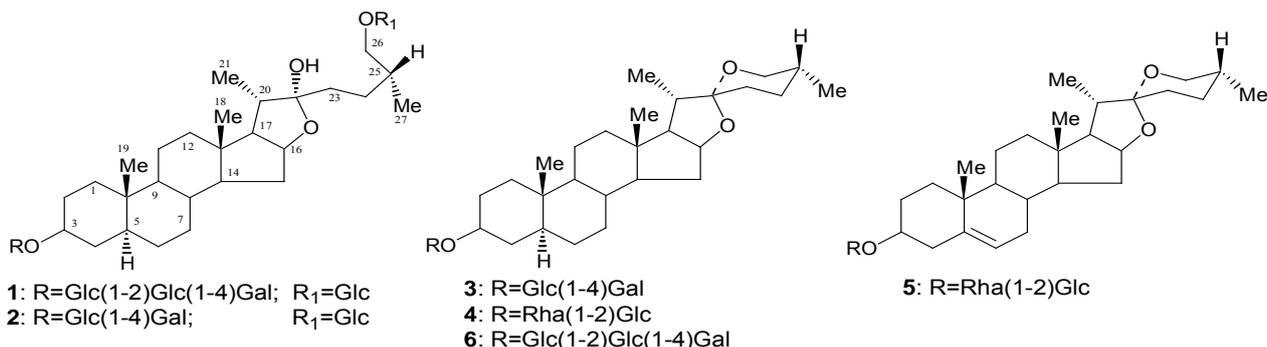
Species	Extract	Spirost. fraction	Compounds						Ref. Drug.*
			1	2	3	4	5	6	
Yeast									
Candida albicans ATCC 90029	0.125	50	>100	>100	25	25	25	25	1.56
C.albicans 38248	0.125	50	100	100	12.5	12.5	12.5	12.5	1.56
C.albicans 40109	0.125	50	>100	>100	25	25	25	12.5	1.56
C. tropicalis IP 1275-81	0.06	25	>100	>100	12.5	12.5	12.5	12.5	12.5
C. parapsilosis ATCC 220	0.06	50	>100	>100	12.5	12.5	12.5	12.5	3.12
C. glabrata ATCC 90030	0.06	25	100	100	12.5	12.5	12.5	12.5	0.78
C. kefir Y0106	>1	>100	N.T.	N.T.	>100	>100	>100	100	0.78
C. kruse ATCC 6258	0.25	>100	>100	>100	25	25	25	25	3.12
C. lusitaniae CBS 6936	>1	>100	N.T.	N.T.	>100	>100	>100	100	1.56
Cryptococcus neoformans	0.06	25	>100	>100	12.5	12.5	12.5	12.5	0.78
Dermatophytes									
Trichophyton rubrum	0.5	7.8	>100	>100	6.25	6.25	6.25	3.12	1.56
Trichophyton mentagraphytes	0.5	15.6	>100	>100	6.25	6.25	6.25	6.25	1.56
Trichophyton soudanense	1.0	15.6	N.T.	N.T.	12.5	12.5	12.5	12.5	1.56
Microsporum canis	0.5	7.8	>100	>100	6.25	6.25	6.25	3.12	1.56
M. gypseum	0.5	7.8	>100	>100	6.25	6.25	6.25	3.12	1.56
Epidermophyton floccosum	0.5	7.8	>100	>100	6.25	6.25	6.25	6.25	1.56
Filamentous fungi									
Aspergillus funigatus	10	100	N.T.	N.T.	75	75	75	50	1.56
A. flavus	7.5	31.2	N.T.	N.T.	6.25	6.25	6.25	3.12	0.78
Scopulariopsiss brevicaulis	15	250	N.T.	N.T.	125	125	125	100	3.12

*The reference antifungal agents were amphotericin B and ketoconazole for yeasts and filamentous fungi, and dermatophytes, respectively.

The point of attachment of these sugars was then deduced from the HMBC of **2** which showed a cross peak between the H-1 Gal at δ 4.39 and C-3 of the aglycon at δ 78.9. Other correlations between H-1 GlcI at δ 4.27 and C-26 of the aglycon at δ 75.8; and between H-1 Glc at δ 4.54 and C-4 Gal at δ 79.0. On the basis of the above results the

structure of **2** was determined as 3-*O*-β-D-glucopyranosyl -(1→4)-*O*-β-D-galactopyranoside-[(25*R*) - 5α- furostan- 3β, 22α, 26-triol]-26-*O*-β-D-glucopyranoside. The presented chemical structures have been previously reported in *Petunia hybrida* by Shvets et al. in 1995 [10].

The ^{1}H NMR spectrum of **3** showed signals for four methyl groups at δ 0.89 (3H, s, Me-19), 0.82 (3H, s, Me-18), 1.00 (3H, d, Me-21), 1.12 (3H, d, Me-27). The HMBC correlation of methyl groups clearly showed that the aglycon moiety was similar of compound **1** and was identified as (25*R*)- 5α-spirostan-3β, 26-diol. Glycoside spirostanol skeleton is suggested according to the presence of primary alcoholic function at δ 67.7 (C-26). The ^{1}H NMR spectrum showed signals for two anomeric protons at δ 4.39 (1H, d, *J* = 7.5 Hz), 4.56 (1H, d, *J* = 7.5 Hz). The HMBC, 1D-TOCSY and DQF-COSY spectral analysis of the sugar moiety was similar to those of compound **2.** According to these data, compound **3** was assigned the structure 3-*O*-β-D-glucopyranosyl -(1→4) -*O*-β-D-galactopyranoside-(25*R*) - 5α- spirostan - 3β-ol, described with Gutsu et al. in year 1987 from *Capsicum annum* [11].

1: R=Glc(1-2)Glc(1-4)Gal; R$_1$=Glc
2: R=Glc(1-4)Gal;   R$_1$=Glc

3: R=Glc(1-4)Gal
4: R=Rha(1-2)Glc
6: R=Glc(1-2)Glc(1-4)Gal

5: R=Rha(1-2)Glc

The structures of compounds **4**, **5** and **6** were elucidated by us earlier [2]. For compound **4** -3-*O*-α-L-rhamnopyranosyl-(1→2) - β - D-glucopyranoside-(25*R*)- 5α- spirostan - 3β-ol; for compound **5-** *O*-α-L-rhamnopyranosyl-(1→2) - β - D-glucopyranoside -(25*R*)- 5-ene -spirostan -3β-ol and for compound **6** -3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl -(1→4)-*O*-β-D-galactopyranoside-(25*R*) - 5α- spirostan - 3β-ol.

Table 2 shows that extract had a broad spectrum of antifungal activity *in vitro*. It was active against all the tested strains except for two (*C. lusitaniae* and *C. kefir*). The best antifungal activity was observed against dermatophytes and against yeasts, to a lesser extent. The least sensitive yeast species were *C.tropicalis, C. parapsilosis, C. glabrata* and *Cryptococcus neoformans*. Its activity against filamentous fungi was weak to moderate. The furostanol glycosides were devoid of any antifungal activity. The antifungal activity was found to reside entirely in the spirostanol fraction and individual glycosides with minimum inhibitory concentrations (MIC) from 7.8 to 250 µg/mL. The MICs for the dermatophytes were between 3.12 and 125 µg/mL. The most sensitive filamentous species was *A. flavus* (MIC = 3.12 µg/ mL).

EXPERIMENTAL

The seeds of *Hyoscyamus niger L.* have been collected in the scientific research field of the Institute of Scientific Researches and Technological Constructions for Tobacco and Tobacco Products of Moldova in November 2001 year. The voucher specimen has been deposited in laboratory of botanic by leading of Doctor in Biology Kalkei E.D. The air-dried seeds were extracted three times in 50°C with water saturated n-buthanol. After evaporation of n-buthanol, water extract was purified with chloroform and finally the steroidal glycosides were crystallised in acetone. The sum of steroidal saponins has been obtained in yellow powder in the yield 3,7%. Extract has been chromatographied on silica gel column (30-500mm, 60-100µm, Merck). The column was eluted with the system of solvents chloroform-methanol-water (8:2:0→20:10:1). The polar subfractions were obtained, which was further separated on a C$_{18}$ column (7,8x300mm, LiChroprep RP18, 25-40µm, XTerra Waters) using a MeOH/H$_2$O (60-65% MeOH) gradient. Three single compounds were obtained.

Structures were determined by 1D- (^{1}H, ^{13}C, TOCSY), 2D (COSY, HSQC, HMBC) NMR, ESIMS and HRMS experiments. Samples were analyzed by matrix assisted laser desorption ionization (MALDI) mass spectrometry. ESIMS analyses were performed using a ThermoFinnigan (San Josè, CA, USA) LCQ Deca XP Max ion trap mass spectrometer equipped with Xcalibur software. NMR experiments in CD$_3$OD were performed on a Bruker DRX-600 spectrometer at 300 K. Column chromatography was performed over Silica gel (Merck). HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC$_{18}$ column, and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

1. HRMS, *m/z* 1081.643 [calculated for C$_{51}$H$_{84}$O$_{24}$ (M)$^+$]; 919.6 [M-162]$^+$**;** 757 [M-2x162]$^+$**;** ^{1}H NMR (aglycon)

δ 4.39 (1H, m, H-16), 3.68 (1H, m, H-3), 3.41 (1H, m, H-26a), 3.29 (1H, m, H-26b), 0.89 (3H, s, Me-19), 0.84 (3H, s, Me-18), 1.02 (3H, d, H-21), 0.98 (3H, d, Me-27). (sugars) δ4.40 (d, *J*=7.4 Hz, H-1Gal), 3.60 (dd, *J*=7.4 and 9.0 Hz, H-2Gal), 3.53 (dd, *J*=4.0 and 9.0 Hz, H-3Gal),   4.02 (dd, *J*=2.5 and 4.0 Hz, H-4Gal), 3.69 (ddd, *J*=2.5, 2.5 and 4.5 Hz, H-5Gal), 3.62 (dd, *J*=4.5 and 12.0 Hz, H-6aGal), 3.95 (dd, *J*=2.5 and 12.0 Hz, H-6bGal), 4.56 (d, *J*=7.5 Hz, H-1Glc), 3.55 (dd, *J*=7.5 and 9.0 Hz, H-2Glc), 3.60 (dd, *J*=9.0 and 9.0 Hz, H-3Glc), 3.35 (dd, *J*=9.0 and 9.0 Hz, H-4Glc), 3.25 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5Glc), 3.60 (dd, *J*=4.5 and 11.5 Hz, H-6aGlc), 3.93 (dd, *J*=2.5 and 11.5 Hz, H-6bGlc), 4.69 (d, *J*=7.5 Hz, H-1GlcI), 3.29 (dd, *J*=7.5 and 9.0 Hz, H-2GlcI), 3.36 (dd, *J*=9.0 and 9.0 Hz, H-3GlcI), 3.40 (dd, *J*=9.0 and 9.0 Hz, H-4GlcI), 3.42 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5GlcI), 3.69 (dd, *J*=4.5 and 11.5 Hz, H-6aGlcI), 3.82 (dd, *J*=2.5 and 11.5 Hz, H-6bGlcI), 4.26 (d, *J*=7.5 Hz, H-1GlcII), 3.20 (dd, *J*=7.5 and 9.0 Hz, H-2GlcII), 3.37 (dd, *J*=9.0 and 9.0 Hz, H-3GlcII), 3.33 (dd, *J*=9.0 and 9.0 Hz, H-4GlcII), 3.28 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5GlcII), 3.69 (dd, *J*=4.5 and 11.5 Hz, H-6aGlcII), 3.85 (dd, *J*=2.5 and 11.5 Hz, H-6bGlcII). For ^{13}C NMR see Table 1.

2. HRMS, *m/z* 919.342 [calculated for $C_{45}H_{74}O_{19}$ (M)$^+$]; 757.53 [M-162]$^+$; 595 [M-2x162]$^+$; ^{1}H NMR (aglycon) δ 4.38 (1H, m, H-16), 3.68 (1H, m, H-3), 3.41 (1H, m, H-26a), 3.29 (1H, m, H-26b), 0.89 (3H, s, Me-19), 0.84 (3H, s, Me-18), 1.02 (3H, d, H-21), 0.98 (3H, d, Me-27). (sugars) δ4.39 (d, *J*=7.4 Hz, H-1Gal), 3.52 (dd, *J*=7.4 and 9.0 Hz, H-2Gal), 3.53 (dd, *J*=4.0 and 9.0 Hz, H-3Gal),   4.08 (dd, *J*=2.5 and 4.0 Hz, H-4Gal), 3.69 (ddd, *J*=2.5, 2.5 and 4.5 Hz, H-5Gal), 3.70 (dd, *J*=4.5 and 12.0 Hz, H-6aGal), 3.92 (dd, *J*=2.5 and 12.0 Hz, H-6bGal), 4.54 (d, *J*=7.5 Hz, H-1Glc), 3.55 (dd, *J*=7.5 and 9.0 Hz, H-2Glc), 3.38 (dd, *J*=9.0 and 9.0 Hz, H-3Glc), 3.35 (dd, *J*=9.0 and 9.0 Hz, H-4Glc), 3.24 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5Glc), 3.69 (dd, *J*=4.5 and 11.5 Hz, H-6aGlc), 3.89 (dd, *J*=2.5 and 11.5 Hz, H-6bGlc), 4.27 (d, *J*=7.5 Hz, H-1GlcI), 3.20 (dd, *J*=7.5 and 9.0 Hz, H-2GlcI), 3.37 (dd, *J*=9.0 and 9.0 Hz, H-3GlcI), 3.32 (dd, *J*=9.0 and 9.0 Hz, H-4GlcI), 3.29 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5GlcI), 3.69 (dd, *J*=4.5 and 11.5 Hz, H-6aGlcI), 3.84 (dd, *J*=2.5 and 11.5 Hz, H-6bGlcI). For ^{13}C NMR see Table 1.

3. HRMS, *m/z* 741.452 [calculated for $C_{39}H_{64}O_{13}$ (M)$^+$]; 579.3 [M-162]$^+$; ^{1}H NMR (aglycon) δ 4.41 (1H, m, H-16), 3.68 (1H, m, H-3), 3.46 (1H, m, H-26a), 3.33 (1H, m, H-26b), 0.88 (3H, s, Me-19), 0.81 (3H, s, Me-18), 0.98 (3H, d, H-21), 1.10 (3H, d, Me-27). (sugars) δ4.38 (d, *J*=7.4 Hz, H-1Gal), 3.51 (dd, *J*=7.4 and 9.0 Hz, H-2Gal), 3.53 (dd, *J*=4.0 and 9.0 Hz, H-3Gal), 4.08 (dd, *J*=2.5 and 4.0 Hz, H-4Gal), 3.58 (ddd, *J*=2.5, 2.5 and 4.5 Hz, H-5Gal), 3.63 (dd, *J*=4.5 and 12.0 Hz, H-6aGal), 3.90 (dd, *J*=2.5 and 12.0 Hz, H-6bGal), 4.53 (d, *J*=7.5 Hz, H-1Glc), 3.29 (dd, *J*=7.5 and 9.0 Hz, H-2Glc), 3.39 (dd, *J*=9.0 and 9.0 Hz, H-3Glc), 3.24 (dd, *J*=9.0 and 9.0 Hz, H-4Glc), 3.31 (ddd, *J*=2.5, 4.5 and 9.0 Hz, H-5Glc), 3.62 (dd, *J*=4.5 and 11.5 Hz, H-6aGlc), 3.93 (dd, *J*=2.5 and 11.5 Hz, H-6bGlc). For ^{13}C NMR see Table 1.

Antifungal assays. Whatever the tested product (extract, spirostanol fraction, individual glycosides) and the test microorganisms (yeasts, dermatophytes, filamentous fungi), the antifungal activity was evaluated with an agar dilution method, as previously described [12]. Yeast nitrogen base (Difco, Becton Dickinson, Sparks, MD) supplemented with 2% agar (Difco) was used as a test medium for yeasts. For dermatophytes and filamentous species, the tests were performed on Sabouraud dextrose agar (Difco). Eight reference yeast strains were used: *Candida albicans* ATCC 90029, *Candida albicans* Y0109, *Candida albicans* 38248, *Candida tropicalis* IP 1275-81, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 90030, *Candida kefyr* Y 0106, *Candida krusei* ATCC 6258 and *Candida lusitaniae* CBS 6936. Dermatophytes (one isolate of each species: *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton soudanense*, *Microsporum canis*, *Microsporum gypseum*, *Epidermophyton floccosum*) and *Cryptococcus neoformans* were all clinical isolates. Filamentous fungi (one isolate of each species: *Aspergillus fumigatus*, *Aspergillus flavus*, *Scopulariopsis brevicaulis*) were environmental isolates. All samples were dissolved in dimethyl sulphoxide (DMSO). The tested concentrations were 0.03 mg/mL for extract, and from 0.0625 to 10 μg/mL for pure compounds and the fractions. The final concentration of DMSO never exceeded 1%. The minimum inhibitory concentration was defined as the first concentration showing no visible growth after the incubation time (48 h for the yeasts, 5–15 days for the dermatophytes and the filamentous fungi according to the species). The tests were performed in duplicate and in two separate experiments. Amphotericin B and ketoconazole (Sigma Chemical Co., St Louis, MO) were included in the assays as reference antifungal agents, for yeasts and filamentous fungi, and dermatophytes, respectively.

CONCLUSION

During the investigation six steroidal glycosides have been isolated from the seeds of *Hyoscyamus niger L.* for the first time. In this work the following structures have been suggested for three of them: compound **1** - 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-[(25*R*)- 5α- furostan- 3β, 22α, 26-triol]- 26-*O*-β-D-glucopyranoside; compound **2** - 3-*O*-β-D-glucopyranosyl -(1→4)-*O*-β-D-galactopyranosyl-[(25*R*) - 5α- furostan- 3β, 22α, 26-triol]-26-*O*-β-D-glucopyranoside; compound **3** - 3-*O*-β-D-glucopyranosyl -(1→4) -*O*-β-D-galactopyranoside-(25*R*) - 5α- spirostan - 3β-ol. Structural determination of steroidal glycosides **4, 5** and **6** have been reported in previous work.

The results of biological tests revealed that antifungal steroidal glycosides are present in *Hyoscyamus niger* seeds. *In vitro* spirostanol fraction and glycosides showed a broad spectrum of antifungal activity. Only slight differences in their fungicidal profiles were observed. Not surprisingly, these compounds are monodesmosidic spirostanol glycosides

like the majority of biologically active steroid saponins [9]. The antifungal properties of saponins are usually ascribed to their ability to complex with sterols in fungal membranes, thereby causing pore formation and loss of membrane integrity [13]. Interestingly, the tested extracts and compounds were inactive against *C. lusitaniae* ATCC 6936 although its sterol composition was shown to be similar to that of *C. albicans* ATCC 90029 [14]. Because antifungal agents must pass through the polysaccharidic cell wall first before reaching the target, this finding could be related to an unusual cell wall structure of this emerging yeast pathogen.

Thus, our study revealed the therapeutic potential of some spirostanol glycosides from *Hyoscyamus niger* seeds. Extract, which showed good activity against dermatophytes and which is readily available in large quantities, is a promising active principle for the topical treatment of dermatophytosis. Therefore, it is wortwhile to further study these antifungal compounds and, in a broad sense, the structure-activity relationships of more steroidal saponins to explore the therapeutic potential of this important class of natural products as antifungal leads for drug discovery.

REFERENCES
[1]   Kirtikar, K.R.; Basu, B.D.; Indian Medicinal Plants, Reprint ed., Periodical Experts, New Delhi, 1975, 3, pp 1794-1795.
[2]   Lunga, I.; Kintia, P.; Shvets, S.; Bassarello, C.; Pizza, C.; Piacente, S. Three spirostanol glycosides from the seeds of Hyoscyamus niger L., Chemistry Journal of Moldova, 2006, 1, pp 123-127.
[3]   Dimoglo, A. S.; Choban, I. N.; Bersuker, I. B.; Kintia, P. K.; Balashova, N. N. Structure-activity correlations for the antioxidant and antifungal properties of steroid glycosides. Bioorg. Khim., 1985, 11, pp 408–413.
[4]   Imai, S.; Fujioka, S.; Murata, E.; Goto, M.; Kawasaki, T.; Yamauchi, T. Bioassay of crude drugs and oriental crude drug preparations. XXII. Search for biologically active plant ingredients by means of antimicrobial tests: 4. Antifungal activity of dioscin and related compounds. Takeda Kenkyusho Nenpo, 1967, 26, pp 76–83.
[5]   Wolters, B. The share of the steroid saponins in the antibiotic action of *Solanum dulcumata*. Planta Med, 1965, 13, pp 189–193.
[6]   Wolters, B. Antimicrobial activity of plant steroids and triterpenes. Planta Med, 1966, 14, pp 392–401.
[7]   Sparg, S. G.; Light, M. E.; Staden J. V. Biological activities and distribution of plant saponins. J. Ethnopharmacol. 2004, 94, pp 219–243.
[8]   Lacaille-Dubois, M. A.; Wagner, H. A review of the biological and pharmacological activities of saponins. Phytomedicine, 1996, 2, pp 363–386.
[9]   Hostettman, K. A.; Marston, A. Saponins. Cambridge University Press: Cambridge, 1995.
[10]   Shvets, S. A.; Naibi, A. M.; Kintia, P. K. Steroidal glycosides from Petunia hybrida seeds. II. Structure of petuniosides I, L and N. Inst. Genet., AN Respub. Moldova, Chisinau, Moldova. Khimiya Prirodnykh Soedinenii, 1995, 2, pp 247-52.
[11]   Gutsu, E.V.; Kintia, P.K.; Lazur'evskii, G.V. Steroidal glycosides of Capsicum annuum root. Part II. Structure of capsicosides. Khimia Prirodnykh Soedinenii, 1987, 2, pp 242-246.
[12]   Favel, A,; Steinmetz, M.D.; Regli, P. et al. In vitro antifungal activity of triterpenoid saponins. Planta Med, 1994, 60, pp 50–53.
[13]   Morrissey, J.P.; Osbourn, A. E. Fungal resistance to plant antibiotic as a mechanism of athogenesis. Microbiol. Mol., Biol. Rev., 1999, 63, pp 708–724.
[14]   Peyron, F.; Favel, A.; Calaf, R. et al. Sterol and fatty acid composition of Candida lusitaniae clinical isolates. Antimicrob Agents Chemother, 2002, 46, pp 531–533.