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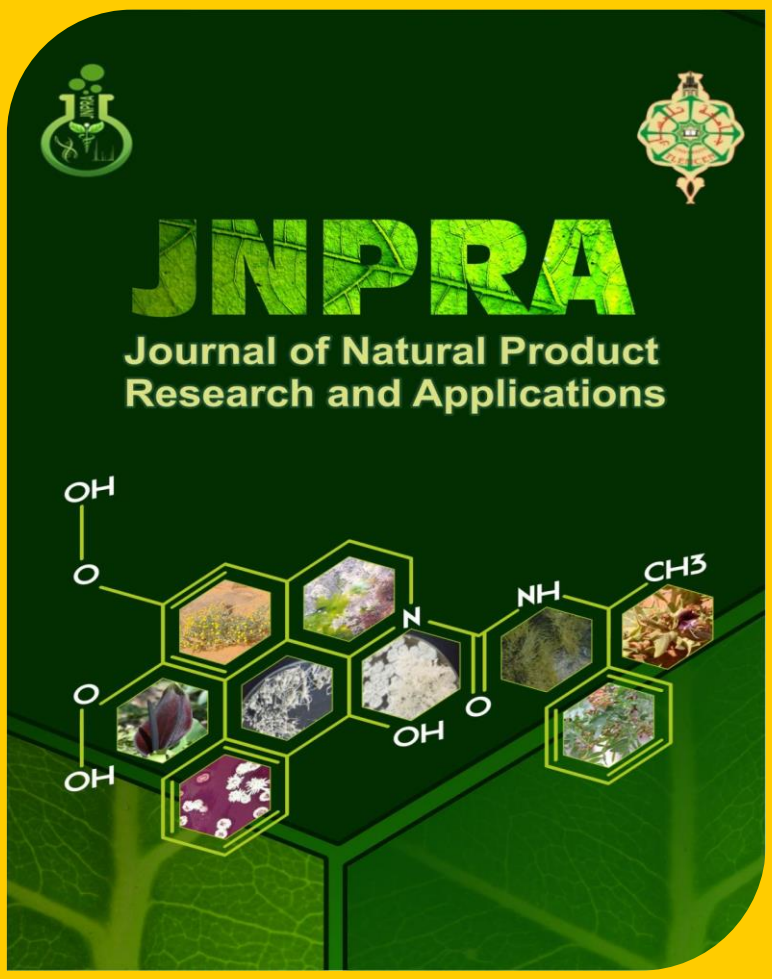

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Research Article

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Rapid identification analysis of chemical constituents of *Sedum villosum* L. (Orpin.) by UHPLC-DAD-HRSM

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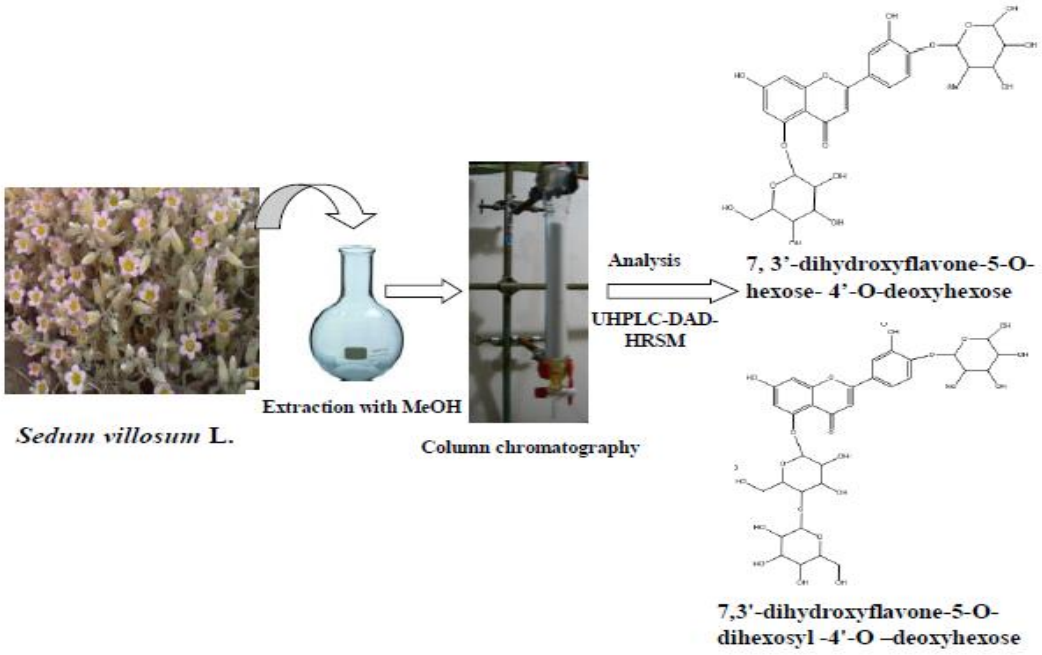
Highlights

- Crude methanolic extract of *Sedum villosum* L.flowers was fractionated on column chromatography.
- 24 fractions were separated.
- F16 showed more antioxidant activity to scavenge DPPH.
- F16 provided two major molecules of glycosyl the flavones.

Graphical Abstract



Abstract
In the present paper, we are interested, for the first time, to separate and identify two glycosyl the flavones from *Sedum villosum* L, family Crassulaceae collected in the flowering phase in Lakhdar Oued, village of Tlemcen (Algeria). The crude methanolic extract of the flowers part was fractionated on column chromatography, and eluted with dichloromethane/methanol each time with increasing polarity of methanol; 24 fractions were separated. One of these fractions named F16 showed more antioxidant activity to scavenge DPPH free radical with percentage inhibition of 94.849 %. F16 was separated by thin-layer chromatography (TLC) to give 10 compounds. We were chosen the sub-fractions F16.8, which has antioxidative activity of 77.02 %, provided two major molecules of glycosyl the flavones, analysed by ultra-high-performance liquid chromatography coupled to mass spectrometry (UHPLC-DAD-HRSM). Compound 1 was identified as 7, 3'-dihydroxyflavone-5-O-dihexosyl-4'-O-deoxyhexose and compound 2 was 7, 3'-dihydroxyflavone-5-O-hexose- 4'-O-deoxyhexose.

Keywords: *Sedum villosum*; column chromatography; preparative TLC; HPLC/SM; antioxidant activity; DPPH.

1. Introduction

The genus Sedum (Crassulaceae) consists of about 470 species, mainly distributed in the northern hemisphere, some located in the southern part of Africa and Latin America (Niu et al., 2011). The phytochemical constituents of *Sedum* species have been extensively reported and some *Sedum* plants have been documented as either vegetables or folk medicines for treatment of many diseases. These plants have been used for a long time in traditional medicine as an anti-inflammatory, keratolytic and analgesic agent, due to its beneficial effect on treating tooth pain or tonsillitis (Szewczyk et al., 2012).
Sedum (Crassulaceae) encompasses a large number of species used in pharmacy. Chemical studies of *Sedum* species have led to the isolation of several classes of substances such as alkaloids, tannins, flavonoids and cyanogenic compounds (De Melo et al., 2009). Among *Sedum* species, *S. villosum* has received great attention in our laboratory. Only two works realized by our research group (Belyagoubi-Benhammou et al., 2014; Ghembaza et al., 2014) described for the first time the phytochemical and the antioxidant properties of *S. villosum* extracts from Algeria. However, there has been no report regarding the chemical composition of this species and its others biological activities so far. The aim of the present study was to separate by column chromatography (CC) on silica gel and thin-layer chromatography (TLC) the active compounds in methanolic extract of the flowers part of *S. villosum.* 24 fractions were obtained. The sub-fraction F16.8 was identified by ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-DAD-HRSM), two major molecules have been identified.

2. Materials and methods

2.1. Plant material

The flowers part of *Sedum villosum* was harvested in April 2010 from Lakhdar Oued in Tlemcen region (Algeria). Specimen was identified in the Laboratory of "Plant Ecology" and voucher specimen was deposited at the Herbarium of the Department of Biology, Tlemcen University, Algeria. The plant samples were dried at room temperature and store for future use.

2.2. Extraction and separation

The powder of the flowers part (50 g) was extracted with 300 ml methanol 96.6° for 24 h at room temperature. The crude extract was filtrated through Whatman N° 0.45 µm, and the filtrate was evaporated to dryness using a rotary evaporator type HAHNVAPOR R-200 at 50 °C. The quantity of 2019 mg of crude methanol extract of *S. villosum* was chromatographied on a chromatographic column (CC) (d= 2.5; l = 44 cm) using 46 g of silica gel and eluted with dichloromethane/methanol gradient (Figure 1).

The eluted fractions were recovered in the UV lamp. The fractions obtained (24 fractions) have been tested for their antioxidant capacity using DPPH radical scavenging activity according to the technique of Sanchez-Moreno et al. (1998). The most active fraction (F16) was separated by preparative thin layer chromatography (stationary phase-silica gel, mobile phase ethyle acetate / formic acid / water; 65/15/20).Ten sub-fractions obtained were tested for their antioxidant capacity using DPPH radical test (Table 1; Photo 1). The sub-fraction F16.8 was purified using column chromatography packed with Sephadex LH-20.

Ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-DAD-HRSM) was used to analyse two major molecules of glycosyl the flavones (Figures 2, 3, 4).

2.3. C18 column gradient UHPLC of isolated compounds

The sub fraction F16.8 was analysed by gradient elution Ultra-High Performance Liquid Chromatography using Agilent 1290 infinity series system consisting of a solvent delivery pump (G4226A) an autosampler (G1316C) a column oven (G4212A) connected to a diode array detector (DAD, G4212A) and a quadrupole mass spectrometer coupled to a time of flight analyser (QTOF, Agilent 6530) electrospray (ESI) positive ion (Agilent Technologies).

Five microliters of extract were separated on column Poroshe 120 C18 (3.0*150 mm, 2.7 µm), with a flow rate of 1.2 ml / min. Mobile phases consist of 0.4% formic acid in water (A) and acetonitrile (B). Phenolic compounds were eluted using a gradient elution, the program was as follows:1% of B ; 0-2 min, 1-7 % of B; 2-15 min, 7-20 % of B ; 15-25 min, 20-40 % of B; 25-35 min, 40-100 % of B; 35-46 min and 100% of B ;46 with 47 min. Detection was performed by a diode array detector (190-600 nm).

The peak areas of the extracts were measured at a wavelength of 280 nm.

2.4. Spectrometry analysis

Mass spectrometry analysis was performed with electrospray ionization (ESI) source. Sample was scanned in both positive ion modes to get the complementary information for structural identification. Electrospray positive (ESI^+), under the following conditions: The ESI $^+$ fashion: voltage to 70V fragmenter, the capillary to 4000V; set gas sources (N_2) at 40 psi with a flow rate of 11 L min at a temperature of 320 °C.

The full analysis of mass spectra (MS) was performed at high resolution (R = 12000) with a scanning from 100 to 3000 m/z (mass / charge ratio) for accurate mass and MS2 scans were performed automatically (acquisition self MSMS) for structural study.

2.5. Data analysis

The results of these experiences were processed using the MassHunter software (Agilent Technologies). The experimental masses and the UV spectra were compared with the available literature to identify the nature of the metabolites.

3. Results and discussion

The most active fractions which have an important antioxidant activity to scavenge DPPH free radical, are F1, F2, F6, F9, F10, F13 , F14, F16, F17, F18, F19, F20, F21, F22, F23 and F24 (Figure 1). The percentages of inhibition are limited between 75.97 and 98.77 %.

The fraction F16 was chosen depending on its high anti-radical activity to neutralize the radical DPPH (94.84 %). This fraction was analysed on TLC plate (Photo 1). It showed better separation with ethyl acetate/formic acid/water (65/15/20) and gave 10 sub-fractions (F16.1-F16.10) of different colors under UV lamp (365 nm) (Table 1). The sub-fraction active F16.8 (77.02 %) obtained from the flowers part of *S. villosum* was analysed by UHPLC/DAD/HRMS in positive ionisation mode, the chromatograms UV and MS are shown in Figure 2.

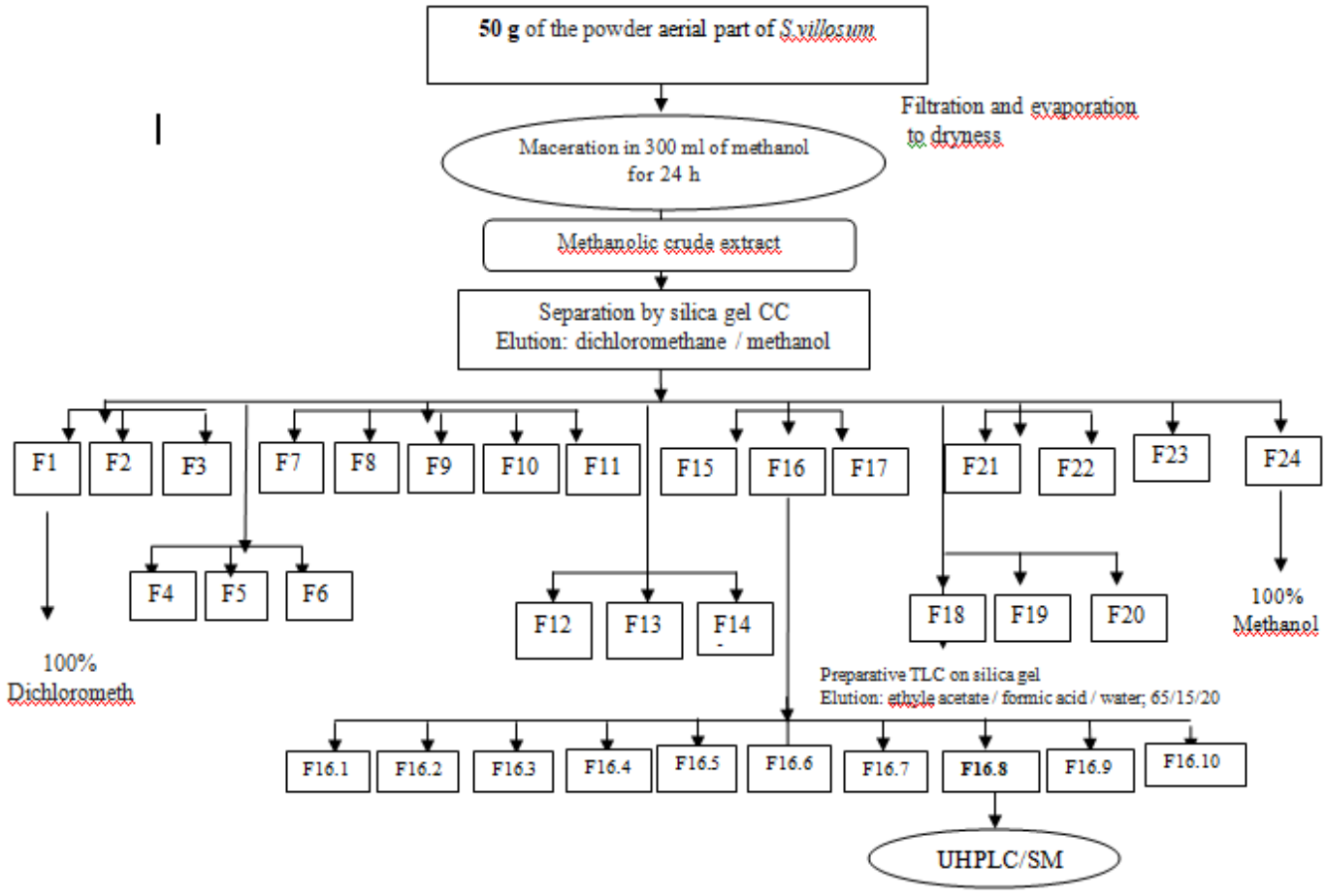


Figure 1. Schematic representation of extraction and separation of methanolic crude extract of *S. villosum.*

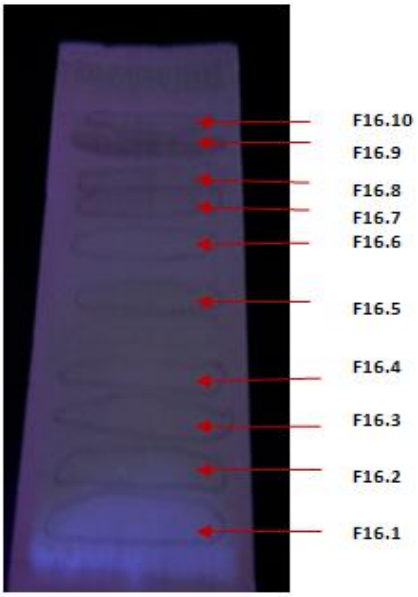


Photo 1. TLC separation on silica gel of the fraction F16 of *S. villosum.*

Table 1. Compounds obtained by the preparative TLC of fraction F16.

Sub-fractions	Color under UV (365 nm)	Rf	Percentage inhibition (%)	Concentration (mg)
F16.1	blue	0.09	11.57	4.08
F16.2	brown	0.22	6.54	2.94
F16.3	blue	0.24	10.87	6.66
F16.4	brown	0.32	8.58	7.38
F16.5	purple	0.46	12.89	8.04
F16.6	pink	0.58	14.84	7.44
F16.7	purple	0.66	15.66	4.20
F16.8	brown	0.71	77.02	4.20
F16.9	purple	0.80	11.01	10.40
F16.10	blue	0.85	90.29	10.20

Two major molecules of the sub-fraction have been identified. Compounds 1 and 2 were eluted at 7.275 and 7.544 min and have masses at m/z 757.2196 and 595.1649 respectively in positive mode $[M + H]^+$. Calculated mass corresponds to the formula $C_{33}H_{40}O_{20}$ for compound 1 and $C_{27}H_{30}O_{15}$ for compound 2 (Figures 3, 4). Compound 1 was identified as 7,3'-dihydroxyflavone-5-O-dihexosyl -4'-O -deoxyhexose and compound 2 was 7, 3'-dihydroxyflavone-5-O-hexose 4'-O-deoxyhexose, the positions of the sugars being allocated on the basis of UV spectra and literature.

No work has been done on the identification of phenolic compounds from the flowers part of *S. villosum*. For other species, 14 phenolic constitutents were isolated from the Korean endemic species *S. takesimense* Nakai. Eight compounds like the ferulic acid, caffeic acid, gallic acid, methyl gallate, myricetin, quercetin, luteolin, and luteolin-7-O-b-D-glucoside are

widely distributed compounds in the plant kingdom, and extensively known to have antioxidant activity. Other five compounds were also identified such as 1-(4-hydroxyphenyl)-2-(3,5-dihydroxyphenyl)-2-hydroxyethanone, rhodalin, rhodalidin, arbutin, gossypetin-8-O-b-D-xylopyranoside and 2,6-di-O-galloylarbutin (Thuong et al., 2007).

In *S. dendroideum* leaf juice, the chemical investigation of this species led to isolate and to identify seven flavonoids: kaempferol 3-O-α- rhamnopyranoside-7-O-α-rhamnopyranoside (kaempferitrin), kaempferol 3-O-β-glucopyranoside-7-O-α-rhamnopyranoside, kaempferol 3-O-neohesperidoside-7-O-α-rhamnopyranoside, kaempferol 3-O-neohesperidoside-7-O-β-glucopyranoside , kaempferol 7-O-α-rhamnopyranoside (α-rhamnoisorobin), kaempferol 3-O-α-rhamnopyranoside (afzelin) and kaempferol (De Melo et al., 2005, 2009). Investigation of a methanol extract from the Chinese endemic species *S. aizoon* L. led to the isolation of two new isoflavone derivatives that were named sedacin A and B (Li et al., 2011).

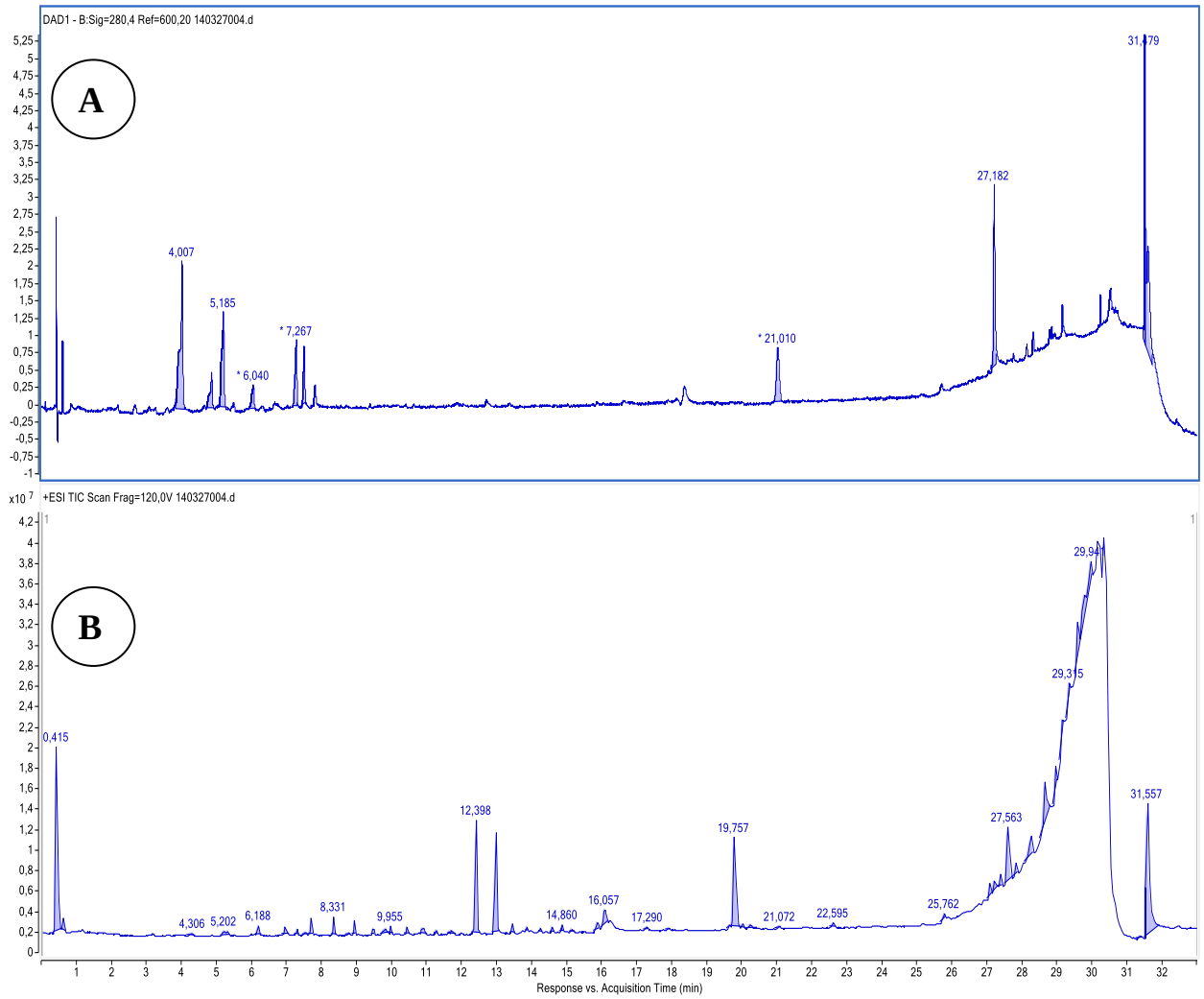


Figure 2. Chromatogram of the sub-fraction F16.8 of the flower part of *S. villosum*: UV recorded at 280 nm (A) and total ion current (TIC) in positive electrospray mode (ESI $^{+}$) (B).

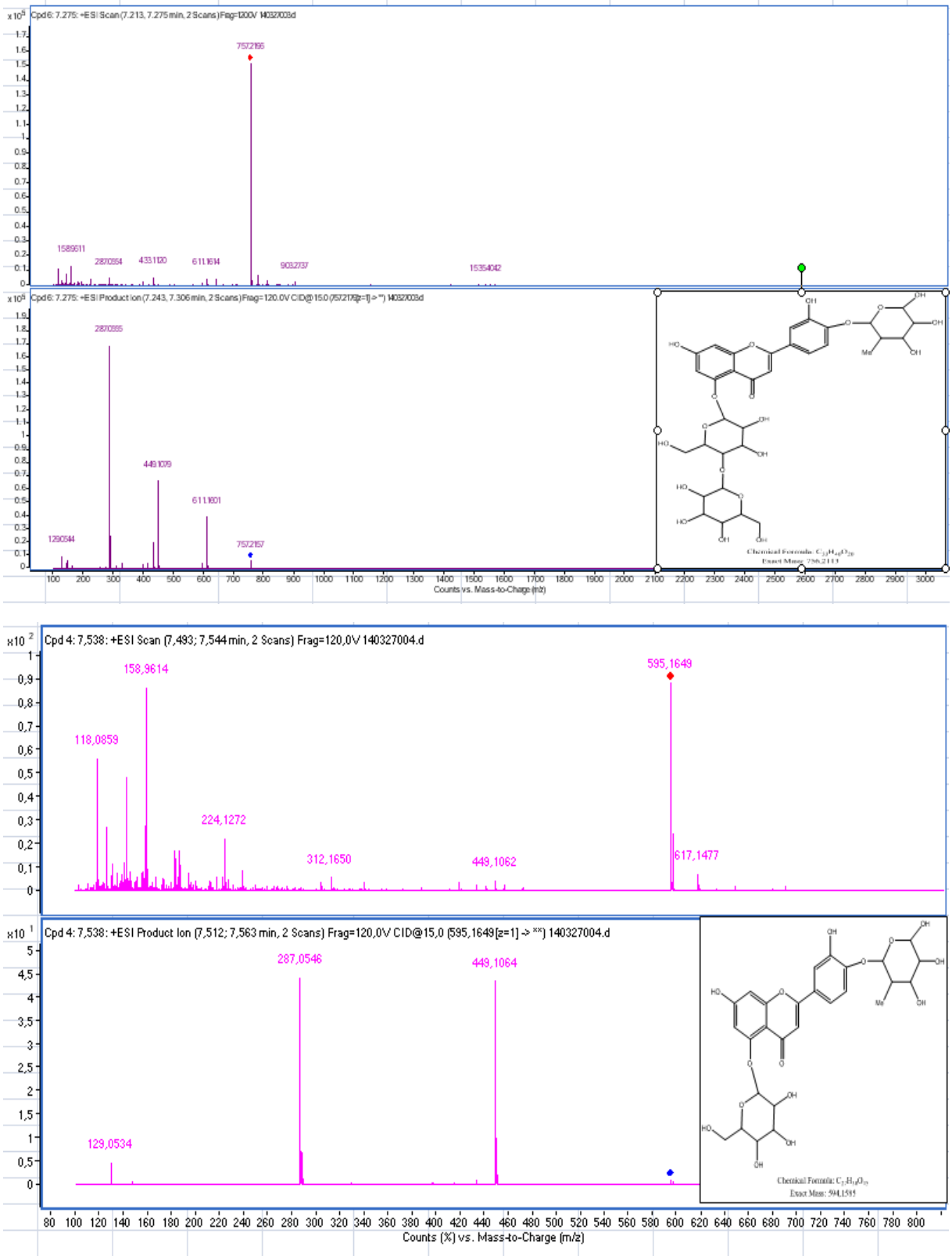


Figure 3. Spectra of fragmentation in positive mode [M + H] + and structures of isolated compounds. (**1**) 7,3'-dihydroxyflavone-5-O-dihexosyl -4'-O –deoxyhexose; (**2**): 7, 3'-dihydroxyflavone-5-O-hexose- 4'-O-deoxyhexose.

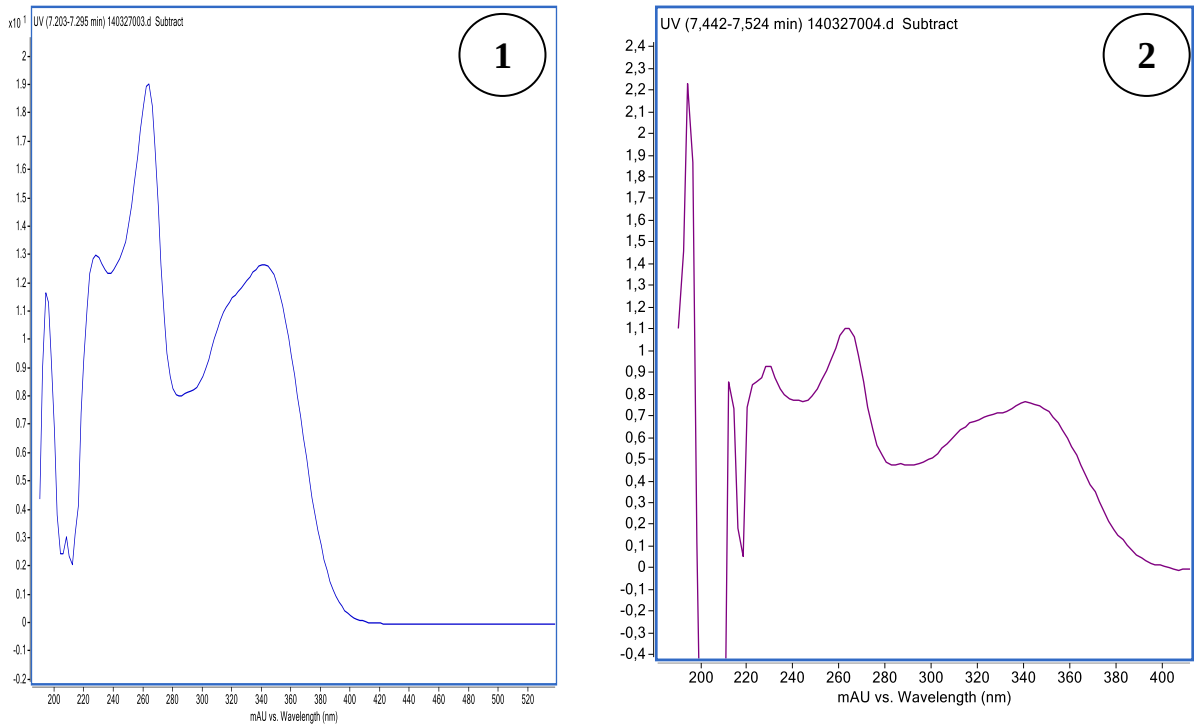


Figure 4. UV Spectra of isolated compounds. (**1**) 7,3'-dihydroxyflavone-5-O-dihexosyl -4'-O –deoxyhexose; (**2**): 7, 3'-dihydroxyflavone-5-O-hexose- 4'-O-deoxyhexose.

3. Conclusions

The results obtained in the present paper demonstrated that the separation of the compounds from the crude methanolic extract of the flowers part of *S. villosum* by column chromatography (CC), and analysis by Ultra-High Performance Liquid Chromatography using Agilent 1290 infinity series system, showed that the compound 1 was identified as 7,3'-dihydroxyflavone-5-O-dihexosyl-4'-O-deoxyhexose and compound 2 was 7,3'-dihydroxyflavone-5-O-hexose-4'-O-deoxyhexose. Other identification is required for the compounds of the same sub-fraction to determine responsible the compound or compounds for the antioxidant activity.

Conflicts of interest

The authors declare no conflicts of interest.

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