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Saponin Rich Fractions (SRPs) from Soapwort Show Antioxidant and Hemolytic Activity

Idris Arslan[a,]*, Ali Çelik[b]

[a]*Pamukkale University, Faculty of Technology, Biomedical Engineering, TR20070, Denizli, Turkey*
[b]*Pamukkale University, Faculty of Science and Arts, Biology, TR20070, Denizli, Turkey*

Abstract

The present study established baseline data on hemolytic and antioxidant capacity of saponin rich fractions (SRFs) of *Gypsophila arrostii, G.pilulifera* and *G.simonii* (Caryophyllaceae) naturally found in Turkey. The antioxidant activity of the each SRF was carried out using 2 different methods: free-radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) and ABTS assay. Hemolytic activity of SRFs was tested using diluted sheep bloods and saline/distilled water as control groups. Also, total phenolic contents of each fraction were determined. Our results demonstrated that *G.arrostii*, *G.pilulifera* and *G.simonii* possessed strong antioxidant and the slight hemolytic activity when comparing the other saponin containing extracts.

Keywords: SAPONIN RICH FRACTION, ANTIOXIDANT, HEMOLYTIC ACTIVITY

1. Introduction

Saponins are a widespread class of natural compounds which are found in a lot of plant species. Saponins consist of a hydrophobic triterpenoidal C30 or steroidal C27 backbone and one or two hydrophilic glycoside moieties attached to the backbone. Due to this structural composition, saponins are amphiphilic glycoconjugates which give soap-like foams in water. Crude saponins appear as white powder, with bitter

* Corresponding author. Tel.: +90 2582963026; fax: +90 2582963263.
E-mail address: iarslan@pau.edu.tr, idris.arslan@yahoo.com.

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taste and easily provoke sneezing. The triterpenoid *Quillaja* saponins obtained from the bark of *Quillaja saponaria* MOL. stimulate Th1 type immunity and are the only adjuvants reported to date that are capable of stimulating the production of antigen-specific cytotoxic T-lymphocyte (CTL) against exogenous or soluble protein antigens [1]. With desirable adjuvant characteristics, *Quillaja saponins* have high toxicity, hemolytic effect, and instability. All these features have restricted their use in human vaccination [2-3]. Roots from *Gypsophila* species are an especially rich source of triterpene saponins [4]. They are exploited commercially for a variety of purposes including medicines, detergents, adjuvants and cosmetics [5]. The genus *Gypsophila* is known as soapwort (Turkish name *çöven* or *sabunotu)* in Aegean Region and generally used as ornamental plant all over the world. The present study was aimed to investigate antioxidant and hemolytic properties of selected *Gypsophila* species from Turkish flora.

2. Materials and Methods

2.1. Plant sample

Plant species was collected from Korkuteli-Yazır Village, Antalya (*Gypsophila arrostii* Guss.var. *nebulosa*), Lara Beach, Antalya (*G.pilulifera*) and Ankara-Çankırı road (*G.simonii*). A voucher specimen was identified by Dr. Celik and deposited at the Herbarium of Biology, Pamukkale University, Denizli, Turkey.

2.2. Extraction of saponin rich fractions (SRFs)

Roots of *Gypsophila* species crushed and powdered and defatted by petroleum ether. Samples were extracted with MeOH for 3 h with mild heating. In order to get the saponin rich fraction extract was again dissolved in methanol and acetone was added (1:5 v/v) to precipitate the saponins as described by Yan [6]. The precipitate was dried under vacuum. The whitish amorphous powder, thus obtained was named as a saponin rich fraction (SPF).

2.3. ABTS assay

The effect of the saponin rich fraction (SRFs) on ABTS radical was detected using a modified ABTS decolorization assay applicable to both hydrophilic and lipophilic compounds [7-8]. The $ABTS^{+}$ radical cation was produced by reacting a 7.0 mmol L^{-1} stock solution of ABTS with 2.45 mmol L^{-1} potassium persulfate. The mixture was stood in the dark for at least 16 h at room temperature before use. The ABTS radical solution was diluted with ethanol 80% (*v/v*) to obtain an absorbance of 0.70±0.05 at 734 nm and equilibrated at 30 °C. Diluted ABTS solution, prepared as described above, was mixed with soapwort extract and measured at 734 nm after 6 min. The results were corrected for dilution and expressed in μM trolox per 100g dry weight. All determinations were performed in triplicate.

2.4. DPPH Assay

The free radical scavenging activity of the SRFs was measured by the DPPH assay [9]. A 0.1 ml aliquot of each extract (0.1, 1.0 and 5.0 μg/ml) and BHA in MeOH was added to 3.9 ml of $6x10^{-5}$ M MeOH solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured at 517 nm. All measurements were made in triplicate. The ability to scavenge DPPH radical was calculated by the following equation:

Scavenging Effect (%) = $1 - [(A_1 / A_o) \times 100]$, where A_1 and A_0 are absorbance of sample and control.

2.5. Determination of total phenolic content (TOC)

Total phenolic content was determined by the Folin–Ciocalteu micro-method [10]. A 20 μL aliquot of extract solution was mixed with 1.16 mL of distilled water and 100 μL of Folin– Ciocalteu's reagent followed by 300 μL of 200 g L−1 Na_2CO_3 solution. The mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance at 760 nm was measured. Gallic acid was used as standard for the calibration curve and total phenolic content expressed as gallic acid equivalent (GAE).

2.6. Hemolytic assay

Red blood cells were obtained from Sabanoglu Integrated Cattle Farm (Denizli, Turkey). Blood was collected with BD Vacutainer™ (NH 143 IU, Belliver Industrial Estate, Plymouth, UK). Aliquots of 7 mL of blood were washed three times with sterile saline solution (0.9 % w/v NaCl, pyrogen free) by centrifugation at 180×g for 5 min. The cell suspension was prepared by finally diluting the pellet to 0.5% in saline solution. A volume of 0.5mL of the cell suspension was mixed with 0.5mL diluent containing raw saponin 5, 10, 25, 50, 100, 250, 500, or 1000 μg/mL in saline solution. The mixtures were incubated at 37 ºC for 30 min, and centrifuged at 70×g for 10 min. Free hemoglobin in the supernatants was measured at 412 nm [11]. Saline and distilled water were used as minimal and maximal hemolytic controls.

2.7. Statistical analysis

The data were expressed as mean±SD and examined for their statistical significant difference with analysis of variance.

3. Results and Discussion

As stressed by Frenkel and Meyer, no single method is adequate for evaluating the antioxidant capacity of foods, since different methods can yield widely diverging results. Several methods based on different mechanisms must be used [12]. Here, we applied assays of ABTS and DPPH radical-scavenging activity to each saponin rich fraction. ABTS assay was not only a rapid and reliable test of total antioxidant capacity but also an advantageous assay applicable to both hydrophilic and lipophilic antioxidants/systems. In addition, proton radical scavenging is an important attribute of antioxidants. $ABTS^+$, a protonated radical, has a characteristic absorbance maximum at 734 nm that decreases with the scavenging of proton radicals [13]. As it can clearly be seen Table 1, the ABTS values of saponin rich fractions from *G.pilulifera, G. simonii,* and *G.arrostii* were found as 166.89, 84.41 and 42.44 μM trolox/100 g DW, respectively. The ABTS assay results demonstrated that *G.pilulifera* compared to those of and *G.arrostii*, *G.simonii* possessed the strongest antioxidant activity. However, the DPPH assay showed that *G.pilulifera* had the highest free radical scavenging activity at the concentrations of 1 and 5μg/mL. Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases [14]. DPPH is considered to be a model of a stable lipophilic radical and a chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH, reducing the number of DPPH free radicals to the number of their available hydroxyl groups. The antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, preventing of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts [15]. It is generally accepted that the use of plants and plant-derived dietary supplements with high antioxidant potential in the regular diet plays an important role in the prevention of a

number of health disorders associated with free radical formation during aerobic metabolism [16].

Table 1. ABTS and DPPH assays and total phenolics (TPs) of *Gypsophila* species

Plant species	Total phenolics (TPs)[a]	ABTS[b]	DPPH radical scavenging activity (%)		
			0.1[c]	1	5
G.arrostii	02.68±0.7	042.44±1.2	2.3±0.6	10±1.1	40±1.0
G.pilulifera	05.40±1.1	166.89±0.9	0.1±0.2	21±1.3	70±1.6
G.simonii	15.15±0.1	084.41±1.1	*nt*	12±2.1	63±1.6
BHT (control)			58	92	92

[a]mg GAE g–1 DW, [b](μM trolox/100 g DW), [c]: μg/mL, *nt*: not tested

Antioxidants have been widely used as additives to provide protection against oxidative degradation of foods and cosmetics initiated by free radicals. In particular, synthetic antioxidants such as BHA and BHT are extensively used in the food and pharmaceutical industries as stabilisers and inhibitors of lipid peroxidation in fatty products. However, ever since their first introduction to the food and related industries, questions about their safety and efficiency have frequently arisen, mainly owing to their instability, toxicity and potential carcinogenicity [17]. Therefore, plant extracts are in increasing demand from the manufacturers of foods and pharmaceuticals, and numerous extracts have already been recognised and widely used as effective preservative agents. Since the phenolic compounds are very important constituents of plants and known as powerful chain-breaking antioxidants.

Table 2. Hemolytic activities of *Gypsophila arrostii, G.pilulifera* and *G.simonii*. Hemolytic percents of saline and distilled water were used as minimal and maximal hemolytic controls. (p<0.05, p>0.05)

	Gypsophila arrostii		*Gypsophia pilulifera*		*Gypsophila simonii*		Control groups	
Groups[a]	Absorbance value	Hemolytic activity (%)	Absorbance value	Hemolytic activity (%)	Absorbance value	Hemolytic activity (%)	Absorbance value	Hemolytic activity (%)
500	0.59±0,2	30.12[b]	0.48±0,1	23.49	0.31±0.3	13.25		
250	0.35±0,3	15.66	0.20±0,2	6.62	0.18±0.3	5.42		
125	0.23±0,1	8.4	0.08±0,1	-0.6	0.10±0.2	0.6		
50	0.19±0,1	6.02[c]	0.06±0,2	-1.8	0.06±0.2	-1.8		
25	0.11±0,1	12	*nt*	*nt*	0.06±0.2	-1.8		
dH_2O							1,66±0.04	100
Saline							0.09±0.03	0

[a)]μg/ml, *nt*: not tested, n=3 tests

The data in Table 1 show that total phenolics of *G.simonii*, *G.pilulifera* and *G.arrostii* with respective values of 15.15, 5.40 and 2.68 mg GAE g^{-1} DW. The highest level of phenolics was found in *G.simonii*, while the lowest was in *G.arrostii*. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids. Roots from *Gypsophila* species are an especially rich source of triterpene saponins [4]. The unique capacity of *Quillaja* saponins to stimulate both the Th1 immune response and the production of cytotoxic Tlymphocyte against exogenous or soluble protein antigens makes them ideal for use in subunit vaccines and vaccines directed against intracellular pathogens and additionally for therapeutic cancer vaccines [1]. As part of the ISCOM (Immune stimulating complex) *Quillaja* saponins were shown to promote the antibody and immune response [18]. In fact, there are a series of commercial veterinary vaccines as well as human vaccines formulated with this kind of adjuvant undergoing clinical evaluation. QS-21 has been evaluated in a large number of vaccines in Phase I and Phase II human clinical trials [11]. These vaccines include cancer immunotherapeutics [19], HIV recombinant envelope [20]. However, *Quillaja* saponins have serious disadvantages. Firstly, they are instable in aqueous phase because of hydrolysis of their ester moieties on the C-28 fucose Secondly, *Quillaja* saponins especially the three most predominant saponins

(QS-17, QS-18, and QS-21) are acylated at the 4-hydroxyl position of fucose with two linked 3,5-dihydroxy-6-methyloctanoic acids containing a glycosylation site at the 5-OH position of the acyl chains. The acylation is highly critical to Th1 type response and the production of cytotoxic T-lymphocyte [2]. However, these saponins might be easy deacylated under mild condition and then may lose both their capacity to stimulate the Th1 immune response and to produce antigen-specific cytotoxic T-lymphocyte. Morever, *Quillaja* saponins have high toxicity and tendency to induce tissue damage partly associated with their hemolytic activity [3]. To surpass these obstacles, non-toxic, non-hemolytic and stable saponins have to be selected fullfiling criteria. Therefore, *Gypsophila* species was screened in this study for hemolytic activity on cattle erythrocytes. As it can be clearly seen Table 2. hemolytic percents of red blood cell treated with Ga-500, Gp-500 and Gs-500 (µg/ml) were 30.12%, 23.49%, and 13.25%, respectively. *G.pilulifera* showed no hemolytic activity at the concentration of 50- 125 µg/ml. Our results showed that *G.arrostii*, *G.pilulifera* and *G.simonii* had the hemolytic activity in lower level when comparing the other saponin containing extracts. Thus, it might be said that *Gypsophila* saponins were safer than Quil A and its components in clinical use.

Acknowledgements

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