UHPLC-DAD-ESI-MS ANALYSIS OF THE *CENTAURIUM ERYTHRAEA* INFUSION

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(ORIGINAL SCIENTIFIC PAPER)
UDC 582.923.1:543.5:547.9
DOI 10.5937/savteh2201013N

Chemical composition was estimated in a tea sample, i.e. infusion prepared from dried aerial herb of *Centaurium erythraea*. The plant was harvested in the Stara Planina mountain (Serbia, 42°43′00″N; 24°55′04″E) during the flowering stage. The flowering tops of the plant were dried to the moisture content of 7.89 (*w/w*) in a dark place. Qualitative analysis was done by Ultrahigh performance liquid chromatography – diode array detector – electrospray mass spectrometry (UHPLC–DAD–ESI–MS/MS) method. The aim of this study was to detect and identify phytochemicals in the infusion prepared in a traditional way and present it as a good source of biologically active substances and bio-antioxidants. The analysis of *C. erythraea* infusion primarily indicated the presence of secoiridoid glycosides (sweroside, gentiopicroside, secologanoside, swertiamarin), xanthones and flavonoids, which promises good quality of tea from Eastern Serbia.

Keywords: *Centaurium erythraea*, UHPLC–DAD–ESI–MS/MS analysis, secoiridoid glycosides, xanthones, flavonoids

Introduction

Common centaury (*C. erythraea*) is a biennial or annual plant from Gentianaceae family, which covers almost all Europe, North Africa and Southwest Asia. It grows mostly on calcareous soils and dry grassy places, including sand dunes and chalky uplands [1-3]. It is a plant with a smooth, erect stems (10–50 cm) with small, tubular, pink flowers, branched inflorescence at the top of the stem [3]. *C. erythraea* is a medicinal plant with a long tradition. Tinctures, tonics, lotions or tea, made from extracts of different parts of the plant, have been traditionally used for treating gastrointestinal disorders, dyspepsia, constipation, fever, anemia, acute jaundice, chronic active hepatitis anorexia, rheumatism, wounds and sores, to stimulate appetite, and to cleanse blood and kidneys [4-6]. *C.erythraea* extracts also exhibit antimutagenic, hepatoprotective, diuretic, antitumorigenic, analgesic, antipyretic, anti-inflammatory gastroprotective, antiulcer, antioxidative, antibacterial and antifungal properties [4,7-10]. The plant is reach in terpenoids, xanthones and phenolic acids [11].

C. erythraea extracts are rich in secoiridoid glycosides, bitter components, which are characteristic for Gentianaceae family, primarily gentiopicroside, sweroside, swertiamarin [3]. According to Molina and co-workers [12], gentiopicroside is responsible for inhibition of the spontaneous contraction of the ileum smooth muscle, as well as, for some analgesic, anti-inflammatory and anti-acetylcholinesterase activities of this plant [13,14]. The aerial parts of plant are rich in sterols (β-sitosterol, stigmasterol, campesterol, brassicasterol, Δ-7-stigmastenol (aerial parts) [15]; phenolic acids (p-coumaric acid, ferulic acid, sinapic acid) [16]; coumarins (isocumarin) [17]; flavonoids (kaempferol) [16] and xanthones (methoxylated and tetraoxygenatedxanthones) [7,18].

Herbal teas made from various medicinal plants are consumed by many cultures all around the world. Medicinal plants prepared for tea infusions have been studied for their different biological ability, such as antioxidant, anti-inflammatory and antimicrobial activity [19]. Herbal teas have also been reported to exhibit synergistic antioxidant effects, which increases their value as beverages for potential health benefits. The aim of this study is to identify the key compounds present in *C. erythraea tea* infusion, a common way of consuming herbal beverages.

Experimental

Material and methods

C. erythraea was harvested in Stara Planina (Serbia, 42°43′00″N; 24°55′04″E) during the flowering stage and the flowering tops of the plant were dried to the moisture content of 7.89 (*w/w*) in a dark place. The infusion was made by using traditional way (1 tablespoon of dried herbs was poured over by 200 mL of boiling water to steep and covered for 15 min, after which the tea was poured through a strainer).

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The manuscript received: November, 23, 2021.
Paper accepted: March, 18, 2022.

UHPLC-DAD-MS/MS analysis

The chromatographic separation was performed by liquid chromatography with ultra-high performances (UHPLC) on Hypersil gold C18 column (50×2.1 mm, 1.9 μm) at 25 °C using a Dionex Ultimate 3000 UHPLC+ system equipped with a diode array (DAD) detector and LCQ Fleet Ion Trap Mass Spectrometer, Thermo Fisher Scientific, USA. Mobile phase composed of two solvents, 0.1% formic acid in water (A) and methanol (B) at 0.250 mL/min flow rate yielding linear gradient program: 10–30% (B) for first two minutes, then 35–40% (B) for 4–5 min and 60–90% (B) for 8–11 min, followed by isocratic run at 90% for 11–14 min and 90–10% (B) from 14 to 14.01 min with the isocratic run at 10% (B) to 20th min. Injection volume was 5 μl. Absorption spectra were recorded on DAD-detector with total spectral range between 200 and 800 nm. Mass spectrometric analysis was performed using a 3D–ion trap with electrospray ionization (ESI) in both negative and positive ion mode.

The ESI–source parameters were as follows: source voltage 4.5 kV and 5 kV, capillary voltage −41 V and 49 V, tube lens voltage −95 V and 100 V, for negative and positive polarity mode, respectively and capillary temperature 350 °C, nitrogen sheath and auxiliary gas flow 32 and 8 arbitrary units for both modes. Additional source ionization was MS−spectra were acquired by full range acquisition of *m/z* 100–900, with a tandem mass spectrometry analysis performed by a data dependent scan – the collision-induced dissociation of detected molecular ions peaks ($[M-H]^-$ and $[M+H]^+$) tuned at 30 eV. Xcalibur software (version 2.1) was used for instrument control, data acquisition and data analysis.

The assignation of the detected compounds was based on their retention times, UV–Vis and MS/MS spectra from corresponding UHPLC chromatograms. Identification of detected compounds was also provided by using reference standards for some compounds (citric acid, chlorogenic acid andrutindihydrate). The corresponding data were compared with the available literature, *i.e.* using absorption UV–Vis and mass spectra.

Results and discussion

The typical UHPLC chromatogram recorded from DAD-signal at λ=300 nm is shown in Figure 1. List of detected compounds in the sample with the corresponding chromatography, UV-Vis, MS/MS data and assignments are shown in Table 1.

The aerial parts of *C. erythraea* are rich in the secoiridoid glycosides (swertiamarine, gentiopicroside and sweroside) and phenols (xanthones, flavonoids and phenolic acids). Citric acid, 5-*O*-caffeoyl-quinic acid and rutin were identified by using reference standard method based on the retention times and overlay the corresponding UV-Vis and MS/MS spectra (compounds No. 1, 5 and 15, respectively, Table 1, Figure 1).

Secologanoside and sweroside as derivatives of the secoiridoid glycosides were also detected in the *C. erythraea* infusions(Table 1). Secologanoside with molecular ion $[M-H]^-$at *m/z* 389 with also intensive ions at m/z 345(100%), 209,165, and 121 in the MS/MS spectrum, was detected under the peak No. 10 in UHPLC chromatogram (Table 1, Figures 1 and 2a) [1]; the corresponding UV/Vis spectrum with absorption maximum at λ_{max}= 232 nm was shown in Figure 2b. Sweroside with molecular ion ($[M+H]^+$ at 359 *m/z* was detected as a peak No. 11 in UHPLC chromatogram (Table 1, Figure 1). An appropriate MS/MS spectrum (Figure 3a) confirmed the presence of this compound, with corresponding fragment ions detected at *m/z* 197(100%), 179 and 127 [1] in positive ion mode. Corresponding UV/Vis spectrum (Figure 3b) contains absorption maximum at λ_{max} = 248 nm.

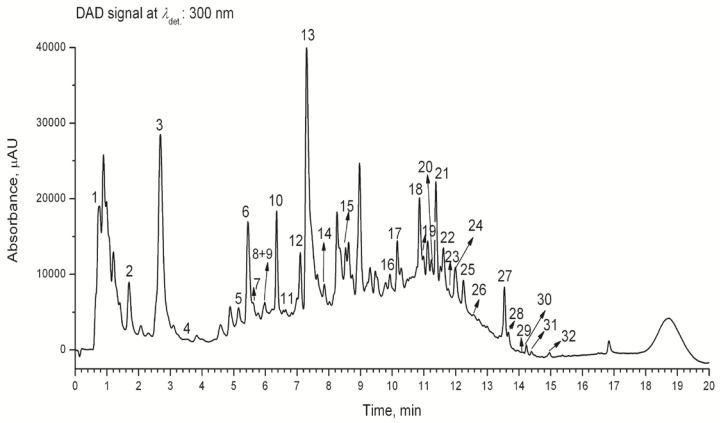


Figure 1. UHPLC chromatogram of *C. erythraea* infusion estimated from DAD signal at λ_{max}= 300nm.

Table 1. List of detected compounds in *C. erythraea* infusion by UHPLC-DAD-MS/MS analysis

Peak No.	t_R, min (MS signal)	λ_{max}, nm	Molecular ion $[M-H]^-$ /$^a[M+H]^+$ *m/z*	MS/MS fragment ions	Assignment [reference]
1	0.90	-	191	173,111(100%),85	Citric acid (standard)
2	2.09	-	161	143(100%),115,71	3- or 4-Hydroxy-2-oxoglutaric acid [20]
3	2.75	297	[a]199	[a]155(100%)	[b]Syringic acid [21]
4	3.50	-	407	179(100%),161,143,131	n.i.
5	5.28	328	353	191(100%)	5-*O*-caffeoyl-quinic acid (standard)
6	5.53	328 300sh	325	193(100%),134	Hydroxy-methoxy-cinnamate derivative [c](MB: KO001109) [22]
7	5.72	240	581	535(100%),517,341	carboxyl derivative of p-Coumaroyl-secologanoside [23]
8	6.01	-	419	355,179(100%),161,143	n.i.
9	6.04	-	[a]771	[a]397(100%)	n.i.
10	6.40	232	389	345(100%),209,165,121	Secologanoside [1]
11	6.76	248	[a]359	[a]197(100%),179,127	Sweroside [1]
12	7.22	297	433	179(100%)	Swertiamarin derivative [23]
13	7.37	329 300sh	473	311,293(100%),179	Caftaric acid hexoside [24]
14	7.85	320	403	371(100%),227,179,165,121	[b]Secoxyloganin [21]
15	8.96	365	609	301(100%),255	Rutin (standard)
16	9.91	-	447	301(100%),285	Quercetin-pentoside [c](MB: FIO00586) [22]
17	10.36	336	551	507(100%),489,389,220	Caffeoyl-O-secologanoside[1]
18	10.73	-	551	389,357(100%),161	Gentioside, isomer [25]
19	10.95	317	885	739(100%)	Kaempferol-glycoside derivative [23]
20	11.25	321	551	357(100%)	Gentioside, isomer [25]
21	11.37	309	[a]349	[a]334(100%),319,301	Dihydroxy-tetramethoxy-xanthone, isomer 1 [1]
22	11.47	329	551	389,357(100%),329,179	Gentioside, isomer [25]
23	12.10	332	273	258(100%)	Trihydroxy-monomethoxy-xanthone, isomer 1[23]
24	12.34	314	273	258(100%)	Trihydroxy-monometoxy-xanthone, isomer 2 [1]
25	12.62	330	273	258(100%)	Trihydroxy-monometoxy-xanthone, isomer 3 [1]
26	12.80	315	287	272(100%),257	Dihydroxy-dimethoxy-xanthone, isomer 1 [1]
27	13.53	316	[a]349	[a]334(100%),319,301	Dihydroxy-tetramethoxy-xanthone, isomer 2 [1]
28	13.75	321	287	272(100%)	Dihydroxy-dimethoxy-xanthone, isomer 2 [1]
29	14.05	-	[a]303	[a]288(100%),271,227	Monohydroxy-trimetoxy-xanthone [1]
30	14.10	-	303	288(100%),273	Trihydroxy-dimethoxy-xanthone [23]
31	14.47	315	333	318(100%),303	Trihydroxy-trimethoxy-xanthone [23]
32	15.04	334	[a]349	[a]334(100%),319,301	Dihydroxy-tetramethoxy-xanthone, isomer 3 [1]

[a] - values obtained by positive ESI MS analysis
[b] - https://pubchem.ncbi.nlm.nih.gov
[c] - https://massbank.eu
sh - shoulder

In the infusion sample, carboxyl derivative of p-coumaroyl-secologanoside, swertiamarin, secoxyloganin, caffeoyl-*O*-secologanoside derivatives of secoiridoid glycosides with molecular ions were also detected in negative ion mode at *m/z* 581, 433, 403, and 551 respectively (peaks No. 7, 12, 14 and 17, Table 1) [23]. High content of bitter substances, the secoiridoid glycosides (mostly used in the treatment of dyspeptic disorders and loss of appetite), has a great impact on medicinal efficiency of the plant [26].

Citric acid ([M-H]–, *m/z* 191) was detected as a peak No. 1 (Table 1, Figure 1), 3-or 4-hydroxy-2-oxoglutaric acid [20], syringic acid, 5-*O*-caffeoyl-quinic acid, hydroxy-methoxy-cinnamate derivative and caftaric acid hexoside were detected in the infusion sample (peaks No. 2, 3, 5, 6 and 13), as shown in Table 1, Figure 1. For syringic acid (comp. No. 3) and caftaric acid glycoside derivative (comp. No. 13) the corresponding MS/MS spectrum in positive and negative ion mode and UV-Vis spectrum were shown in Figures 4 and 5, respectively. Syringic acid is phenolic compound with antioxidant, antimicrobial, antiinflammatory, antiendotoxic, neuro and hepatoprotective activities. It`s also an effective free radical scavenger and alleviates the oxidative stress markers [27]. Syringic acid was detected in positive ion mode at *m/z*155, (peak No. 3, Table 1, Figure 4,). Caftaric acid hexoside was detected as peak No.13 (Table 1) in negative ion mode at *m/z* 311 and 179 (Figure 5). These phenolic derivatives possess anti-oxidant, antimicrobial, anti-inflammatory, antimutagenic activity, and in-

crease the absorption of the acid in the intestinal Caco-2 cells [28].

Flavonoids such as rutin and quercetin-pentoside (peaks No. 15 and 16) with molecular ion peaks in negative ion mode at *m/z* 609 and 447 respectively, were also detected (Table 1, Figure 1). Kaempferol glycoside was identified under the peak No. 19 ([M-H]–, *m/z* 551) [23].

Infusion also contains xanthones and their derivatives, such as isomers of gentioside, dihydroxy-tetramethoxy-xanthone, trihydroxy-monomethoxy-xanthone, dihydroxy-dimethoxy-xanthone, dihydroxy-tetramethoxy-xanthone, monohydroxy-trimetoxy-xanthone, trihydroxy-dimethoxy-xanthone, trihydroxy-trimethoxy-xanthone, dihydroxy-tetramethoxy-xanthone (peaks No. 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 and 32 respectively, Table 1) [1,23,24]. The corresponding MS/MS spectrum in positive ion mode and UV-Vis spectrum were shown in Figure 6 for the compound No. 27 assigned as isomer of dihydroxy-tetramethoxy-xanthone. Xantones, including its glycoside derivatives, have shown several biological effects, including antioxidant, antitumor and antiinflammatory activities [1,29].

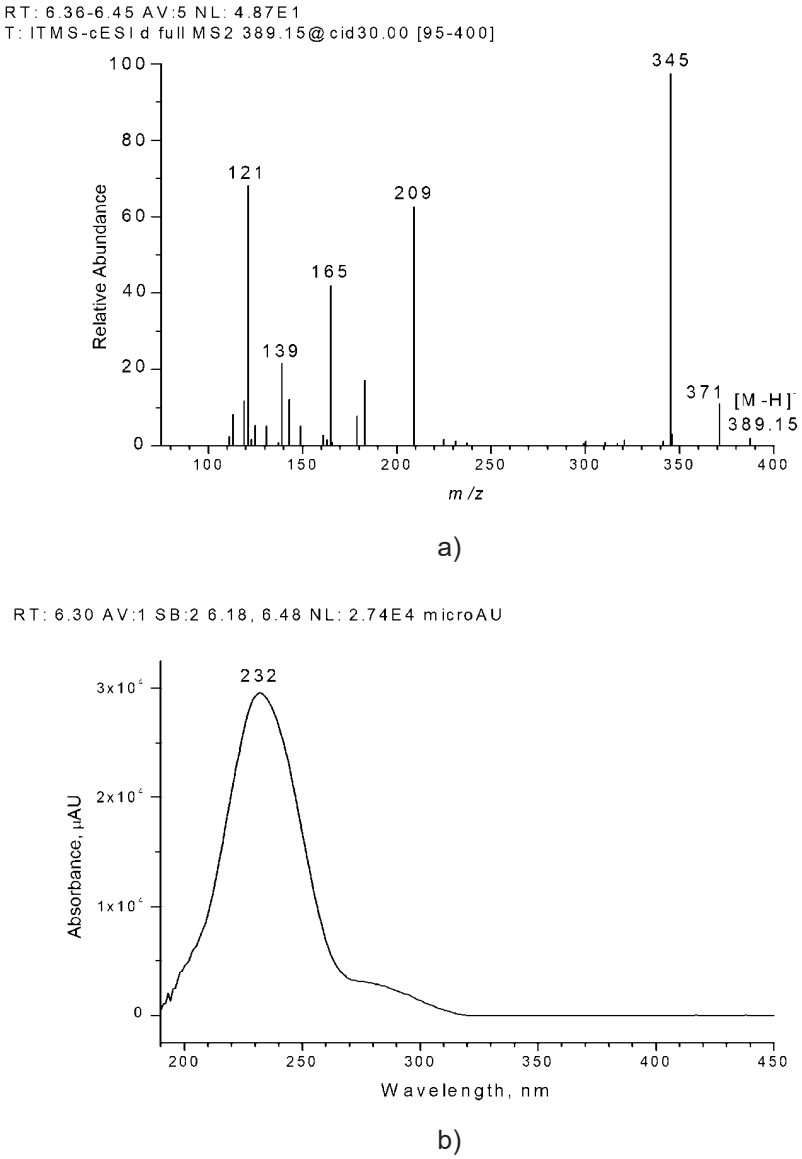
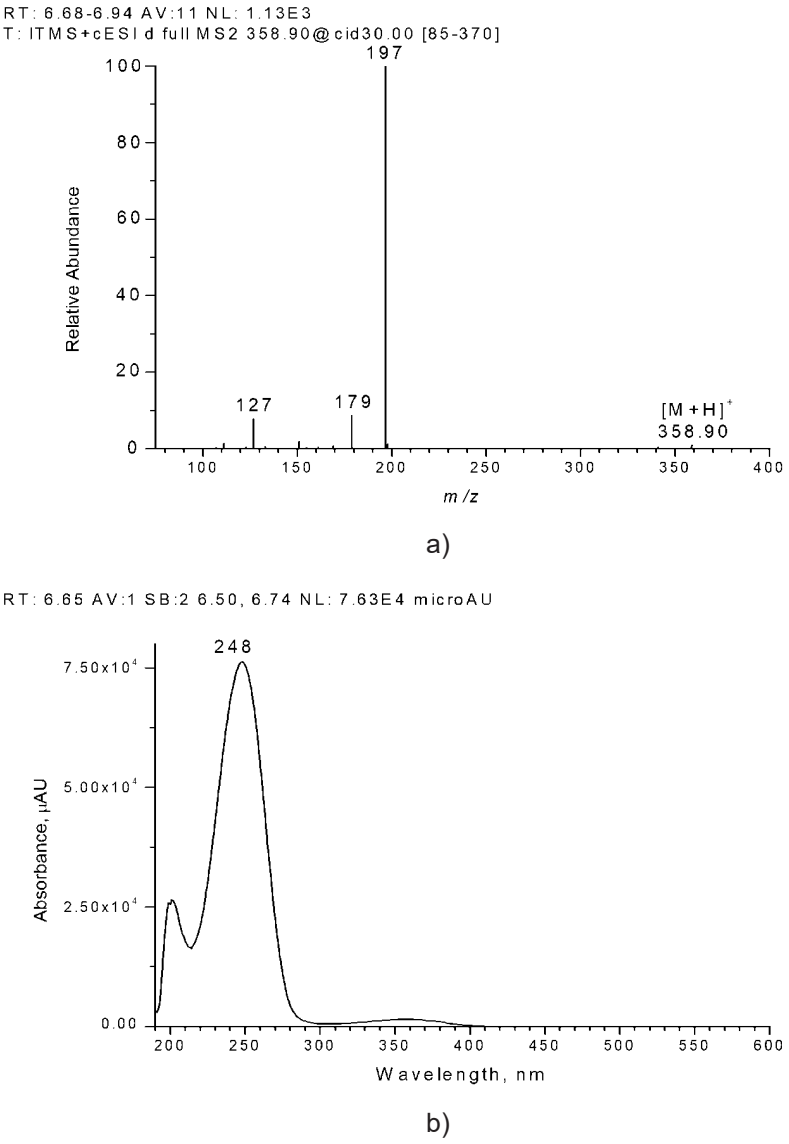


Figure 2. (-)ESI MS/MS (a) and UV-Vis (b) spectra of compound No. 10 assigned as secologanoside (Table 1).



a)

b)

Figure 3. (+)ESI MS/MS and UV-Vis spectra of compound No. 11 assigned as sweroside (Table 1).

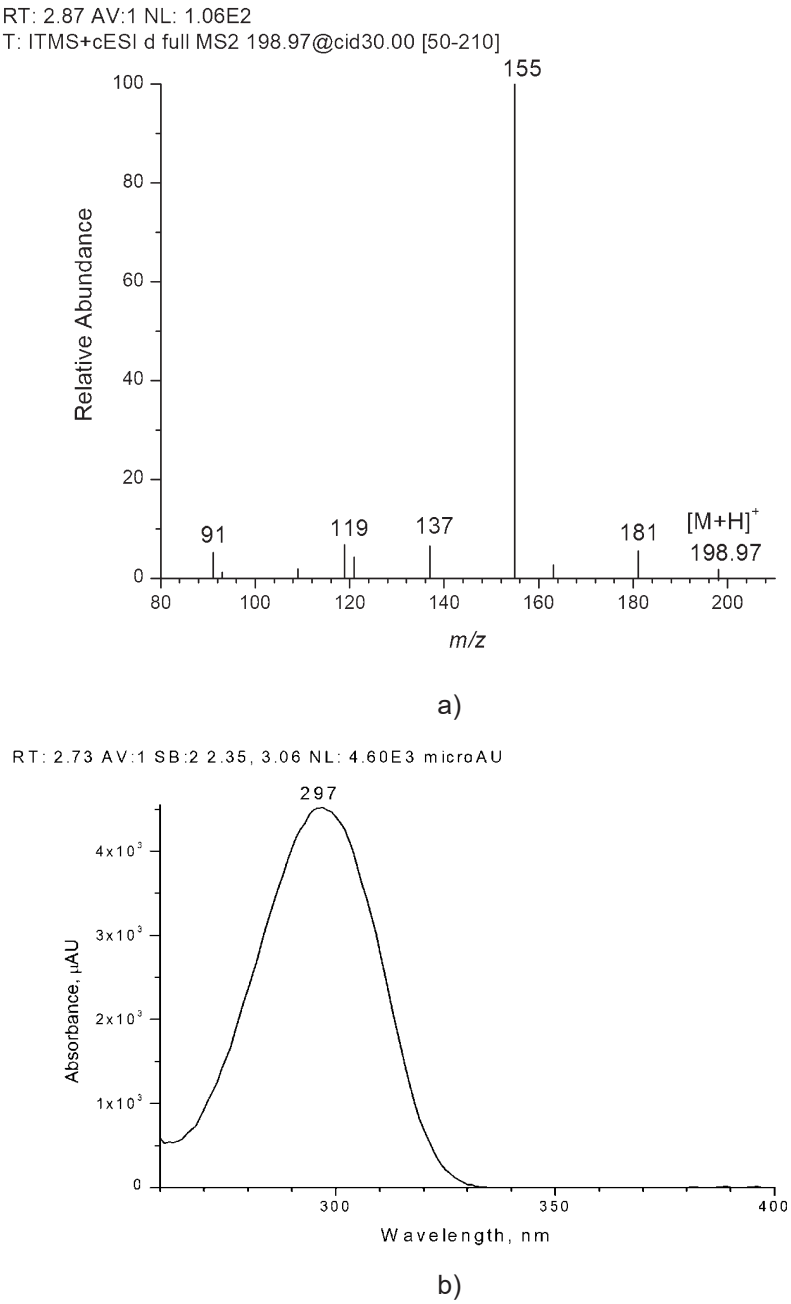
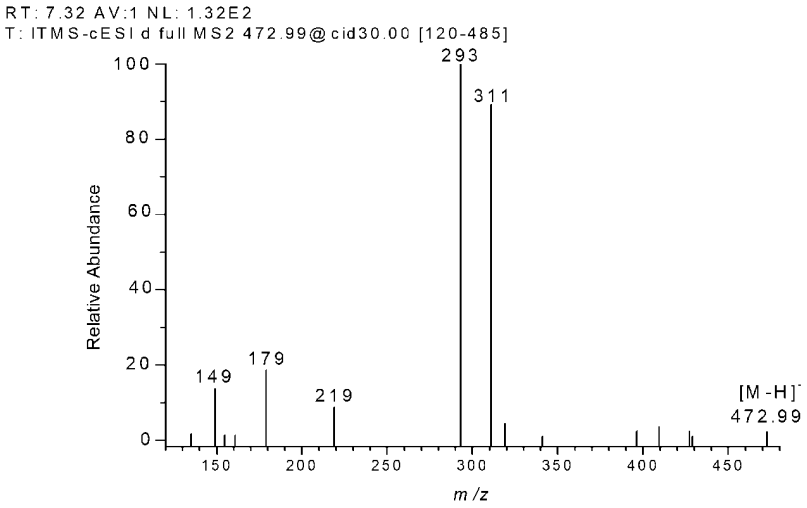
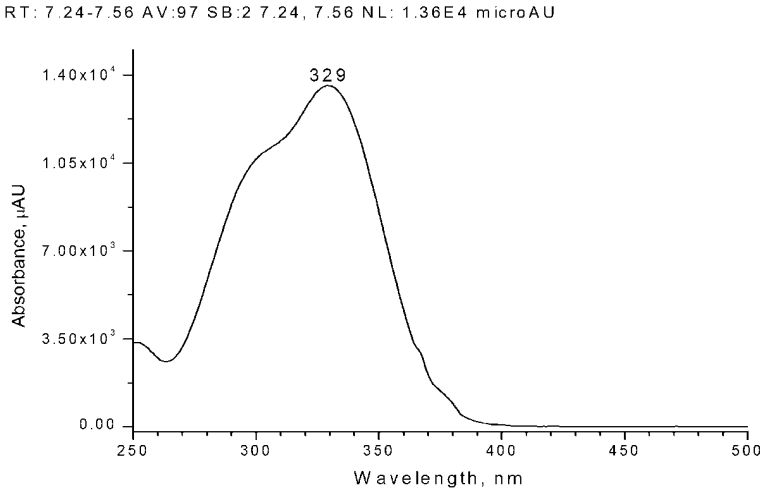


Figure 4. (+)ESI MS/MS (a) and UV-Vis spectrum (b) of compound No. 3 identified as syringic acid (Table 1).



a)



b)

Figure 5. (-)ESI MS/MS (a) and UV-Vis spectrum (b) of compound No. 13 identified as caftaric acid hexoside (Table 1).

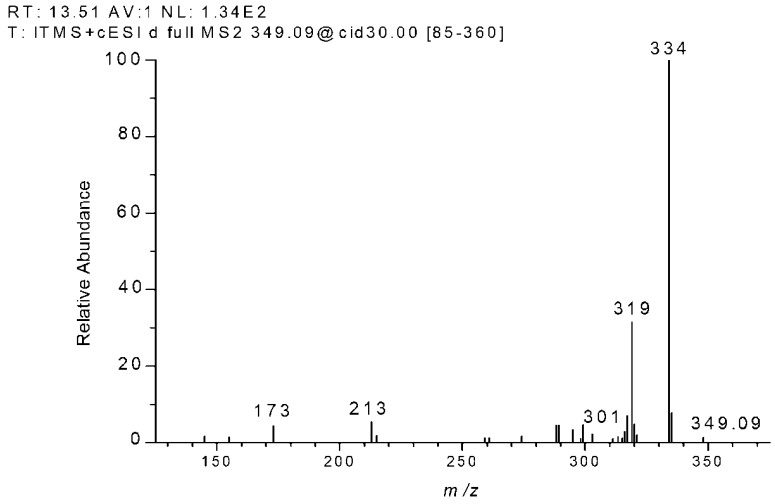


Figure 6. (+)ESI MS/MS spectrum of compound No. 27 identified as isomer of dihydroxy-tetramethoxy-xanthone (Table 1).

Conclusion

The results of the study reveal that compounds of *C. erythraea* infusion could be recommended as a good source of biologically active substances and bio-antioxidants with a potential beneficial effects. The UHPLC-DAD-ESI-MS/MS analysis show presence of secoiridoid glycosides (swertiamarine, gentiopicroside and sweroside) and phenols (xanthones, flavonoids and phenolic acids) in the aerial parts of *C. erythraea*, which are also rich in xanthones and their derivatives, such as isomers of gentioside, dihydroxy-tetramethoxy-xanthone, trihydroxy-monomethoxy-xanthone, dihydroxy-dimethoxy-xanthone, dihydroxy-tetramethoxy-xanthone, monohydroxy-trimetoxy-xanthone, trihydroxy-dimethoxy-xanthone, trihydroxy-trimethoxy-xanthone, dihydroxy-tetramethoxy-xanthone. Further, the infusion possesses flavonoids (rutin and quercetin-pentoside), acids (citric, 3- or 4-hydroxy-2-oxoglutaric, syringic, 5-*O*-caffeoyl-quinic) and caftaric acid glycoside derivative. All identified compounds promise good quality of tea from Eastern Serbia.

Acknowledgment

This work was supported by the Republic of Serbia - Ministry of Education, Science and Technological Development, Program for financing scientific research work, number 451-03-68/2022-14/ 200133.

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(ORIGINALNI NAUČNI RAD)
UDK 582.923.1:543.5:547.9
DOI 10.5937/savteh2201013N

U radu su prezentovani rezultati koji su dobijeni analizom hemijskog sastava infuzije, pripremljene na tradicionalni način od sušenih delova biljke kičice (*Centaurium erythraea*). Biljka je skupljana na Staroj planini (Srbija, 42°43′00″N; 24°55′04″E) za vreme cvetanja i sušenja na tamnom mestu do sadržaja vlage od 7,89 (*w/w*). Kvalitativna analiza je urađena pomoću tečne hromatografije i masene spektrometrije (UHPLC–DAD–ESI–MS/MS) sa ciljem detekcije i identifikacije fitohemijskih jedinjenja u infuziji *C.erythraea*. Rezultati pokazuju da je analizirani uzorak bogat sekoiridoidnim glikozidima (sverozid, genciopikrozid, svercijamarin), ksantonima i flavonoidima, koji ukazuju na dobar kvalitet čajeva Istočne Evrope.

Ključne reči: *Centaurium erythraea,* UHPLC–DAD–ESI–MS/MS analiza, sekoiridoidni glikozidi, ksantoni, flavonoidi