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Steroidal saponins from *Yucca gloriosa* L. rhizomes: LC–MS profiling, isolation and quantitative determination

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ABSTRACT

The occurrence of steroidal saponins in the rhizomes of *Yucca gloriosa* has been detected by LC–MS. On the basis of the LC–MS analysis, five steroidal glycosides, including three spirostane, one furostane and one cholestane glycosides, along with seven known compounds have been isolated and characterized by ESI-MS and by the extensive use of 1D- and 2D-NMR experiments. Quantitative analysis of the steroidal glycosides in *Y. gloriosa* rhizomes was performed by an LC–MS method validated according to European Medicines Agency (EMEA) guidelines. The dried BuOH extract obtained from rhizomes contains more than 25% w/w of glycosides, thus *Y. gloriosa* rhizomes can be considered a rich source of steroidal glycosides.

1. Introduction

Yucca gloriosa L. is largely cultivated in Eastern Georgia, where industrial plantations of this species occupy a total area of about 150 ha (Benidze et al., 1991). *Y. gloriosa* produces a strong underground system, whose main part is represented by rhizomes. In the rhizomes, biosynthesis of 5β steroids occurs, whereas in leaves and flowers only 5α steroids (Skhirtladze et al., 2006; Kemertelidze and Benidze, 2001) have been detected.

The roots and bark of *Y. gloriosa* are free from saponins, but they are rich in phenolic constituents related to resveratrol, with high antioxidant and antiproliferative activities (Bassarello et al., 2007a,b; Nigro et al., 2007; Montoro et al., 2008).

Due to the high content of steroidal saponins (5–6%), the rhizomes of *Y. gloriosa* are used as raw material for the semisynthesis of steroidal hormones. Continuing our studies on *Yucca* spp. (Montoro et al., 2008, 2010; Skhirtladze et al., 2006), here we report on the phytochemical investigation of the steroidal saponins of the rhizomes of *Y. gloriosa*.

A preliminary analysis of the BuOH enriched saponin fraction obtained by *Y. gloriosa* rhizomes was performed by LC–ESI-MS in order to detect the presence of saponins in this part of the plant. Successively, five new steroidal glycosides, including three spirostane, one furostane and one cholestane glycosides, along with seven known compounds were isolated. Finally, an LC–MS/MS method was developed for the quantitative analysis of steroidal glycosides in the extract of *Y. gloriosa* rhizomes.

2. Results and discussion

From the analytical perspective, it is clear that steroidal saponins are not detectable by HPLC–UV analysis for the lack of a strong UV chromophore and that HPLC analysis of all compounds requires gradient elution. Mass spectrometry represents an effective detection method, and in addition improvement in selectivity and specificity can be raised by using tandem mass spectrometry. LC–MS is selective and sensitive enough to carry out the analysis of saponins (Yan and Guo, 2005; Tor et al., 2005; Montoro et al., 2010). On the basis of these observations, a preliminary analysis of the extract obtained by *Y. gloriosa* rhizomes was performed by LC–ESI-MS in order to detect the presence of saponins in this part of the plant.

The ESI-MS spectra of saponins show adducts together with pseudomolecular ions; in the ESI-MS spectra in positive-ion mode, mainly $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, and $[M+K]^+$ ions can be observed (Miao et al., 2002).

Column and mobile phase selection is generally determined by the combination of the compounds to be analyzed and the matrix where they occur. In our work the use of an Atlantis C18 column and a gradient elution allowed us to obtain a good separation of the steroidal glycosides occurring in the BuOH enriched saponin fraction of *Y. gloriosa* rhizomes, without any other compound interfering with the analyte detection.

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The profile of the Total Ion Current (TIC) showed 12 main peaks. For these peaks MS spectra were obtained by extracting them from the total profile. Successively, on the basis of the *m*/*z* values, Reconstructed Ion Chromatograms (RIC) were extracted.

Positive-ion electrospray LC–MS analysis obtained in the acquisition range of 190–1200 amu, TIC profile and RICs are shown in Fig. 1. Twelve main compounds were detected: at *m*/*z* 917, compounds **1** and **3**; at *m*/*z* 915, compound **2**; at *m*/*z* 1199, compound **4**; at *m*/*z* 903, compounds **5** and **7**; at *m*/*z* 757, compounds **6** and **9**; at *m*/*z* 755, compound **8**; at *m*/*z* 741, compounds **10**–**12**.

On the basis of the results of the on-line screening and identification by LC–MS, compounds **1**–**12** were isolated and their structures were unambiguously elucidated from NMR spectroscopic data (Figs. 2 and 3).

The BuOH extract was fractionated by silica gel column chromatography to yield compounds **10** and **11**. The fractions obtained were chromatographed by reversed-phase HPLC to yield compounds **1**–**9** and **12** (see Section 4).

The absolute configurations of the sugar units were assigned after acid hydrolysis of the crude saponin mixture and identification with authentic samples by TLC, followed by preparative separation of each sugar. The D configuration of galactose and glucose and the L configuration of rhamnose were established by comparison of their optical rotation values with those reported in the literature (Belitz et al., 2009; Wang et al., 2008).

The HRMALDITOF mass spectrum of **2** (*m*/*z* 937.4412 $[M+Na]^+$, calcd. for $C_{45}H_{70}O_{19}Na$, 937.4409) supported a molecular formula of $C_{45}H_{70}O_{19}$. The ESI-MS mass spectrum showed the major ion peak at *m*/*z* 937.4 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed a peak at *m*/*z* 775.4 $[M+Na-162]^+$, corresponding to the loss of an hexose unit. In the MS^3 spectrum a peak at *m*/*z* 613.4 $[M+Na-162-162]^+$, corresponding to the loss of a second hexose unit, was observed. Finally, in the MS^4 spectrum a peak at *m*/*z* 451.4 $[M+Na-162-162-162]^+$, indicating the loss of a third hexose unit, was observed. The ^1H NMR spectrum of compound **2** showed signals for two tertiary methyl groups at δ 1.11 (3H, *s*) and 1.12 (3H, *s*), a secondary methyl group at δ 1.05 (3H, *d*, *J* = 6.6 Hz), exomethylene protons at δ 4.81 and 4.72 (each 1H, *br s*), two methine proton signals at δ 4.43 (1H, *m*) and 4.12 (1H, *m*), two methylene proton signals at δ 4.31 and 3.87 (each 1H, *d*, *J* = 12.1 Hz), along with three anomeric protons at δ 4.96 (1H, *d*, *J* = 7.5 Hz), 4.68 (1H, *d*, *J* = 7.5 Hz) and 4.51 (1H, *d*, *J* = 7.5 Hz). The ^{13}C NMR spectrum displayed, for the aglycon moiety, signals ascribable to a keto group at δ 215.6, a ketal function at δ 110.1, two secondary alcoholic functions at δ 75.4 and 80.7, and one primary alcoholic function at δ 65.3, suggesting as aglycon a spirostanol skeleton characterized by the occurrence of a keto group (Table 1). On the basis of the HSQC and HMBC correlations, the aglycon moiety of compound **2** was identified as 5β-spirost-25(27)-en-3β-ol-12-one or schidigera-genin B, previously isolated from *Y. schidigera* (Miyakoshi et al., 2000). The chemical shifts of all the individual protons of the three sugar units were ascertained from a combination of 1D-TOCSY and DQF-COSY spectral analysis, and the ^{13}C chemical shifts of their relative attached carbons were assigned unambiguously from the HSQC spectrum (Table 2). These data showed the presence of three β-glucopyranosyl units (δ 4.96, 4.68 and 4.51). Glycosidation shifts

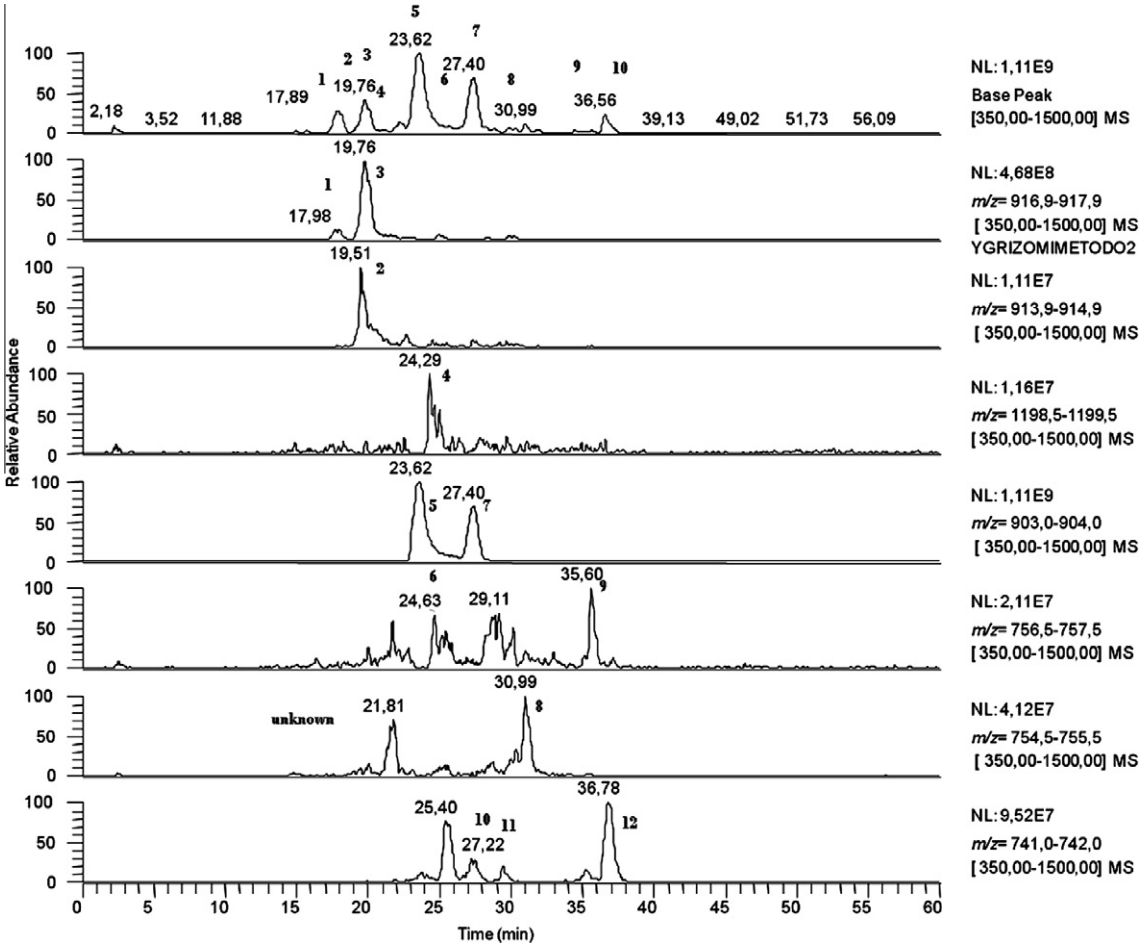

Fig. 1. Positive-ion mode LC–MS analysis of the BuOH extract of *Yucca gloriosa* rhizomes. Total ion current and reconstructed ions chromatogram for compounds **1**–**12**.