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ISOLATION OF NEW SAPONINS FROM *BELLIUMBELLIDIOIDES* L. FAMILY ASTERACEAE

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ABSTRACT

Belliumbellidioides is a small herbal plant (9-15 cm). The origin of this plant is Corsica. Due to the presence of high concentration of Saponins in this plant, *Bellium bellidioides*was the aim of this study. Saponins have special structural features, therefore extraction and isolation of saponins poses a serious challenge. However, in general it is difficult to use a single technique for isolation of saponins, but using Column chromatography on Sephadex 20 and on Silica gel (0.2-0.063 mm) in addition to HPLC on RP18 it could be isolated about 20 –30 and 50 mg of Saponins Agha BS2, AghaBS3 and AghaBS4 in a semi pure form. Using H1NMR and C13NMR Spectrometry in addition to acid hydrolysation and TLC methods it could be determined that the Aglycone is Polygalic acid and the sugars are Rhamnose and glucose.

KEYWORDS: Saponins, extraction, HPLC, H1-NMR, C13-NMR.

INTRODUCTION

Saponins are phytochemicals, found mainly but not exclusively in plants, which exhibit foaming characteristics, and consist of polycyclic-aglycones attached to one or more sugar side chains. The aglycone part, which is also called a sapogenin, is either a steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins characteristically have a bitter taste and some are known to be toxic. The number of saccharide chains attached to the sapogenins (aglycone) can vary as can the length of each chain. The saccharide chain length, so far, varies from 1 to 11 sugar residues, with the numbers 2–5 being most frequently encountered with both linear and branched chains being represented.[1,2,10]

All saponins have attachment of at least one sugar chain to the aglycone and can be described as mono, di, or tridesmosidic depending on the number.

Bellium bellidioides is one of these Plants, which contains Saponins, and it belong the Genus of *Belliumm*, flowering plants in the daisy family, Asteraceae, native to the Mediterranean region. Species ***Bellium bellidioides*** L. is especially native in Balearic region (3-9)

Bellium belidioides L. is a Clump to small mat-forming plant, spreading by short stolon. Leaves elliptic, 6-12mm long, narrowing to a long stalk. Flower heads are 1.2-2cm across, the ray florets have white color (often tinted red beneath), solitary, on stems 5-10cm tall, which appeared in midsummer to autumn.[3,9]

The habitat of the plant is Islands of the western Mediterranean, in damp or shady origin. Not totally hardy in bad winters and must not be allowed to dry out; grows well in tufa. It spreads in south of Europe especially in the west of Balearic such as Sardinia and Corsica. It found in the wet origin in the height of 0 to 2000 m.[5,9]

Bellium bellidioides has 1-4 creping stems. The flowers-stem is about 2-14 cm long. The Plant flowers could be noticed from April to August. The leaves have small and fine hairs (4-7) (Figure 1).

Figure 1- Bellium belidioides Aim of the Study.

The aim of this study was the isolation of Saonins from areal part of the plant *Bellium bellidioedes*, family Asteraceae.

MATERIAL AND METHODS

HPLC, Hitachi/ Merck UV, CC; RP18 (250X16 mm/7micron)
NMR-spectrometer: Bruker, AM 600, in CD3OD, 150.905 MHz, H1-NMR in
600.149 MHz- Braunschweig-Germany
Melting Point microscope (Franz Kuster Nachf. KG Dresden) TLC silica gel 60F254, Merck, Silica gel 60 F264, Merck
Mixtures of fluids consist of Methanol, Chloroform used in Column Chromatography (CC), Thin Layer Chromatography (TLC) and High performance chromatography (HPLC)
Detections: Water, Anisaldehyde/ Sulfuric acid, Blood gel, Thymole/Sulfuric acid.

Plant material

Plant material was gathered from the mountains of island Corsica, dried in the sun and the aerial part was grounded.

Extraction

Extraction was done using Soxhlet extraction method (Methanol 80%) and the extract concentrated, defatted with chloroform. The aqueous extract eluted with n-Butanol four times. N-Butanol extract dried in vacuum (temp. 50 degree centigrade). The dried n-Butanol-Extract eluted with methanol, and the methanol elution dropped in Ether to precipitate the raw Saponins. The indication of Saponins is carried out using TLC si F254 (Detection water), the indicated Saponins showed the hemolysis effect (TLC, Blood gel as detection).[1,3,9]

Raw Saponins are purified using column chromatography on Sephadex LH20 and on Silica gel (0.2-0.063 mm, Solution; Chloroform 40%, Methanol 60% and water 5%).

The detection of saponins was done using TLC on silica gel F254 (Solution Chloroform 60%, Methanol 40%, 2% water), and the following detections were used: water, Anisaldehyd/Sulfuric acid, and blood gelatin on the Plate. The investigation of the purity of isolated saponin fractions was checked out using TLC, Si254, using Anisaldehyd/Sulfuric acid detection- solution. To ensure the purity of isolated saponins HPLC RP18 was used (eluent; Methanol 80%, detection UV 207 nm.).[3,9]

Determination of the Chemical structure

In order to determine the chemical structure H1 NMR and C13 NMR – Spectrometry was carried out. The chemical structure of isolated Saponins could be compared with isolated Saponins from *Bellis perennis* L.[3,9,10], (table 1, 2).

Table 1: C13 NMR-Data of the Aglycons in Bellis-saponin BA1 and Agha Saponins ASB2, ASB3 and ASB3.

Aglycons									
	BA1	**ASB2**	**ASB3**	**ASB4**		**ASBA1**	**ASB2**	**ASB3**	**ASB4**
C-1	45.06	45.12	45.91	45.12	C-16	74.63	74.63	74.60	74.64
C-2	71.85	71.97	71.96	71.93	C-17	50.20	50.36	50.34	50.26
C-3	82.42	82,61	82.59	82.44	C-18	42.30	42.39	41.85	41.83
C-4	43.38	43.48	43.46	43.45	C-19	47.90	48.05	48.03	48.00
C-5	47.95	48.05	48.03	48.05	C-20	31.24	31.29	31.28	31.31
C-6	18.94	19.03	19.02	19.02	C-21	36.46	36.49	36.52	36.56
C-7	33.59	33.73	33.69	33.66	C-22	31.85	31.90	31.89	32.02
C-8	40.85	40.98	40.96	40.90	C-23	65.75	65.97	64.96	65.82
C-9	48.33	48.36	48.36	48.39	C-24	14.85	14.93	14.93	14.93
C-10	37.69	37.79	37.78	37.76	C-25	17.99	18.02	18.01	18.01
C-11	24.58	24.69	24.67	24.66	C-26	17.94	17.88	17.88	17.88
C-12	123.45	123.51	123.52	123.51	C-27	27.13	27.26	27.24	27.21
C-13	144.63	144.59	144.70	144.71	C-28	177.28	177.34	177.33	177.25
C-14	42.94	43.04	42.36	42.33	C-29	33.29	33.31	33.32	33.36
C-15	36.32	36.49	36.52	36.42	C-30	24.91	25.07	25.06	24.97

From table 1 we could determined the chemical structure of the Aglycone in the isolated Saponins as Polygalic acid (figure 2)

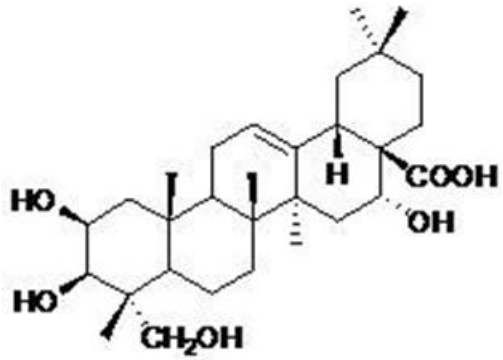

Figure 2: Polygalic acid, the Aglycone Part of the isolated Saponins.

Table 2: C13 NMR-Data of the glycons (sugars) in *Bellis saponin* BA1 and Agha Saponins ASB2, ASB3 and ASB4.

Glycons (Sugars)											
		BA1	**ASB2**	**ASB3**	**ASB4**			**ASBA1**	**ASB2**	**ASB3**	**ASB4**
Rha. A	C-1	102.43	102.53	102.50	102.49	Fuc.	C-1	94.92	95.03	95.01	94.94
	C-2	72.19	72.31	72.28	72.30		C-2	75.51		75.11	75.43
	C-3	72.19	72.31	72.38	72.24		C-3	74.58			
	C-4	73.93	74.04	74.02	73.99		C-4	74.84			
	C-5	69.93	70.09	70.06	70.00		C-5	71.18		71.03	71.03
	C-6	17.94	18.02	18.01	17.98		C-6	16.49	16.64	16.64	16.65
Xyl	C-1	107.00	107.08	107.04	107.08	Rha. C	C-1	104.08	104.06	104.04	104.12
	C-2	76.32	76.34	76.32	76.40		C-2	72.19	72.31	72.28	72.30
	C-3	84.22	84.50	84.47	84.31		C-3	72.19	72.31	72.28	72.22
	C-4	68.88	68.97	68.96	68.90		C-4	73.93	74.04	74.02	73.99
	C-5	67.10	67.16	67.14	67.18		C-5	70.28	70.43	70.41	70.34
Rha. B	C-1	101.62	101.50	101.49	101.50		C-6	17.81	18.02	18.01	17.88
	C-2	72.19			72.22						
	C-3	72.19			72.24						
	C-4	84.39	84.50	84.47	84.47						
	C-5	69.78	69.91	69.88	69.86						
	C-6	18.35	18.41	18.41	18.42						

From the table- 2 it could be determined the Sugars, which found attached to the aglycons, which using TLC on silica gel, and Standards of Sugars ensure the presence of detected Sugars in C13-NMR Spectrometer, whereas the following sugars in the hydrolyzed Saponin Fractions are detected: Rhamnose, Xylose and Fucose. (TLC on Si-F254, detection Thymol detection solution).

Using TLC after alkali hydrolyzation determined the acyl-attached sugars as L. Rhamnose, the results of C13-NMR data determined also the above chemical structure of the isolated saponins as di-glycoside triterpenoid saponins (bisdesmoside Saponins).

The melting ranges of the isolated Saponins were determined also the UV-max of each Saponins and the Rf. using TLC si-F254/ Chloroform 40%, Methanol 60% and Water 5% in room temp.) Table 3.

Table 3: The specific characterizations of isolated Saponins.

Saponin	Melting range	UV-max	RF
ASB2	163-165	206	0.71
ASB3	163-165	206	0.68
ASB4	159-162	206	0.80

DISCUSSION

Three Triterpen Saponine were isolated. Methods of Chromatography (TLC, CC, and HPLC) were used in the Isolation and purification of the Saponins.

To determine the chemical structure of isolated saponins C13-NMR and H1- NMR spectrometry were applied. The Data of NMR Spectrometry could be compared with the Data of *Bellis* Saponins.[3,9,11] The Aglycone Part was triterpen Polygalic acid and the Sugars were determined as Rhamnose (three units) Fucose (one unit) and Xylose (one unit).

The investigation of the isolated saponins is carried out after acid and alkali hydrolyzation on TLC (using Comparable standards of aglycone and sugars) showed the presence of Polygallic acid and the sugars (Rhamnose, Xylose and Fucose), which ensure the results of NMR-Data.

The Isolated saponins are Di Glycoside with two sugar chains in tow positions 3 and 28. Where the position 28 attached with one Sugar (Rhamnose) and the attached sugar chain in the position 3 is consist of L-Rhamnose, D-Xylose and L-F ucose.

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