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Gas chromatographic-based techniques for the characterization of low molecular weight carbohydrates and phenylalkanoid glycosides of *Sedum roseum* root supplements

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ABSTRACT

An extensive characterization of low molecular weight carbohydrates (LMWC) and phenylalkanoid glycosides (PAG) of *Sedum roseum* root supplements has been carried out for the first time by gas chromatography coupled to mass spectrometry (GC–MS) and by comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-ToF MS). Optimization of the required derivatization procedure for improved determination of PAG showed the combined use of trimethylsilylimidazole and trimethylchlorosilane as the most appropriate reagents. Up to 37 compounds were qualitative- and quantitatively characterized in different dietary supplements of *S. roseum* by GC–MS. In addition to the well-known rosin, rosarin, rosavin and salidroside, other carbohydrates, polyalcohols, acids, etc. were determined. Among them, several seven-carbon monosaccharides such as coriose and 2,7-anhydro-*β*-D-*altro*-heptulose were detected for the first time in *S. roseum* root supplements. Sedoheptulose was found to be the most abundant compound (9–151 mg g^{-1}), followed by rosiridin (20–81 mg g^{-1}) and rosavin (11–56 mg g^{-1}). The use of GC × GC-ToF MS allowed the detection and tentative assignation of 48 additional compounds mainly belonging to the phenylalkanoid glycoside, pentosyl-hexose and hexosyl-hexose families.

1. Introduction

Sedum roseum (L.) Scop. (also called *Rhodiola rosea*) belongs to the Crassulaceae family and it is cultivated worldwide mainly to obtain dietary supplements [1], which usually consist of alcoholic extracts from *S. roseum* roots, traded alone or in combination with other plants [2]. It is defined as an adaptogen, showing a favourable influence on a diversity of physiological functions [3–6]. Thus, several beneficial activities have been attributed to this plant such as antioxidant, anti-hypoxic, immunomodulatory, cardioprotective and neuroprotective, among others [1,7–9]. Recently, hypolipidaemic and hypoglycemic activities have also been attributed to *S. roseum* [10,11] and, therefore, supplements from this plant could potentially be used as therapeutic agents for diabetes mellitus treatment.

The presence of different compounds in *S. roseum* roots such as polyphenols, terpenes, phenylalkanoids, carbohydrates and glycosides has been reported to be related to the biological activities of this plant [4,11–13]. Several manuscripts have been devoted to the analysis of polyphenols, terpenes and phenylalkanoids in *S. roseum* extracts [14–16]. Regarding the last compounds, a global estimation by high performance liquid chromatography (HPLC) of selected bioactives [mainly the phenylpropanoid glycosides rosarin, rosavin and rosin, which are reported as 'rosavins', and their relationship with salidroside (Table 1)] is usually considered to evaluate *S. roseum* bioactivity. Nevertheless, each of these active compounds shows a specific physiologic effect and their individual determination is highly recommended.

Studies regarding *S. roseum* roots carbohydrate composition are scarce and mainly aimed to the estimation of total and reducing sugars [11], or the rough determination of polysaccharides [17] and free monosaccharides [18]. Therefore, there is a need to develop analytical methodologies that allow the detailed study of the low molecular weight carbohydrate (LMWC) composition of *S. roseum*

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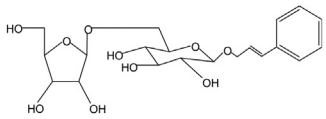
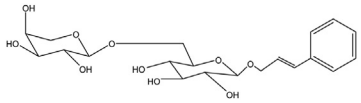
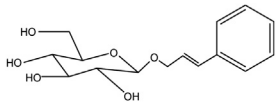
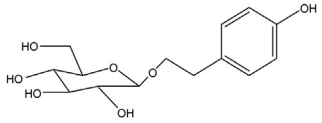
Table 1
Nomenclature and chemical structure of some bioactive compounds of *Sedum roseum*.

Common name	IUPAC name	Structure
Rosarin	**(2E)-3-Phenyl-2-propen-1-yl 6-O-α-L in l-arabinofuranosyl-β-d-glucopyranoside**	
Rosavin	**(2E)-3-Phenyl-2-propen-1-yl 6-O-α-L-arabinopyranosyl-β-D-glucopyranoside**	
Rosin	**(2E)-3-Phenyl-2-propen-1-yl** β-D-glucopyranoside	
Salidroside	**2-(4-Hydroxyphenyl)ethyl** β-D-glucopyranoside	

extracts for the required characterization of these food supplements prior to their consumption.

Considering the advantages in terms of resolution, sensitivity and identification capability provided by gas chromatography coupled to mass spectrometry (GC–MS), this technique is an interesting choice for the comprehensive characterization of the complex mixtures of LMWC and phenylalkanoid glycosides (PAG) present in *S. roseum* supplements. Moreover, the use of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-ToF MS) could significantly improve the peak capacity and average sensitivity over monodimensional GC (1D GC), resulting in an improved characterisation of less abundant compounds. Although GC–MS has been mainly used to analyse the volatile composition of essential oils from *S. roseum* roots [19], to our knowledge, no previous reference has addressed the study by these GC-based techniques of the PAG present in *S. roseum* root supplements or the comprehensive characterization by GC × GC of the LMWC in this particular type of matrix.

At the sight of the above exposed, the main objective of the present study was the exhaustive characterization of LMWC and PAG of *S. roseum* root supplements by developing and applying GC–MS and GC × GC-ToF MS methodologies with a view to provide a deeper understanding of the composition of these food supplements. To this aim, efficiency of the derivatization procedure, mandatory for GC analysis of these compounds, was first evaluated.

2. Materials and methods

2.1. Standards and samples

Analytical standards of 2,7-anhydro-*β*-D-*altro*-heptulose, arabinose, arabitol, cellobiose, fructose, galactose, gallic acid, glucose, 1-kestose, mannitol, mannose, D-*altro*-2-heptulose (sedoheptulose), sorbitol, sucrose, *myo*-inositol, gentiobiose, raffinose, ribose, xylitol, and xylose were obtained from Sigma-Aldrich (St. Louis, USA). Rosin, rosarin, rosiridin, rosavin and salidroside were purchased from Chengdu Biopurify Phytochemicals Ltd. (Sichuan, China). Leaves from *Coriaria myrtifolia* and *Coriaria ruscifolia* were supplied by Dr. R. Morales from Real Jardín Botánico of CSIC (Madrid, Spain).

A total of eight *S. roseum* root supplements were analysed: Z1 – Z6 were kindly provided by Biosearch Life (Madrid, Spain); C1 and C2 were purchased from Lamberts Plus (Lamberts Española SL, Madrid, Spain) and Solgar España (Las Rozas, Madrid, Spain), respectively. As a reference, a *S. roseum* root sample (R1) was also analysed. The concentrations of 'rosavins', as indicated in the labels of the containers, of the different *S. roseum* root supplements under study are shown in **Table S1** of Supplementary Material.

2.2. Extraction procedure

Commercial supplements C1 and C2 (1 g) and Z1-Z6 (0.3 g) were dissolved into 10 mL of an ethanol (Scharlau, Barcelona, Spain):water mixture (70:30, v/v). Water of ultra-pure quality (18.2 MΩ cm) was produced in house using a Milli-Q Advantage A10 system from Millipore (Billerica, MA, USA).

S. roseum dried roots (R1) were ground using a mill (Moulinex, Barcelona, Spain) and sieved (< 500 μm) before extraction. One gram of ground root sample was extracted with 10 mL of ethanol:Milli-Q water (70:30, v/v) for 2 h under constant stirring. Three successive extractions from the same sample were carried out and extracts thus obtained were further combined.

All extracts were filtrated through Whatman No. 4 filter paper (Sigma-Aldrich) and kept in the dark at −20 °C until analysis.

2.3. Analysis

2.3.1. Derivatization procedure

S. roseum root extracts (1 mL) were mixed with 0.5 mL of a 70% ethanolic solution of phenyl-*β*-D-glucoside (1 mg mL^{-1}), employed as internal standard, and then dried under vacuum at 38–40 °C before derivatization. Trimethylsilyl oximes (TMSO) were chosen as derivatives considering that the formation of oximes before trimethylsilylation reduces the number of possible tautomers of reducing sugars to only two forms (the *syn* (*E*) and *anti* (*Z*) isomers), whereas non-reducing compounds are converted into trimethylsilyl ethers [20]. Oximes were formed using 2.5% hydroxylamine chloride in pyridine (350 μL) after heating at 75 °C for 30 min [21]. Different silylation reagents [hexamethyldisilazane (HDMS), trimethylsilylimidazole (TMSI) + trimethylchlorosilane (TMCS)], and silylation temperatures (22 and 45 °C) were considered in the optimization of the derivatization procedure. Efficiency of this process, not previously evaluated for phenylpropanoid glycosides, was also determined.

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