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Determination of Plant Saponins and Some of *Gypsophila* Species: A review of the literature

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Abstract

In this study, chemical and physical properties of unripe saponins obtained by extraction from the roots of Çöven *(Gypsophila simonii)*, an endemic plant, were investigated and isolated. Unripe saponins were separated into its components by thin layer chromatography and the R_f values of spots obtained under this study were calculated. The structure of the component in the spot which was expected to be pure was investigated by spectral methods such as ^{1}H NMR, ^{13}C NMR, FTIR and EIMS and named Gypsogenin ester saponin. The findings indicate that the proposed structure of that sponin was as a new Gypsogenin ester ($C_{31}H_{51}O_3$) [1].

Key Words: *Gypsophila*, *Gypsophila simonii*, Saponin, Sapogenins.

INTRODUCTION

Gypsophila

Gypsophila (family Caryophyllaceous), annual, biennial or perennial herbs, often suffrutescent, glabrous, with eglandular or more frequently with glandular hairs. Leaves linear-subulate to lanceolate, rarely broader, and often subfleshy [2].

Gypsophila, botanical name with authority and synonyms:

Gypsophilae radix is presently obtained from 7 plant species in Turkey as described below;

Gypsophila bicolor (Freyn & Sint.) Grossh.; Van Çöveni, Tarla Çöveni,

Gypsophila arrostii Guss. var. *nebulosa* (Bois & Heldr.) Bark.; Beyşehir Çöveni,

Gypsophila eriocalyx Boiss.; Çorum-Yozgat Çöveni,

Gypsophila perfoliata L.; Niğde Çöveni,

Gypsophila graminifolia Bark.; Dağ Çöveni, Başkale Çöveni,

Gypsophila venusta Fenzl; Konya Çöveni,

Ankyropetalum gypsophiloides Fenzl.; Siirt Çöveni, Helvacı Kökü [3],

Gypsophila simonii **Hub. Mor.** roots are found in Turkey and those are endemic. Its roots are endemic species growing in Çankırı (Turkey) [1].

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Gypsophila species are distributed all over Turkey mostly in steppe regions, maximum amounts is collected in eastern Anatolia, and lesser quantities come from Isparta and Konya provinces [2]. The genus *Gypsophila* is well known to contain saponins of industrial interest with various applications [4]. For example, the saponins from the roots of G. paniculata and G. *arrostii* have been used as detergent and expectorant [5]. G. *struthium* is known as a source of saponins since the antiquity and is also used in gastronomy in Arabic countries [6]. The structures of saponins isolated from G. *paniculata*, G. *arrostii*, G. *struthium*, G. *pacifica* and G. *capillaris* have been investigated [6-10]. Plants of the genus *Gypsophila* mainly grow in the Mediterranean zone. G. *bermejoi* is a Spanish endemism, found in Cuenca, Segovia and Madrid. A lot of saponins [11-16] from various *Gypsophila* species [17,18] were investigated and reported by many researchers. However, there have been no literature reports on Gypsogenin ester saponin of *Gypsophila simonii*.

GENERAL CHARACTERISTICS OF SAPONINS

Saponin is an important class of natural products that can be found primarily in roots, petals and foliage of many plants, as well as in some marine animals. [19]. A saponin molecule consists of an aglycone (or sapogenin) and on or two sugar moieties. According to the structures of the aglycones, saponins can be classified into two types: triterpenoid and steroidal. The most common sugar residues are hexoses (glucose, galactose), 6-deoxyhexoses (furanose, quinovose, rhamnose), pentoses (arabinose, xylose), and uronic acids (glucuronic acid, galocturonic acid) [20]. The sugar moiety is linked to the aglycone through an ether or ester glycosidic linkage at one or two glycosylation sites. The glycosides, frequently occurring in complex mixtures, are widely distributed in the plant kingdom. According to the nature of the aglycone they can be classified into steroidal or triterpene groups. All classes of aglycones may have a number of functional groups (–OH, –COOH, –CH) causing big natural diversity only because of aglycone structure. Over 100 steroidal and probably even larger numbers of triterpene sapogenins have been identified. These structures and their amounts may differ depending on the plant part studied. This structural diversity and resulting wide range of polarities makes determination of individual saponins very difficult [19].

Saponins occur constitutively in a great many plant species, in both wild plants and cultivated crops. In cultivated crops the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties [20]. Triterpenoid saponins have been detected in many legumes such as soyabeans, beans, peas, lucerne, etc. and also in alliums, tea, spinach, sugar beet, quinoa, liquorice, sunflower, horse chestnut, and ginseng. Steroid saponins are found in oats, capsicum peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. One example of an extensively studied group of triterpenoid saponins is produced from Quillaja saponaria, a tree native to the Andes region. Yucca schidigera is the most common commercial source of steroid saponins [21].

Saponins are generally known as non-volatile, surface active compounds that are widely distributed in nature, occurring primarily in the plant kingdom [22-24]. The name ‘saponin’ is derived from the Latin word sapo, which means ‘soap’, because saponin molecules form soap-like foams when shaken with water. Saponins have a diverse range of properties, which include sweetness and bitterness [25-27] foaming and emulsifying properties [28] pharmacological and medicinal properties [29] strong haemolytic properties, as well as

antimicrobial, insecticidal, and molluscicidal activities [30]. Saponins have found wide applications in beverages and confectionery, as well as in cosmetics [31] and pharmaceutical products [32]. Saponins have a potential as pharmaceutical synthons and have been used in hormone synthesis [33].

Saponins are a diverse group of compounds commonly found in legumes, e.g. chick peas, soya beans, lentils, peanuts, lentils, *Phaseolus* beans and alfalfa sprouts; and in some plants commonly used as flavourings, herbs or spices, e.g. ginseng, fenugreek, sage, quillaja bark, thyme, sarsaparilla and nutmeg [34]. Their structures are characterised by the presence of a steroid or triterpene group, referred to as the aglycone, linked to one or more sugar molecules. The presence of both polar (sugar) and non-polar (steroid or triterpene) groups provide saponins with strong surface-active properties which then are responsible for many of its adverse and beneficial biological effects [35].

Sapogenins and Saponins: Chemical Structure and Bioactivity

Although the study on plant saponins started in the 1970s, only recently they have received an increasing attention in consideration of the involvement of saponins in important biological processes and of their ability to act as natural drugs in many diseases. In fact several papers have demonstrated antifungal, antitumor, cytotoxicity, blood coagulability, anti-spasmodic and cholesterol-lowering effects of saponins isolated from onion and garlic [36-38]. Most saponins possess a variety of bioactivities (e.g., cardiac, antifungal, hemolytic activities and abilities to affect metabolism and biosynthesis); they are among the major effective components in nutriceutical products [39].

Several biological effects have been ascribed to saponins. Extensive research has been carried out into the membrane-permeabilising, immuno-stimulant, hypocholesterolaemic and anti-carcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agents. These compounds can thus affect animals in a host of different ways both positive and negative [21].

Some of *Gypsophila* species are also used for medical treatment purposes, as drug in response to certain diseases such as an expectorant [40] and diuretic [41], for treatment of hepatitis, gastritis and bronchitis [42]. Due to its content of saponin glycosides it is used as foaming agent in detergents, and in film emulsions, fire extinguishing tubes and in leather industry. In Turkey it is mainly used in the manufacturing of Turkish delight and helva, Turkish speciality sweets [3]. Its infusion is used as expectorant and diuretic. Roots and barks of some saponin bearing plants have been reported to be used in Turkey against dysentery, diarrhea and stomach ulcer, and as analgesic, antipyretic, anti-inflammatory, sedative, emetic and insecticidal [43].

Isolation of Saponins

The unique chemical nature of saponins demands tedious and sophisticated techniques for their isolation, structure elucidation and analysis. The task of isolating saponins from plant material is complicated also by the occurrence of many closely related substances in plant tissues, and by the fact that most of the saponins lack a chromophore. Thus,

for many years, the complete characterisation of saponins from even well-known saponin-containing plants was not achieved. However, recently renewed interest in medicinal plants and foods alongside the dramatic evolution of analytical tools has resulted in a burst of publications presenting numerous novel saponins. The modern methods available for the separation and analysis of saponins have been well reviewed in literature [44-46].

As a general rule, they begin with the extraction of the plant material with aqueous methanol or ethanol. Further processing of the extract is carried out after evaporation under reduced pressure, dissolution in a small amount of water and phase separation into n-butanol. It is currently recognised that this step is sometimes undesirable, since only those saponins with short oligosaccharide side chains will eventually be extracted into the butanolic phase. A further purification is then carried out, which involves liquid chromatography over a silica gel column, or a gradient elution from a polymeric support or liquid–partition chromatography, or, as most commonly employed, HPLC (High Performance Liquid Chromatography) separation. In most cases, certain of the above steps have to be repeated with a change of support or eluent to achieve high purity. Once the saponin has been purified, it may be subjected to analytical methods including MS (Mass Spectrometry), proton and carbon NMR (Nuclear Magnetic Resonance) spectroscopy, and FTIR (Fourier Transform Infrared Spectroscopy). Other classical methods are used to ascertain the presence of saponins in a crude plant extract, and to elucidate their composition throughout purification steps. TLC (Thin Layer Chromatography) and staining with dehydrating reagents containing aromatic aldehydes (such as anisyl aldehyde in sulfuric acid) are commonly used. The pure saponin may also be hydrolysed to verify the nature of its glycosidic moieties [21]. The saponins were hydrolyzed and the resulting aglycones were identified by GCMS (Gas Chromatography-Mass Spectrometry), aided by NMR, FTIR and UV (Ultraviolet-Visible spectroscopy) data [47].

The aim of this review is to propose a classification of saponins based of the carbon skeletons of the aglycones.

EXPERIMENTAL

Plant Material

Çöven (*Gypsophila simonii*) was collected in June 1997 near to Çankırı, (Turkey) and identified by Professor Dr. Zeki Aytaç from Department of Biology, Gazi University. The root material was dried in a cool dark place and powdered at the Faculty of Pharmacy of Gazi University.

The ethanolic extract of the dried parts of Çöven (*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].

RESULTS AND DISCUSSION

Gypsogenin ester saponin (Figure 1); mp: 235^{o}C (uncorrupted). Also, molecular ion peak was observed at EIMS; *m/z*, [M $^{+}$] : 472. All these results confirm the proposed structure that saponin is a new one and called as Gypsogenin ester ($C_{31}H_{51}O_3$). The aim of this review is to propose a classification of saponins based of the carbon skeletons of the aglycones.

Then, this compound compared with similar compounds that have been explained on previous researches [48-52]. The data were identical with

those of authentic samples reported in the literature. The ^{13}C NMR spectrum gave peaks for 31 carbons and supported, the deductions derived from the FTIR and EIMS. From these results we proposed that Gypsogenin ester [52].

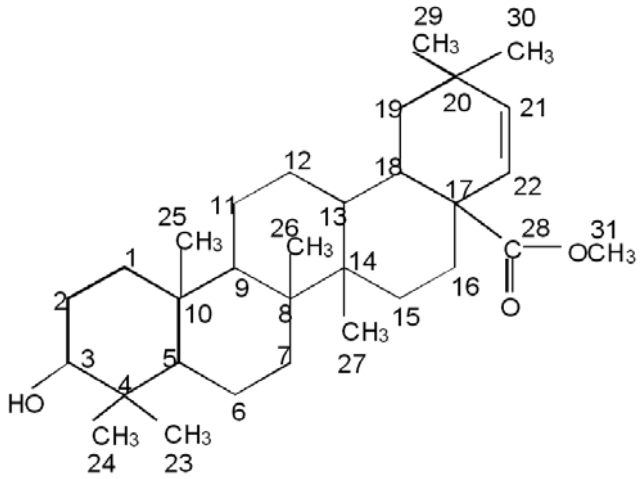

Figure 1. Gypsogenin ester saponin.

Acid hydrolysis of the saponins

The separated saponin (Gypsogenin ester) was heated under reflux in absolute with 5% HCl for reflux 3 hr. Third spot (R_f = 0.28) was worked up as isolation and identification. Sugar components were identified on Paper Chromatograms (PC). The sugar in filtrate was identified as D-Glucose (m.p. 204°C, decmp.) by comparison on PC (ethyl-acetate: pyridine: water, 12: 5: 4) with on authentic sugar [53].

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