Gypsophila *simonii*: Identification, Extraction, Isolation of Sapogenin and X-Ray Crystallographic Structure of Sucrose

Gypsophila *simonii*: Teşhis, Ekstraksiyon, Sapogenin İzolasyonu ve Sakkarozun X-Ray Kristalografik Yapısı

Research Article

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ABSTRACT

The plant Çöven, Gypsophila simonii is widely distributed throughout Çankırı, where it is a native species, and Turkey. In this study, chemical and physical properties of unripe saponins obtained by extraction from the roots of Gypsophila simonii, an endemic plant, were isolated and investigated. Purified aglycones recovered from acid hydrolysis of the saponins were separated by reversed chromatography on a thin layer of silica gel. The structure was characterized methods of analysis by means of ^{1}H-NMR, ^{13}C-NMR, FTIR and EIMS. The findings indicate that the proposed structure of that saponin was as a new Gypsogenin ester ($C_{31}H_{51}O_3$) [1,2]. The obtained sapogenin was crystallized for X-ray diffraction; but X-ray analysis results showed that the compound crystallized was only sucrose ($C_{12}H_{22}O_{11}$). Phytochemical tests showed the presence of terpenoids in the crude extracts. The total saponin content in plant materials and extracts varied to %12.30±0.50. Furthermore in this study, type of ose and total ose (7.0±0.20%), were investigated as qualitative and quantitative.

Key Words

Gypsophila simonii, Sapogenin, Total saponin content %, ose (total sugar), X-ray crystallography, Sucrose.

ÖZET

Çöven bitkisi, Gypsophila simonii yaygın olarak Çankırı çevresinde yetişen yerli bir türdür, Türkiye geneline dağılır. Bu çalışmada, endemik bir bitki olan Gypsophila simonii köklerinden ekstraksiyon sonunda elde edilen ham saponinlerin kimyasal ve fiziksel özellikleri araştırıldı. Saponinler, asit hidrolizi sonucu elde edilen saf aglikon silika jel kaplı ince bir tabaka üzerinde kromatografi yöntemleri ile ayrıldı. Yapısı ^{1}H-NMR, ^{13}C-NMR, FTIR and EIMS analiz yöntemleri ile karakterize edilmiştir. Analiz verilere göre saponinden yeni bir Gypsogenin ester ($C_{31}H_{51}O_3$) yapısı önerilmiştir [1,2]. Elde edilen sapogenin X-ray difraksiyon için kristalize edildi, fakat X-ray analiz sonuçları kristalize bileşikte sadece sakkaroz ($C_{12}H_{22}O_{11}$) olduğunu gösterdi. Fotokimyasal testleri, ekstraksiyon özütlerde terpenoidler varlığını gösterdi. Bitki materyalindeki ekstraktların toplam saponin içeriği % 12.30 ± 0.50 arasında değişmektedir. Ayrıca bu çalışmada, ose ve toplam ose (7.0 ± 0.20%) tipi kalitatif (nitel) ve kantitatif (nicel) olarak incelenmiştir.

Anahtar Kelimeler

Gypsophila simonii, Sapogenin, Toplam saponin içeriği % ose (toplam şeker), X-ışını kristalografisi, Sakkaroz.

Article History: Received October 28, 2011; Revised December 13, 2011; Accepted January 5, 2012; Avaliable Online: March 5, 2012.

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INTRODUCTION

Gypsophila is a member of the Caryophyllaceae family. Saponins, glycosides widely distributed in the plant kingdom, include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains. They consist of aglycones coupled with one or more monosaccharide moieties [3]. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional and industrial applications [4,5].

Saponin glycosides

Saponin glycosides are divided into 2 types based on the chemical structure of their aglycones. Saponins on hydrolysis yield an aglycone known as "sapogenin".

SAPONIN = SUGAR + SAPOGENIN

glycone aglycone

Saponins consist of a sugar moiety usually containing the most common monosaccharides include: glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid or steroid in nature. Saponins have one or more linear or branched sugar chains attached to the aglycone via a glycosidic ether or ester link. In some saponins, the presence of acylated sugars has also been detected. According to the number of sugar chains attached to the aglycone, the saponins can be monodesmosidic saponins (with a single sugar chain), or bidesmosidic saponins (with two sugar chains) [4,6]. Glucose is one of the most important carbohydrates. Its chemical formula is $C_6H_{12}O_6$. Sucrose is a disaccharide formed from glucose and fructose [6].

General properties of saponins

The genus Gypsophila is well known to contain saponins. Some species are used in folk medicine as remedies for cough, and colds also as a source of various products in industry such as detergents, because saponin molecules form soap-like foams when shaken with water. Saponins have a diverse range of properties, which include foaming and emulsifying properties [4], non-volatile, surface active compounds [6], sweetness and bitterness [7], as well as antimicrobial, insecticidal, mol-luscicidal activities and pharmaceutical products [8].

Gypsophila Perfoliata root which is the best and high quality type growing in Isparta. Also reviewed is the recent literature on the effect of processing on saponin content (Table 1).

MATERIAL AND METHODS

Plant material identification: Çöven (Gypsophila simonii) was collected around Çankırı province, Turkey. The root material was dried in a cool dark place and powdered in the Faculty of Pharmacy of Gazi University.

Saponin extraction; Approximately 3.5 kg of dried materials were placed into a cartridge and then extracted with chloroform in Soxhlet apparatus for 24 h. The cartridge was re-extracted with ethanol for extra 8 h and dried completely at the room temperature. The extracts containing saponins were evaporated by using a rotary evaporator at 40 rpm, without solvent the extract was filtrated and dried under reduced pressure. Saponin content (%) of the extract was calculated using the following equation;

$$\text{Saponin content (\%)} = W_{\text{crude extract}} / W_{\text{dried plant}} \times 100$$

$W_{\text{crude extract}}$ = weight of crude extract
$W_{\text{dried plant}}$ = weight of dried plant material

Saponin identification methods

The presence of saponins and terpenoids in the extract was determined using the froth test, Salkowski test and Liebermann- Burchard test for terpenoids.

Froth test; Froth test was done where in 2 g of the powered sample was boiled in 20 mL of distilled water bath and filtered [13]. The filtrate (5 mL) was mixed with 5 mL distilled water and shaken vigorously for a stable persistent froth. The foaming index has been checked on this plant is well-adjusted with literature [14].

Table 1. Saponin content of some selected plant materials

Source	Saponin content (%)	Reference
G. bicolor Grosh.	20-25	Sezik et al., 1986 [9]
G. arostii	11.20-11.41	Sezik et al., 1986 [9]
G. venusta Fenzl.	12.65	Battal et al., 2003 [10]
G. eriocollyx Boiss.	12.39	Battal et al., 2003 [10]
G. perfoliata var. anatolica	14.44	Battal et al., 2003 [10]
G. paniculata	4.00	Henry et al., 1991 [11]
G. simonii	12.30	Yücekutlu, 2007 [2]
Soybean	0.22-0.47	Fenwick et al., 1991 [5]
Quillaja bark	9-10	San Martin and Briones, 1999 [12]
Yucca	10.00	Oleszek et al., 2001 [3]
Alfalfa	0.14-1.71	Fenwick et al., 1991 [5]
Horse chest nut	3.00-6.00	Price et al., 1987 [4]
Sugar beet leaves	5.80	Price et al., 1987 [4]

Hemolytic test; Saponin which has been widely utilized as a standard for hemolytic test was obtained from the roots of several Gypsophila species. On the other hand, hemolytic assay was done by mixing the crude extract with red blood cell solution in microplate wells using the method by Mojica and Merca [15].

Salkowski test; For the test for terpenoids, 5 mL of the crude extact was mixed with 2 mL of chloroform and concentrated sulfuric acid (3 mL) was carefully added to form a layer.

Lastly, the Liebermann-Burchard test for detection of triterpenes was performed as described by Houghton and Raman [16]. 10 drops of the crude extract was placed in dry test tubes. It was added with 3 drops of acetic anhydride and one drop of concentrated sulfuric acid and observed for any color changes [17]. The formation of a red, pink or purple shade color after a few minutes is positive.

Moreover, for the test for terpenoids, 5 mL of the crude extract was mixed with 2 mL of chloroform and concentrated sulfuric acid (3 mL) was carefully added to form a layer.

Separation, Purification and Isolation; The classical methods of isolation of triterpenoid saponins are available. The advent of recent chromatographic techniques has provided valuable means for isolation of pure saponins or their derivatives. The dried extracts content (%) were calculated and dissolved in ethanol afterwards applied on Thin-Layer Chromatograms (TLC) (20x20, silica gel G60 Art. 7731) in the solvent system (1-Bu OH: 1-PrOH: HAc: H_2O) (40: 20: 7,5: 30). Spots on TLC were detected by spraying with 10% H_2SO_4 followed by heating at 110°C for 5-10 min. Spraying was done in order to identify the points of the separated compounds [18]. After derivatization the chromatograms were observed in daylight or in UV light at λ= 366 nm.

Acid Hydrolysis of the Saponins: Each of separated saponins (Gypsogenin ester) was refluxed for 7 h with 5% HCl [19]. Third spot (R_f=0.28) was run up for isolation and identification.

Ose Type: Sugar components were identified on Paper Chromatograms. The sugar in filtrate was identified as D-Glucose by comparison on PC (Whatmann 3 MM) ethyl-acetate; pyridine; water (12; 5; 4) with on authentic sugar [20].

Total Ose: It has been identified with paper chromatography as well as by preparing diphenyl hydrazine. The melting point of the composed 2,4-dinitrophenylhydrazone has been identified by crystallizing it (204 °C). The determination of sugar was used by the Bertrand method [21].

Crystallization and Structure Determination

After hydrolysis the remaining solution was evaporated in the rotary evaporators. The resulting precipitant was solved in a small amount of ethanol at the 78,5 ºC, then it was washed and dried with cold acetone (salt-ice bath). The obtained single crystals were used for the x-ray diffraction experiment.

X-ray measurements were performed on a Enraf-Nonius CAD4 four circle diffractometer equipped with graphite monochromator MoKα radiation. Intensity of peaks was corrected for standard reflections automatically. Structure was solved by direct methods using SHELXS (1) and, refinement was performed with SHELXL (2). Non-hydrogen atom parameters were refined anisotropically. All H atoms, except of the rings H, were placed in idealized positions and refined using a riding model with Ueq (H) = 1.3Ueq(C), and fixed distances of O-H = 0.82Å and C-H = 0.97Å (methyl). The H atoms of the rings were found from a difference Fourier map and refined isotropically. The geometric calculations were performed using the program Platon [3]. The crystal data, intensity data collection parameters and final refinement results are summarized in Table 4. The molecular structure with atom numbering scheme is presented in Figure 2. Selected bond angles and torsion angles are listed in Table 3.

RESULTS AND DISCUSSION

The ethanolic extract of the dried parts of Gypsophila simonii was purified on preparation TLC (R_f= 0.28).

Structure of the isolated Gypsogenin ester was characterized by spectroscopic methods such as FTIR, ^{1}H NMR, ^{13}C NMR and EIMS [1]. The results were compared with the similar studies [22, 23]. It was concluded that form presented in Figure 1.

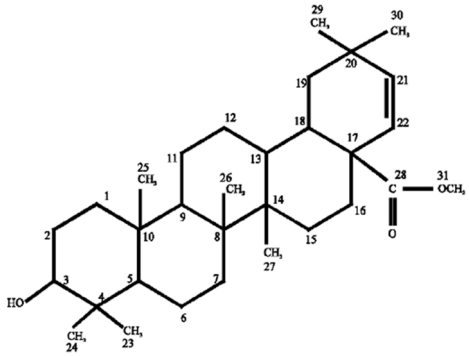

Figure 1. Gypsogenin ester saponin.

Gypsogenin ester saponin, mp: 235°C (uncorrupted); Molecular ion peak was also observed at and EIMS; m/z, [M+]: 472. All these results confirm that the proposed structure of that saponin appears as a new one and is called as Gypsogenin ester ($C_{31}H_{51}O_3$) [2]. PC results showed the presence of D- Glucose by comparing their retention times with those of authentic sugar (R_G=1.00, mp: 204 °C).

Investigating the saponin content and composition of Gypsophila simonii was found %12,30 ± 0,50 of the total saponin content and additional 7,0 ± 0,20% ose. No previous investigation has been reported on this plant but is well-adjusted with literature. The extract also showed positive results with the Liebermann-Burchard test and Salkowski test indicating that the triterpenoid saponins.

X-ray crystallography

X-ray analysis results showed that the compound crystallized was sucrose (I). The bond lengths and angles of (I) exhibit normal values and generally agree with those of sucrose (II), [24]. The disposition of the two sugar rings with respect to the C-O bond of the glycosidic linkage of (I) as shown in Table 2 is also similar to that of (II) Table 3 (Figure 2).

CONCLUSION

Phytochemical tests using the crude extracts showed the presence of saponin and triterpenoid. This is consistent with the concentrations of other soapwort varieties cultivated in our country. Paper chromotograms showed that, in G. simonii, D- Glucose was identified by comparison with on

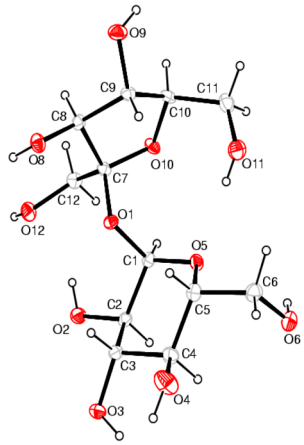

Figure 1. View of the molecule of sucrose (I) showing the atom-labelling scheme

Table 2. Crystal structure data and details of the structure determination of (I)

Chemical formula	$C_{12}H_{22}O_{11}$
Formula weight	342.3
Space group; Crystal system	$P2_1$; Monoclinic
Crystal dimensions (mm)	0.39 x 0.32 x 0.21
a (Å)	7.7493(15)
b (Å)	8.70614(12)
c (Å)	10.8613(16)
α (º)	90.0
β (º)	102.958(14)
γ (º)	90.0
Volume(Å^3); Z	714.1(2); 2
D_c (g cm^{-3}); μ(mm^{-1})	1.592; 0.143
λ (Å); Scan type	0.71073; ω - 2θ
Range of θ(º)	2.7; 52.56
No. refls. measured	1543
No. reflns. [I > 2σ(I)]; R_{int}	1334; 0.0162
No. reflns. used in refinement	1543
No. param. refined	240
R and R_w values	0.0411; 0.1149
Goodness of fit	1.093
Extinction coef.	0.0041(6)
Max. and min. electron density (e Å^{-3})	0.382, -0.356

Table 3. Comparison of selected bond angles and torsion angles (º) for (I) with those of sucrose (II) (Brown & Levy (1973))

	Sucrose (I) this work	Sucrose (II) Brown and Levy (1973)
C_1-O_1-C_7	113.4(3)	114.30(8)
O_1-C_1-O_5	110.2(3)	110.49(8)
O_1-C_1-C_2	110.1(3)	110.33(8)
O_1-C_7-O_{10}	110.9(3)	111.00(8)
O_1-C_7-C_8	107.7(3)	108.43(7)
O_1-C_7-C_{12}	110.5(3)	109.93(8)
C_1-O_1-C_7-C_8	-159.6(3)	-159.81(8)
C_1-O_1-C_7-O_{10}	-45.3(3)	-44.75(11)
C_1-O_1-C_7-C_{12}	73.9(4)	73.70(130)
C_7-O_1-C_1-C_2	-129.0(3)	-129.25(9)
C_7-O_1-C_1-O_5	108.2(3)	107.82(10)
O_5-C_5-C_6-O_6	-57.9(5)	-56.42(13)
C_4-C_5-C_6-O_6	63.8(5)	64.39(13)

authentic sugar. Physiochemical investigation of G. simonii led to identification of sucrose by x-ray crystallography. This is the first report about the presence of these compounds in the roots of the plant.

ACKNOWLEDGMENTS

The author gratefully acknowledges for his valuable guidance to my supervisor Professor Ahmet Yaşar from Department of Chemistry, Gazi University (Turkey). We thank kindly to Professor Zeki Aytaç in Department of Biology, Gazi University (Turkey) for identification of plant material, and we grateful to Professor Yılmaz Yıldırır from Department of Chemistry, Gazi University (Turkey) for help with spectral interpretation.

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