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Determination of phenolic compounds in *Yucca gloriosa* bark and root by LC–MS/MS

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ABSTRACT

On the basis of the biological activities shown by yuccaols and gloriosaols from *Yucca schidigera* and *Yucca gloriosa*, the content of yuccaols and gloriosaols in two different parts of *Y. gloriosa* (roots and bark), was determined for each single compound, and compared with phenolic determination in *Y. schidigera* bark, concluding that *Y. gloriosa* bark and roots are rich sources of phenolic derivatives structurally related to resveratrol.

LC/ESIMS (liquid chromatography coupled to electrospray mass spectrometry) qualitative and an LC/ESIMS/MS (liquid chromatography coupled to tandem electrospray mass spectrometry) quantitative studies of the phenolic fraction of *Y. gloriosa* were performed. LC/ESIMS/MS multiple reaction monitoring (MRM) method previously described for yuccaols in *Y. schidigera* was applied and optimised for separation and determination of gloriosaols and yuccaols in *Y. gloriosa*.

Due to the sensitivity and the repeatability of the assay, we suggest this method as suitable for industrial quality control of raw materials and final products.

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1. Introduction

Among the species of *Yucca* genus (Agavaceae) the best known is *Yucca schidigera*, a plant that grows in California and Mexico, recognized by native Indians as a plant with health-promoting activity. The extract of this plant finds wide commercial utilization in food, cosmetic, pharmaceutical, and feeding staff industry for its high content of saponins [1,2]; it is approved by Food and Drug Administration as a food additive, regarded with the “GRAS” label (generally recognized as safe) [3]. Two different products of yucca are available on market, yucca powder and yucca extract. The main application of yucca products is in animal nutrition, in particular as a food additive to reduce ammonia and fecal odors in animal excreta [4]. The positive effects of dietary supplementation with yucca products on the growth rates, feed efficiency, and health of livestock seem to be due not only to the saponin constituents but also to other constituents. These observations prompted us to investigate the phenolic fraction of *Y. schidigera*, and this study led to the isolation from the methanolic extract of *Y. schidigera* bark of resveratrol (**1**) and *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene (**2**) along with the novel yuccaols A (**3**), B (**4**), C (**5**), D (**6**), and E (**7**) [5,6]. The stilbene derivatives yuccaols A–E present spiro-structures rarely occurring in plant kingdom characterised by a C15 unit, probably derived from a flavonoid skeleton and a stilbenic portion linked by a γ-lactone ring. The multifunctional activities of resveratrol together with the novelty of yuccaols A–E, structurally related to resveratrol, prompted us to carry out a program aimed to evaluate some of the activities exerted by resveratrol for yucca phenolics. In this frame a strong radical scavenging activity was observed for all yucca phenolics [7]. Furthermore, the evaluation of the inhibitory effects of yucca phenolics on thrombin-induced platelet aggregation revealed that these compounds showed even stronger anti-platelet activity than resveratrol [8]. They also had an inhibitory effect on the thrombin-induced enzymic platelet lipid peroxidation and inhibited the generation of free radicals in blood platelets activated by thrombin or by thrombin receptor activating peptide (TRAP) [9]. Furthermore, yuccaol C was found to inhibit the expression of the inducible isoform of nitrogen oxide synthase (iNOS) [10]. Yuccaols in addition were found to inhibit Kaposi’s Sarcoma cells proliferation, migration and PAF synthesis [11].

Based on the biological activities observed for *Y. schidigera*, we deemed it of interest to investigate another species of the same genus, *Y. gloriosa*. This species is largely cultivated in eastern Georgia and was previously studied for its saponin content [12]. Studies

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Table 1
Calibration curve equation and regression obtained for compounds **1**–**12**

Compounds	HPLC–MS/MS	
	Calibration equation	R^2
1	$y = 44.11x - 4.08$	0.996
2	$y = 19.65x - 2.76$	0.994
3	$y = 19.66x - 0.95$	0.998
4	$y = 28.50x - 1.54$	0.990
5	$y = 17.91x - 6.00$	0.998
6	$y = 11.9x - 1.12$	0.997
7	$y = 23.5x - 0.99$	0.996
8	$y = 37.1x - 3.55$	0.995
9	$y = 29.45x - 2.11$	0.998
10	$y = 21.48x - 2.51$	0.993
Mix **11** + **12**	$y = 46.76x - 1.25$	0.992

oriented to the investigation of phenolic compounds led to the isolation of new phenolic constituents named gloriosaols A–E (**8**–**12**) [13,14], along with yuccaols C–E previously isolated from *Y. schidigera* [5–7]. Gloriosaols are spiro-structures made up of the same basic C15 and C14 structural units of yuccaols C–E but differing from yuccaols C–E in the occurrence of two C15 units instead of one. Gloriosaols A and B exhibit the two *p*-hydroxyphenyl rings of the C15 units at the opposite side of the stilbenic moiety, gloriosaol C shows the two *p*-hydroxyphenyl rings at the same side of the stilbenic moiety, and in gloriosaols D and E a *p*-hydroxyphenyl ring is oriented to the same side of the stilbenic moiety and the other one is located to the opposite side.

Concerning the production of phytopharmaceuticals, it is very important to characterise a plant with qualitative and quantitative analyses of representative and specific constituents found only in that plant by using modern technologies [15]. On the other hand, phenolic compounds are of great importance for the food and medicinal use of plants, being closely related to the organoleptic and pharmaceutical properties, which makes their analysis of considerable interest. In particular, the potential of HPLC coupled to tandem mass spectrometry (MS/MS) has gained interest as a rapid and efficient tool for the screening of crude plant extracts, due to its inherent accuracy, excellent sensitivity, and enhanced selectivity [16–19]. The large group of biological activities of resveratrol and of yuccaols A–E, peculiar constituents of this plant, prompted us to develop an analytical method for the quality control of the plant and its products based on these phenolic compounds, and to compare different analytical method for the phenolic fraction of this plant [20]. This paper presents a simple, highly sensitive and specific LC/ESIMS/MS method for the simultaneous quantification of different phenols from *Yucca* species. In order to develop and optimise simple and rapid techniques to determine these compounds for the purposes of quality control of collected material and in order to compare the phenolic content of *Y. gloriosa* with that of *Y. schidigera* (Table 1), crude extracts from *Y. gloriosa* bark and root were investigated by LC/ESIMS and LC/ESIMS/MS.

2. Materials and methods

2.1. Reagents and standards

HPLC grade MeOH, acetonitrile and trifluoroacetic acid were purchased from J.T. Baker (Baker Mallinckrodt, Phillipsburg, NJ, USA). HPLC grade water (18 mΩ) was prepared using a Millipore (Bedford, MA, USA) Milli-Q purification system. Standards of resveratrol (**1**), *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene (**2**), yuccaols A–E (**3**–**7**) gloriosaols A–E (**8**–**12**) and dihydroresveratrol, used as internal standard (IS), were isolated in our previous studies [5,7,13,14]. A standard stock solution (1 mg/mL) was prepared by dissolving each compound in MeOH, and four solutions containing, 5, 25, 50, and 125 μg/mL of resveratrol, *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene (**2**), yuccaols and gloriosaols (as external standard) respectively, and 25 μg/mL of dihydroresveratrol (as IS) were prepared in MeOH.

2.2. Plant material and preparation of extracts

The roots and the barks of *Y. gloriosa* were collected in December 2003 in the experimental field of the Institute of Pharmacochemistry of the Academy of Sciences, Tbilisi, Georgia. A voucher specimen (no. 259) was deposited at the Institute of Pharmacochemistry.

Y. schidigera wild bark was obtained from Desert King Int., Chula Vista, CA, USA. Methanolic extract of *Y. gloriosa* bark and root and *Y. schidigera* bark were obtained by suspending wild material powdered (1 g) added with internal standard (dihydroresveratrol) (10 mg) in MeOH (25 mL), working with Ultrasonic for 1 h and then keeping in dark for a night. Extracts were diluted 1:100 with MeOH before injecting 20 μL in LC or LC/MS system.

In order to obtain the enriched phenolic extract, powdered *Y. gloriosa* bark (100 g) and roots (100 g) were extracted with MeOH (2 × 300 mL) at room temperature. After filtration was evaporated to dryness *in vacuo* at 40 °C, yielding a brown solid that was dissolved in 15% MeOH and loaded onto C18 filled column (30 mm × 70 mm, 60 μm, Baker) equilibrated with water. The column was washed with 40% MeOH to remove phenolics, and then with MeOH to wash on the remaining substances.

0.1 mg of the phenolic fraction was dissolved in 1 mL of MeOH, and diluted 1:10 with MeOH before analysis of 20 μL in chromatographic systems.

2.3. ESIMS analysis

ESIMS analyses were performed using a Finnigan (Thermo Finnigan, San José, CA, USA) LCQ Deca ion trap instrument equipped with Xcalibur software. Samples of isolated compounds were dissolved in MeOH to obtain 1 μg/mL solutions and infused into the ES ionisation source using a syringe pump at a flow rate of 5 μL min^{-1}. For the analysis of yuccaols and gloriosaols, the instrument was operating in the positive ion mode with a capillary voltage of 5 V, a spray voltage of 5 kV, and a tube lens offset of −10 V. Capillary temperature was 220 °C, sheath gas (N_2) flow rate was 60 (arbitrary units) and the data were acquired in the MS1 and MS/MS scanning modes. Scan range was *m*/*z* 150–900, maximum injection time was 50 ms, and the number of microscan was 3, for MS/MS scanning mode percentage of collision energy was 25%.

To tune the LCQ for yuccaols and gloriosaols, the voltages on the lenses were optimised in the TunePlus function of the Xcalibur software both in positive ion mode, whilst infusing a standard solution (1 μg resveratrol/1 mL MeOH) at the flow rate of 3 μL min^{-1}. The infusion of a yuccaol standard did not give relevant differences in parameters.

2.4. LC/ESIMS/MS analysis

Bark crude extract and root crude extract of *Y. gloriosa* were analysed by LC/ESIMS/MS "on-line" using a Thermo Finnigan Spectra System HPLC coupled with an LCQ Deca ion trap. Analyses were performed by using a Waters Symmetry C18 column (150 mm × 2.1 mm i.d.; particle size 5 μm) and as mobile phase a gradient of 0.05% trifluoroacetic acid (TFA) as eluent A and acetonitrile with 0.05% TFA as eluent B. Elution was performed by means of a linear gradient from 80:20 (A:B) to 70:30 over 30 min, then an isocratic portion of 70:30 for 10 min, and a subsequent slope to 60:40

in 30 min, at a flow rate of 0.3 mL min^{-1}. The flow generated by chromatographic separation was directly injected into the electrospray ion source. The positive ion mode for MS and MS/MS analysis was selected, working with the condition described in ESIMS paragraph.

For fragmentation pattern study, two scan events were prescribed to run simultaneously in the LCQ mass spectrometer. The first event was a full scan MS to acquire data on ions in the range 200–900 *m*/*z* and the second event was a MS/MS product scan event

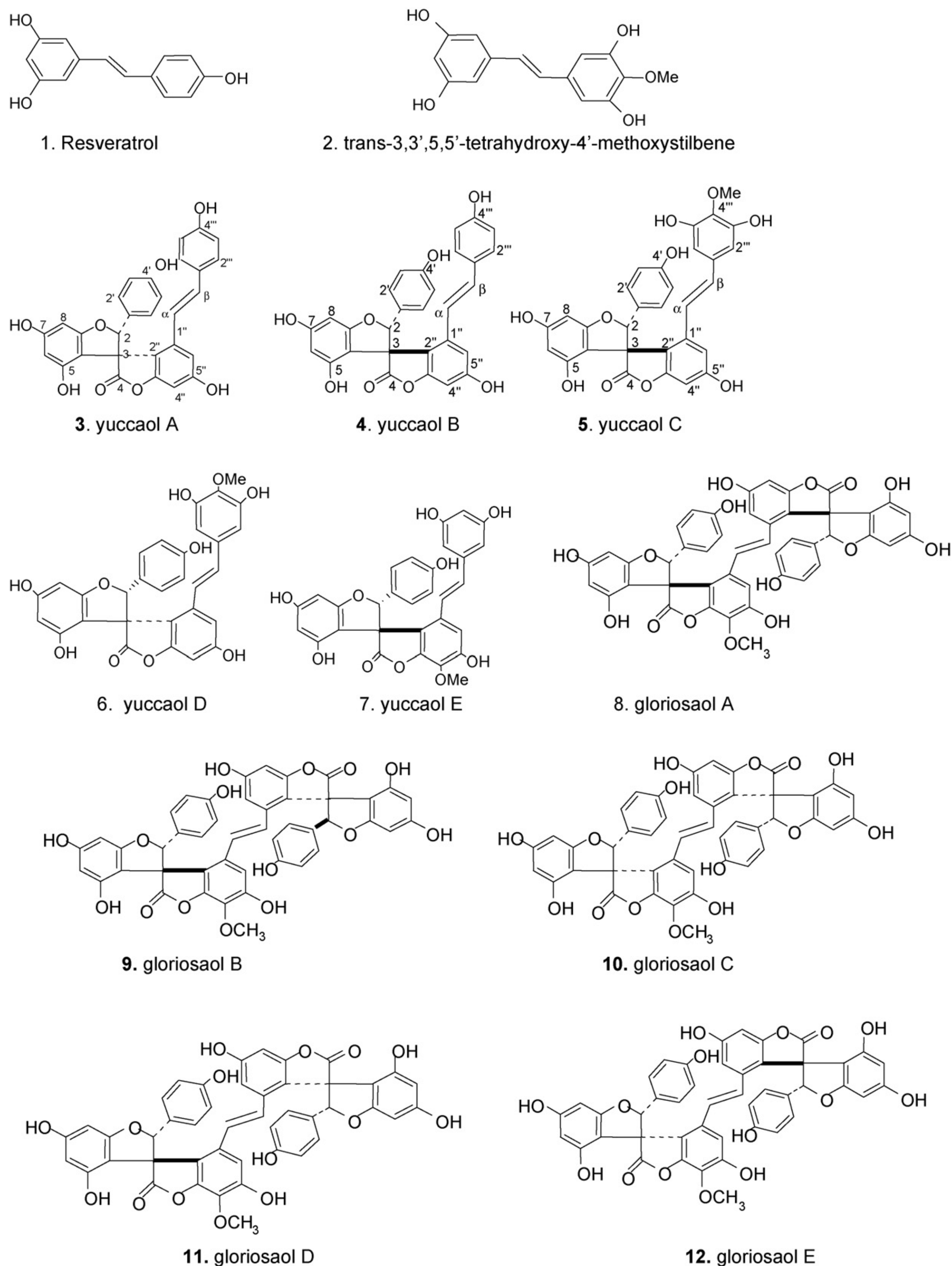

Fig. 1. Phenolic compounds isolated from *Yucca schidigera* and *Yucca gloriosa*.

on selected mass ions, and respectively, m/z 229.0, m/z 275.0, m/z 543.0, m/z 497.0, and m/z 811.0.

Six scan events were prescribed to run simultaneously in the LCQ mass spectrometer. The first event was a full scan MS to acquire data on ions in the range 200–900 m/z. The scan events 2–6 were MS/MS experiments that were carried out on protonated molecule ions of compounds **1**–**12** at the collision energy of 25%. The selected fragmentation reaction was the loss of a phenol neutral fragment for yuccaols, gloriosaols, resveratrol, and internal standard. For compound **1**, the selected fragmentation reaction was the loss of a methoxylated phloroglucinole neutral fragment.

3. Results

Previous phytochemical investigation on methanolic extract of the barks and roots of *Y. gloriosa* led to the isolation of yuccaols C–E (**5**–**7**) and gloriosaols A–E (**8**–**12**). These compounds, along with resveratrol (**1**) and *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene (**2**) and yuccaols A–B (**3**–**4**) reported for *Y. schidigera* [5–7,13,14] are reported in Fig. 1.

3.1. LC/MS

Total ion current profile of positive ion electrospray LC/MS analysis from *Y. gloriosa* barks and roots phenolic extracts (experimental conditions described above) is shown in Fig. 2. Compounds were identified by comparing retention times and m/z values in the total ion current chromatogram to those of the pure isolated compounds. Unfortunately, compounds **11** and **12** were eluted simultaneously and were not separated neither by chromatography neither by mass spectrometry, exhibiting the same molecular weight and the same fragmentation pattern, thus in the subsequent experiments they were quantified as a mixture.

Comparison between the two TIC chromatograms did not show relevant qualitative differences, since the same peaks occurred in the two chromatograms; whereas considerable quantitative differences could be observed. Thus, the application of a quantitative method for a quantitative determination of each single phenolic in *Y. gloriosa* bark and root was applied.

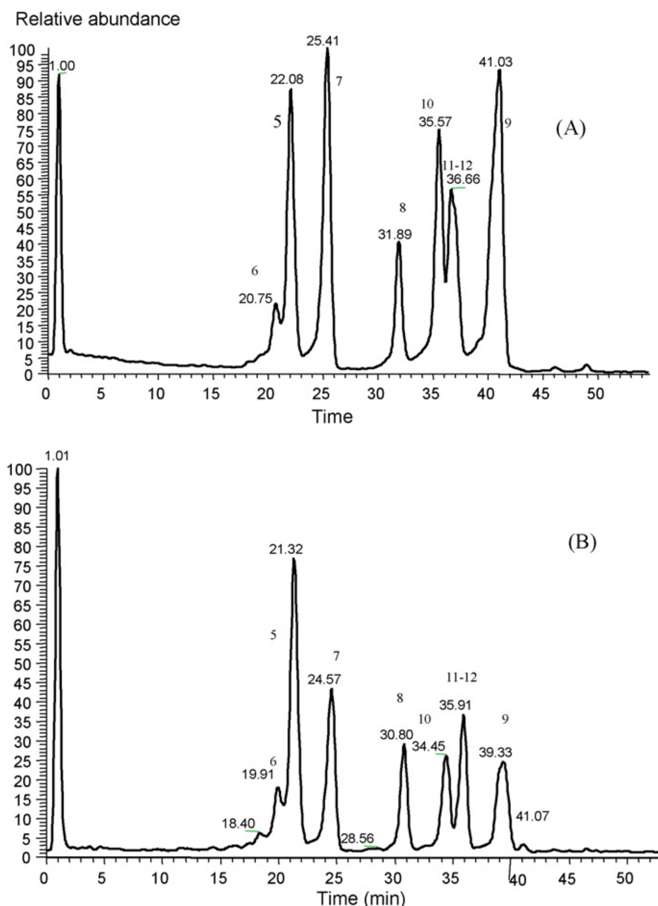

Fig. 2. LC/ESIMS qualitative comparison between *Y. gloriosa* bark (A) and roots (B). Column: Waters Symmetry C18 (150 mm × 2.1 mm i.d.; particle size 5 μm); eluent A: trifluoroacetic acid (TFA) 0.05%; eluent B: acetonitrile + 0.05% TFA. Elution performed by means of a linear gradient from 80:20 (A:B) to 70:30 over 30 min, then an isocratic portion of 70:30 for 10 min, and a subsequent slope to 60:40 in 30 min. Flow rate 0.3 mL min^{-1}. Compounds numbers as seen in Fig. 1.

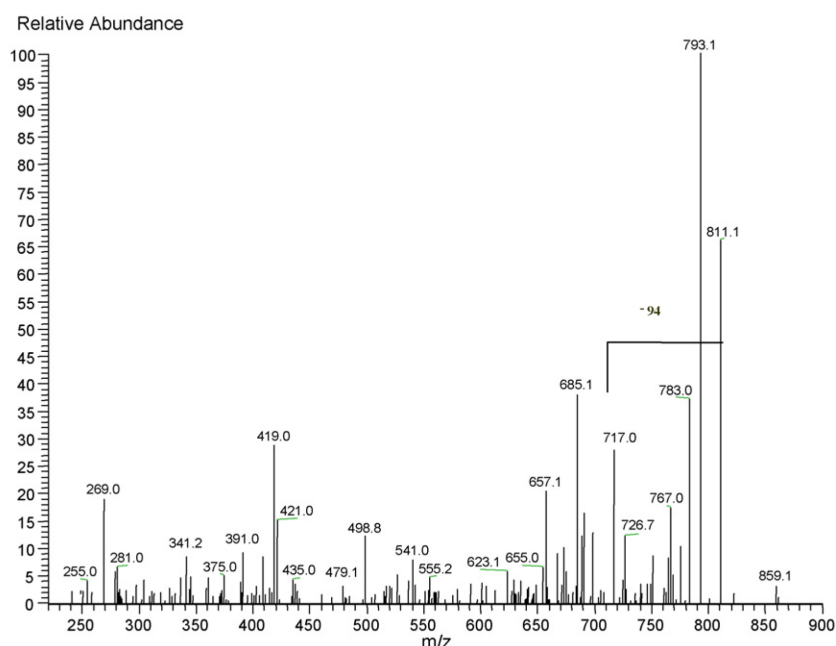

Fig. 3. ESIMS/MS spectrum of gloriosaol C.

3.2. LC/ESIMS/MS product ion scan and multiple reaction monitoring (MRM)

Simultaneous experiments of LC/ESIMS/MS in product ion scan mode, selecting the phenol pseudomolecular ions (m/z 229.0, m/z 275.0, m/z 543.0, m/z 497.0, and m/z 811.0) as precursor ions, were performed. The fragmentation patterns were more informative when spectra were recorded in positive ion mode, and this operative mode was preferred for the LC/ESIMS/MS method development. The loss of a phenolic group (value of m/z 94) was observed in product ion spectra of all compounds occurring in *Y. schidigera*, as reported in our previous paper [20] and for all the gloriosaols occurring in *Y. gloriosa* (Fig. 3).

For these compounds beginning from the selected protonated molecular ions, $[M+H]^+$, the product ions selected were the fragment ions generated after the loss of the phenol group, $[M+H-94]^+$.

The following scheme was used in these experiments:

(1) Compound **1**: precursor ion m/z 229.0, product ion m/z 135.0, and collision energy 25%;
(2) Compound **2**: precursor ion m/z 275.0, product ion m/z 135.0, and collision energy 25%;
(3) Compounds **3** and **4**: precursor ion m/z 497.0, product ion m/z 403.0, and collision energy 25%;
(4) Compounds **5**, **6** and **7**: precursor ion m/z 543.0, product ion m/z 449.0, and collision energy 25%;
(5) Compounds **8**–**12**: precursor ion m/z 811.0, product ion m/z 717.0, and collision energy 25%;
(6) Internal standard: precursor ion m/z 231.0, product ion m/z 137.0, and collision energy 25%.

Since these compounds differ only for stereochemistry they will occur in the same reaction monitoring chromatogram.

The chromatographic profile obtained from the LC/ESIMS/MS MRM experiment did not show all the peaks corresponding to the compounds under investigation, being not present in *Y. gloriosa* compound **1**, neither compound **2**; the other compounds were detected in appreciable intensity for quantitative purpose.

The calibration graphs, obtained by plotting area ratio between external and internal standard versus the known concentration of each compound, were linear in the range of 5–150 μg/mL for all the phenols. In order to build these curves, seven concentration levels of each compound were used. Five aliquots of the methanolic crude extract of the bark of *Y. gloriosa* and five aliquots of the methanolic crude extract of roots were analysed in order to quantify the phenol content. The method resulted to be specific for the phenols and the internal standard, since no interfering compounds could be seen at the elution positions of the phenols compounds. Dihydroresveratrol was selected as a suitable internal standard for the present calibration, since no interfering peaks were seen in any extract sample, thus confirming the advantage in the use of this compound.

3.3. Validation

Validation of the method was realised in agreement with EMEA note guidance on validation of analytical methods [21]. Table 2 shows calibration curve equation and regression obtained for compounds **1**–**12**.

Validation of the LC/MS/MS method included intra- and inter-day precision and accuracy studies on 3 days. Accuracy and precisions were calculated by analysing three samples of each compound at three different concentration levels: 5, 25, and 100 μg/mL. Standard deviations calculated in this assay were <5% for all the three compounds under investigation.

The specificity is the non-interference with other substances detected in the region of interest; the LC/MS/MS method, developed by using a characteristic fragmentation of these peculiar phenolic compounds, resulted to be specific with no any other peak interfering at the retention times of the three marker compounds in the MS/MS detection mode.

Recoveries were determined by the addition of known quantities of the compounds under investigation to known amount of *Y. gloriosa* samples. Quantities were calculated by subtracting total amount of each compound before spiking to the total amount after spiking. Ratio between detected amount and spiked amount was used to calculate the recovery. The mean recovery of the method was 100 ± 2%

The calibration graphs, obtained by plotting area ratio between external and internal standard versus the known concentration of each compound, were linear in the range of 5–100 μg/mL for all compounds.

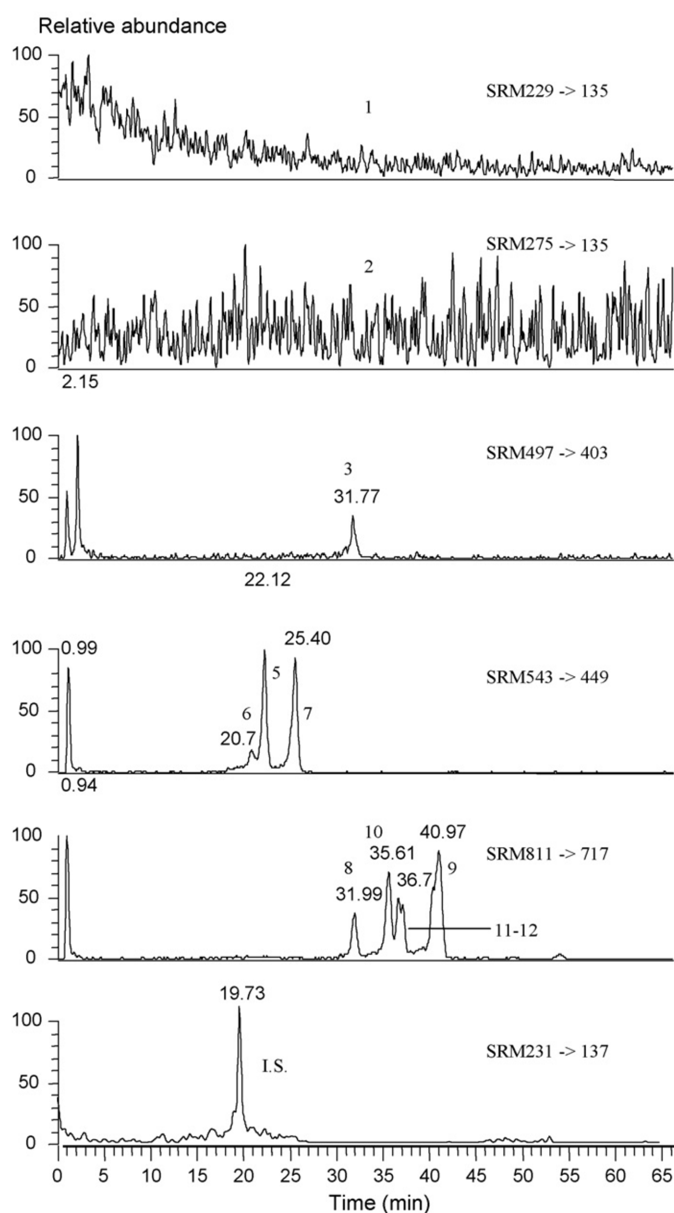

Fig. 4. LC/MS/MS MRM analysis of *Y. gloriosa* roots extract for quantitative purpose. Chromatographic conditions as reported in Fig. 2. Compounds numbers as seen in Fig. 1.

Table 2
Comparison among *Yucca gloriosa* bark, root and *Yucca schidigera* bark, quantitative determination

	Compounds	*Y. gloriosa* bark (mg/g plant)	*Y. gloriosa* roots (mg/g plant)	*Y. schidigera* bark (mg/g plant)
1	Resveratrol	n.d.	n.d.	9.11 ± 2.11
2	*trans*-2,3′,5,5′-tetrahydroxy-4′ methoxystilbene	n.d.	n.d.	9.10 ± 1.10
3	Yuccaol A	2.64 ± 0.80	0.02 ± 0.01	14.72 ± 2.10
4	Yuccaol B	n.d.	n.d.	8.18 ± 4.01
5	Yuccaol C	13.85 ± 1.21	10.68 ± 1.01	12.02 ± 2.28
6	Yuccaol D	6.95 ± 1.59	6.62 ± 2.01	6.63 ± 1.89
7	Yuccaol E	16.19 ± 3.00	1.25 ± 1.02	12.00 ± 3.40
8	Gloriosaol A	11.87 ± 1.32	3.02 ± 1.50	n.d.
9	Gloriosaol B	29.73 ± 2.15	5.30 ± 1.80	n.d.
10	Gloriosaol C	4.53 ± 1.42	3.33 ± 0.91	n.d.
11 + 12	Gloriosaol D, gloriosaol E	10.85 ± 1.58	4.96 ± 1.71	n.d.

n.d. non-detectable.

Quantification limit was measured to establish the sensitivity of the method. Quantification limit is defined as the lowest concentration of compound quantifiable with acceptable accuracy and precision. In the present study it was determined based on the signal to noise ratio, by injection of series of solutions until the signal to noise ratio 10 for LOQ. Each LOQ calculated for each compound was inferior to 10 ng/mL.

3.4. Analyses of Yucca gloriosa samples

Five aliquots of *Y. gloriosa* methanolic extract from bark and five aliquots of *Y. gloriosa* methanolic extract from roots were analysed in order to quantify the phenolic derivatives content.

Fig. 4 shows a representative chromatogram of LC/MS/MS MRM analysis a sample of *Y. gloriosa* roots extract for quantitative purpose. Chromatograms obtained by monitoring the reaction from precursor at 229.0 *m*/*z* to the fragment at 135.0 *m*/*z*, and the reaction from precursor at 275.0 *m*/*z* to the fragment at 135.0 *m*/*z*, selected to investigate the presence of compound **1** and compound **2**, respectively, gave no peak result. The chromatogram obtained by monitoring the reaction from precursor at 497.0 *m*/*z* to the fragment at 403.0 *m*/*z*, selected to investigate the presence of compound **3** and compound **4**, respectively, gave the result of an only peak identified by retention time as compound **3**. The chromatogram obtained by monitoring the reaction from precursor at 543.0 *m*/*z* to the fragment at 499.0 *m*/*z*, selected to investigate the presence of compound **5**, compound **6**, and compound **7** showed three peaks. The chromatogram obtained by monitoring the reaction from precursor at 811.0 *m*/*z* to the fragment at 717.0 *m*/*z*, selected to investigate the presence of compound **8**, compound **9**, compound **10**, and compound **12** showed four peaks. Last chromatogram in the figure is the chromatogram relative to internal standard which presented a peak in the chromatogram obtained by monitoring the reaction from precursor at *m*/*z* 231 to the fragment ion at *m*/*z* 137.

Table 1 reports quantification data for compounds **1**–**12** in samples of *Y. gloriosa* roots and bark compared with *Y. schidigera* phenolic contents.

Resveratrol (**1**) and *trans*-3,3′,5,5′-tetrahydroxy-4′-methoxystilbene (**2**), present in large amount in *Y. schidigera* are not present in *Y. gloriosa* extracts, yuccaol B is not present in *Y. gloriosa*, and yuccaol A is present in the bark extracts from *Y. gloriosa* but not in roots extracts. Quantitative analyses results confirmed that compounds **8**–**12** are major compounds of the plant and, in particular, gloriosaols appear to be more abundant than yuccaols in this species (Table 1).

4. Conclusions

Y. gloriosa bark and roots are rich sources of phenolic derivatives related to resveratrol. Thus *Y. gloriosa* could be alternative to *Y. schidigera* for the isolation of these peculiar compounds that are endowed of different promising biological activities.

LC/MS/MS multiple reaction monitoring quantitative method described for *Y. schidigera* was successfully applied to *Y. gloriosa* optimising it in order to quantify gloriosaols along with yuccaols. The quantitative method described in this paper is also straightforward and convenient because it requires a very fast sample preparation procedure.

References

[1] M. Miyakoshi, Y. Tamura, H. Masuda, K. Mizutani, O. Tanaka, T. Ikeda, K. Ohtani, R. Kasai, K. Yamasaki, J. Nat. Prod. 63 (2000) 332–338.
[2] W. Oleszek, M. Sitek, A. Stochmal, S. Piacente, C. Pizza, P. Cheeke, J. Agric. Food Chem. 49 (2001) 4392–4396.
[3] S. Piacente, C. Pizza, W. Oleszek, Phytochem. Rev. 4 (2005) 177–190.
[4] P. Cheeke, Proc. Am. Soc. Anim. Sci. (1999) 1–10.
[5] W. Oleszek, M. Sitek, A. Stochmal, S. Piacente, C. Pizza, P. Cheeke, J. Agric. Food Chem. 49 (2001) 747–752.
[6] S. Piacente, G. Bifulco, C. Pizza, A. Stochmal, W. Oleszek, Tetrahedron Lett. 43 (2002) 9133–9136.
[7] S. Piacente, P. Montoro, W. Oleszek, C. Pizza, J. Nat. Prod. 67 (2004) 882–885.
[8] B. Olas, B. Wachowicz, A. Stochmal, W. Oleszek, Platelets 13 (2002) 167–173.
[9] B. Olas, B. Wachowicz, A. Stochmal, W. Oleszek, Nutrition 19 (2003) 633–640.
[10] S. Marzocco, S. Piacente, C. Pizza, W. Oleszek, A. Stochmal, A. Pinto, R. Sorrentino, G. Autore, Life Sci. 75 (2004) 1491–1501.
[11] C. Balestrieri, F. Felice, S. Piacente, C. Pizza, P. Montoro, W. Oleszek, V. Visciano, M.L. Balestrieri, Biochem. Pharmacol. 71 (2006) 1479–1487.
[12] A. Skhirtladze, A. Plaza, P. Montoro, M. Benidze, E. Kemertelidze, C. Pizza, S. Piacente, Biochem. Syst. Ecol. 34 (2006) 809–814.
[13] C. Bassarello, G. Bifulco, P. Montoro, A. Skhirtladze, E. Kemertelidze, C. Pizza, S. Piacente, Tetrahedron 63 (2007) 148–154.
[14] C. Bassarello, G. Bifulco, P. Montoro, A. Skhirtladze, M. Benidze, E. Kemertelidze, C. Pizza, S. Piacente, J. Agric. Food Chem. 55 (2007) 6636–6642.
[15] G. Stecher, C.W. Huck, W.M. Stöggl, W. Guggenbichler, R. Bakry, G.K. Bonn, Phytochem. Rev. 1 (2002) 413–426.
[16] M. Carini, G. Aldini, S. Furlanetto, R. Stefani, R.M. Facino, J. Pharm. Biomed. Anal. 24 (2001) 517–526.
[17] M. Chen, F. Song, M. Guo, Z. Liu, S. Liu, Rapid Commun. Mass Spectrom. 16 (2002) 264–271.
[18] A.G. Jensen, K. Ndjoko, J.L. Wolfender, K. Hostettmann, F. Camponovo, F. Soldati, Phytochem. Anal. 13 (2002) 31–38.
[19] P. Hu, Q.L. Liang, G.A. Luo, Z.Z. Zhao, Z.H. Jiang, Chem. Pharm. Bull. 53 (2005) 677–683.
[20] P. Montoro, S. Piacente, W. Oleszek, C. Pizza, J. Mass Spectrom. 39 (2004) 1131–1138.
[21] ICH Q2B International Conference on Harmonisation, London, 1995.